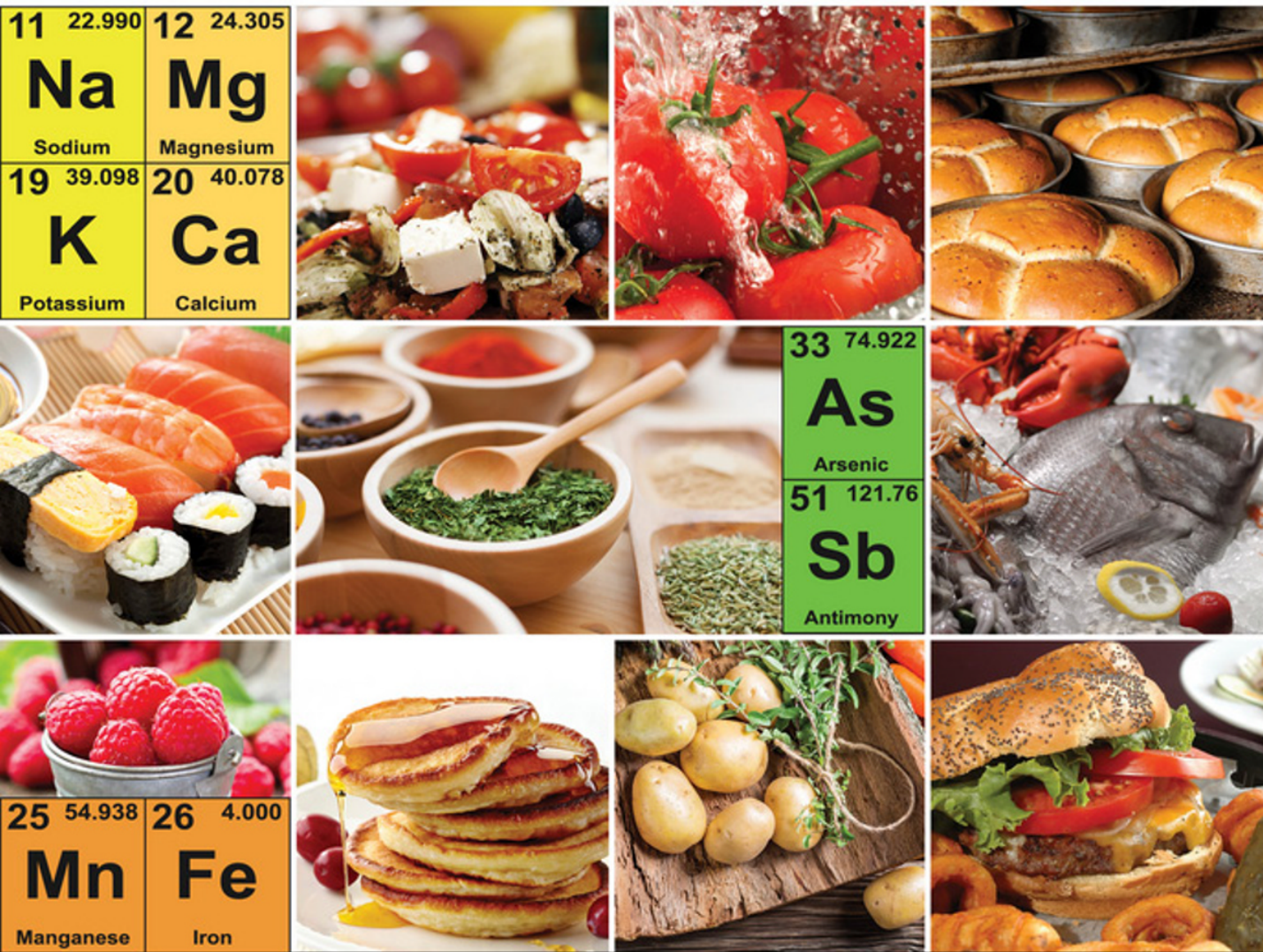
HANDBOOK OF

MINERAL ELEMENTS IN FOOD

Edited by Miguel de la Guardia and Salvador Garrigues



WILEY Blackwell

Handbook of Mineral Elements in Food

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Edited by

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Preface

Publishing another book on the mineral composition of foods could seem to be a redundant task because of the presence in the scientific literature of the many excellent publications on both the nutritional aspects of essential and toxic metals in foods and the analytical approaches for determining and evaluating the concentration of mineral elements in foods.

However, when we proposed this project to Wiley-Blackwell our conception was to assemble all the different aspects of minerals and foods, inviting a series of international specialists in different fields to cover the nutritional and chemical perspectives together with the legal international framework and the specific aspects of different elements and different classes of foods.

Thus this book is not just an update of the data available in the international journals and databases regarding the mineral concentrations of foods produced around the world. It is intended to be a modern reference handbook, with the clear intention to address all aspects of the mineral elements present in the products utilized in human nutrition, from mother's milk to baby food, from water to vegetables, dairy products, meat, fish and sea products, eggs, cereals and pulses, fruits and drinks, and from the analytical tools available to determine the mineral profile of foods to the evaluation of the nutritional aspects of such mineral composition.

Our intention to move from a classical consideration, usually based on exposition element by element, to one focused on the different types of foods can be justified by an appreciation of the contemporary analytical tools available for mineral determination. These have evolved from those based on single-element determination to ones that provide detailed information on multiple elements present in samples at minor, trace and ultratrace levels. Thus modern analytical methodologies for mineral analysis have permitted complete evaluation of the mineral profile of foods, while also allowing the proportion of each element and the content of a specific target element to be determined.

In addition to introducing a change in the presentation of the available data in different fields, we have also considered the nutritional and legal framework within which the reported data can be interpreted and have also added a section on the most useful analytical techniques suitable for evaluating the mineral profile of foods, incorporating the now well-known methods that have emerged in recent years.

In short, we present this book to the reader in the hope of providing a complete picture of the mineral profile of food in order that both nutritionists and developers and users of methodology find inspiration in addressing the main problems related to the presence of minerals in foods and their determination.

Finally, we would like to acknowledge the generous participation of all the authors and their outstanding contributions, together with the continuous support of the professionals at Wiley-Blackwell, who helped to improve our initial proposal and ensured the book's smooth production during this last year.

Miguel de la Guardia and Salvador Garrigues
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CHAPTER 1
The importance of minerals in the human diet

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Abstract: Mineral nutrients are indispensible to the maintenance of life. A mineral element is considered essential when deficient ingestion results in harm or suboptimal function, and if supplementation with physiological levels of this specific element prevents or repairs this damage. Human nutritional requirements demand at least 23 mineral elements, and there are various methods available to establish the nutritional status of minerals. The required daily quantities of mineral nutrients are small, particularly when compared with nutrients such as carbohydrates and lipids. The minimum and maximum mineral contents necessary to produce adverse effects can vary widely between different mineral nutrients. Chosen food regimens are related to geographical availability, and the corresponding biodiversity. Biological adaptive processes, constrained by the regional diversity of diets, have over time established existing nutritional requirements. Nutritional recommendations define mineral consumption values that are not easily achieved with the contemporary Western diet. Scientific evidence suggests that nutrient supplements cannot replace a healthy diet, with the consumption of a wide variety of nutritious foods being the best way to maintain health and prevent chronic disease. There is scope for significant additional study of the role of minerals in the human diet, and their impact on human health.

Keywords: mineral supplements, bioavailability, nutritional recommendations, human diet, food culture, micronutrients

1.1 Historical aspects

Mineral nutrients are essential for the proper functioning of every organism on earth. The interactions between mineral elements in biological systems and their role in mediating the chemical and biological reactions fundamental to life are still being discovered. Archaeological evidence of the feeding habits adopted by our human predecessors has been discovered by fossil studies from different periods and sites. Determination of the minerals in mineralized prehistoric human remains reveals the dietary conditions and food habits, and also the environmental and living conditions, of the population. Understanding the feeding habits adopted by our ancestors helps to elucidate the evolution of the species [1–3].

The availability and distribution of foods and their preparation indicate the preferences of the population and provide knowledge of the social organization practised [1]. The main tool used to unravel the past is archaeological chemistry (archaeochemistry), which has been useful in discovering the practices and lifestyles of past human populations, including their feeding habits [3]. Human remains studied using archaeochemistry, be it via the recovery of tools or paintings or analysis of the concentration of the chemical elements and their isotopic forms, help us understand the role of the biocultural system resulting from the interaction between humans and their environment [4]. The definition of marker elements is essential for archaeochemistry to contribute to the reconstruction of human history, and of the respective feeding practices adopted.

Bones and teeth are body structures considered as indicators of the exposure of humans to minerals [5, 6]. Although bones present numerous analytical difficulties with regard to the separation and characterization of the constituent minerals, they are the target of much research to determine the form of feeding practised by our ancestors. It is known that the geochemistry of

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modern vertebrate bones is directly related to the consumption of food and water: the composition of bone represents the principal food items ingested over the 10–30 years preceding death, varying as a function of the rate of bone renewal of the anatomical part under study. Because of its minimum annual renewal rate of 10%, bone is considered a monitor of some trace elements throughout life. However, because of its morphology, bone exhibits a marked turnover of cortical tissue (32%) and of trabeculated tissue (4.3%) and thus shows greater susceptibility to post-mortem changes [2, 6].

In addition, the form of the mineral incorporated into the bones can be distinct for the various elements. Reconstruction of the diet consumed based on the mineral composition of bones requires that the diagenetic process has not altered bone composition over time. In fossils, the concentrations of the elements Fe, Mn and Cu can be increased due to diagenesis of the soil in which the individuals were buried [2].

The bone concentration of trace elements such as Sr and Ba has been used to discriminate between herbivorous and omnivorous food patterns, where a low Sr level indicates low consumption of foods of animal origin, especially fish [2, 5, 7]. It is worth pointing out that the Sr and F contents suffer progressive post-mortem alterations, and can be enriched due to diffusion–adsorption processes [3], whereas variations in the Sr content of bones of the same individual suggests seasonal or geographical changes in the diet [8].

It is known that the majority of Mg in the human organism comes from foods of animal origin [7]. One of the functions of Mg in the human is its involvement in cell metabolism, and insufficient amounts appear to affect the senescence process negatively [9]. Because of the high level of Zn in blood and meat, a diet based on meat could induce a higher level of this element. However, high levels of Zn can also be found in certain legumes and vegetables and can lead to erroneous interpretation of the diet [2].

The choice of a food regime or types of food is related to the foods available locally and to the biodiversity encountered, and both factors contribute to nutritive mineral values of the diets [10]. In medieval culture, foods shared as presents were an integral part of the diet. Small items such as fruits, offered by anyone, characterized the attributes of commensurability, sociability, hospitality and charity, and also important factors in developing networks of human relationships. The form of donation demonstrated the singularity and adequacy of some food categories and made the intention of the donors obvious [11].

In addition to the diet practiced with selected or donated foods, age and geographical location can lead to variations in the concentration of certain elements in the bones, not only of those classified as nutrients but also of non-nutrient minerals [6]. The levels of the latter in human bones in distinct eras can provide indications of the relative importance of a particular source of contamination. The levels of heavy metals in food, water and air depend predominantly on nature and the local geochemical conditions. The synthetic materials in the immediate environment (e.g. home or workplace) or more distant environment can give useful information concerning the relative importance of these sources, by comparing the levels of contaminating metals in human bones with those in animals not exposed to the same sources of contamination. Additional information can be provided by comparing the temporal alterations in the levels of metal contaminants in human and animal bones with variations in the availability and use of particular elements, and also in the rate of anthropogenic dispersion in the environment [7].

With the objective of reconstructing the diet of our human ancestors, researchers have analysed the variability in the trace element contents of pre-Columbian human bones found in Chupicuaro, Mexico. A comparison of the mineral contents extracted from the skeletons with the mineral content of hydrothermal waters and carbonates available in the Chupicuaro environment provides evidence that Chupicuaro humans very probably consumed these waters, although it is not possible to know if the 'hydrothermal diet' was consumed directly or indirectly or if this diet was the result of cultural habits or the availability of environmental resources [3].

The concentrations of Pb, Cu, Zn, Cd and Fe have been determined in the residual bones of 30 individuals buried in the region of Cartagena (Spain), dated from different historical periods, and also in eight individuals who died in a contemporaneous era. The evolution of the concentrations of the metals over time indicates an increase between the Neolithic period and the period covering the Bronze Age, Roman domination and the Byzantine era. The maximum content for Cu was in the Byzantine era, whereas for Fe it was the Islamic era. Zinc showed a tendency to increase throughout the periods studied, whereas Cd was the only metal showing a

typical tendency to decrease. Of the elements analysed, the lowest values found corresponded with the Neolithic period, showing similar values to the samples of contemporaneous bones with respect to Pb, Cu, Cd and Fe [12].

Another study involved the analysis of Pb by histological light microscopy and synchrotron radiation X-ray fluorescence (SR-XRF) in the bones of individuals who had documented health problems as a function of exposure to this contaminating element between 1793 and 1822 in Antigua (Antilhas). This study indicated agreement between the results obtained by the two techniques [8].

From a historical perspective, dietary intakes of minerals such Fe, Zn, Cu and Mg have fallen in recent years as a function of both reduced energy requirements due to the sedentary lifestyle adopted by most people and changes in dietary patterns combined with lower mineral element densities in the diet. Compositional data indicated a downward trend in the mineral content of foods which has been attributed, at least in part, to intensive farming practices that result in depletion of mineral content of the soil. In the UK, research has demonstrated that the concentrations of Fe, Zn, Cu and Mg in wheat grain remained stable between 1845 and the mid 1960s. However, after this period, and coinciding with the introduction of semi-dwarf high-yielding cultivars, the levels of these minerals decreased significantly, whereas concentrations in the soil either increased or remained stable. Similarly, decreasing trends in mineral concentrations in wheat grain have also been seen when crops were treated with no fertilizers, inorganic fertilizers or organic manure [13].

From the eighteenth century, advances in detemining the chemical composition of the fluids and tissues of animal organisms made it possible to recognize the importance of mineral elements in living organisms. In the nineteenth century, calcium phosphate was the principal constituent of bone, which also included Ba and Mg but in much smaller proportions. Iron was also recognized to be part of haemoglobin and haem, a constituent of blood which also contained P, Na, Cu, Mg, Pb and F. The question derived from this knowledge was whether the diet should provide these inorganic elements [14].

At the end of the eighteenth century, the idea that vegetables were capable of producing inorganic substances was common. An experiment carried out by L.N. Vauquelin with chickens in 1799 aimed to verify whether the animal organism was also capable of generating inorganic material. He showed that the ingress of mineral substances such as calcium phosphate, calcium carbonate and silica via the feed was smaller than the egress via excrement and eggs. It was concluded that there had been a transmutation of the elements by the chickens. These results were accepted for several years. At the end of 1830 it was shown experimentally that vegetables must obtain their organic elements from the soil. A study carried out by Dumas and Prévost with eggs demonstrated conclusively that an embryo did not create the minerals present in its body. Similarly, in 1842 C.J E. Chossat showed that the supply of Ca via the diet was important for maintaining bone composition, and in a publication in 1844 Boussingault considered the nutritional need for minerals to be evident and also noted the importance of knowing the amounts obtained from food [14].

1.2 Types and metabolic function of mineral nutrients

Human nutritional requirements demand at least 49 nutrients to meet organic metabolic needs. Of these, 23 mineral elements are involved in physiological and biochemical activities [15, 16]. The daily quantities of mineral nutrients are, by nature, small, especially when compared with nutrients such as carbohydrates, proteins and lipids. Since minerals are indispensable to functioning of the organism, they must be regularly present in the diet. The macrominerals are those present in greater proportions in the body tissues, leading to greater amounts in the diet. Microminerals are equally essential to the human diet, although required in smaller amounts. A third group also exists, that of the essential trace elements or oligoelements, considered thus when the daily requirement is very small.

Mertz [17] defined as essential trace elements those elements with daily requirements below 18 mg. However, elements such as Zn, Cu, Mb, Fe and Se, which occur in relatively low concentrations in plant and animal tissues (<100 ppm) and in rocks, are also classified as trace elements. Altogether, the trace elements make up less than 1% of the total composition of elements on the Earth and are essential for the correct functioning of many plant, animal and human biological systems [18].

Verdú and Marín [19] used the term 'macrominerals' for elements with a daily requirement equal to or

above 100 mg, and the term 'microminerals' for those with daily requirements generally below 100 mg, which includes the 11 essential trace elements within this group. Of these 11 elements (Va, Cr, Mn, Fe, Co, Cu, Zn, Mo, Se, F and I), eight are in period 4 of the periodic table, and the positive relationship between nucleus size and electron availability of the elements in this period allows them to interact with organic molecules present in biological systems. The remaining trace elements (Se, F and I) are non-metals [16]. Table 1.1 exhibits the principal functions and biochemical modes of action of the macrominerals and microminerals, although not all have defined requirements or even functions.

Table 1.1 Metabolic functions and biochemical modes of action of macrominerals and microminerals.

Minerals	Metabolic functions	Biochemical modes of action	Comments
Microminerals			
Chromium	Carbohydrate metabolism, helps to maintain normal blood glucose levels	Potentiates insulin action, lipoprotein metabolism, gene expression	Based on estimated intake per 1000 kcal
Arsenic	No biological function established in humans, but data in animals indicate requirement	—	No recommendation
Boron	Biological function not clearly established, although animal data indicate a role	—	No recommendation
Cobalt	Part of the vitamin cyanocobalamin	—	No recommendation
Copper	Collagen and elastin synthesis Acts as antioxidant Formation of haemoglobin, red cells and enzymes	Component of the numerous metalloenzymes (lysyl oxidase, Zn/Cu superoxide dismutase) involved in the respiratory chain and in iron metabolism	Plasma copper concentration, serum ceruloplasmin concentration, erythrocyte superoxide dismutase activity and platelet copper concentration
Fluoride	Involved in bone and teeth mineralization Inhibits the initiation and progression of dental caries and stimulates new bone formation	Calcium fluorapatite	Adequate intake
Iodide	Energy metabolism Prevents goiter and cretinism	Component of the thyroid hormones thyroxine and triiodothyronine	Thyroid iodine accumulation and turnover and iodine balance
Iron	Oxygen transport, electron transport Acts in cellular metabolism (as part of cytochrome enzymes) Prevents microcytic hypochromic anaemia	Component of haem/myoglobin and numerous enzymes	Factorial model based on basal iron losses, menstrual losses, fetal requirements in pregnancy, increased requirement during growth, and/or increased tissue and storage iron
Manganese	Not clear, some antioxidant Involved in the formation of bone	Enzymes involved in amino acid, cholesterol, and carbohydrate metabolism (enzyme cofactor, mitochondrial superoxide dismutase)	Based on intake
Molybdenum	Cofactor for enzymes involved in catabolism of sulfur amino acids, purines and pyrimidines	Involved as cofactor and as an electron transfer agent for enzymes (sulfite oxidase, xanthine oxidase)	Molybdenum balance
Nickel	No clear biological function in humans May serve as cofactor of metalloenzymes and facilitate iron absorption or metabolism in microorganisms	—	No recommendation

Table 1.1 (Continued)

Minerals	Metabolic functions	Biochemical modes of action	Comments
Selenium	Cellular antioxidant, regulation of thyroid hormone action, and the reduction and oxidation status of vitamin C and other molecules	Glutathione peroxidase, tyrosine deiodinase, T lymphocyte receptor expression	Estimated average requirement
Silicon	No clear biological function in humans May be involved in bone function based on animal studies	—	No recommendation
Vanadium	No biological function in humans has been identified	—	No recommendation
Zinc	Protein synthesis, control of differentiation, immune system function, acts in many enzymes involved in macronutrient metabolism Involved in sexual maturation Required for normal flavour sensation	Component of multiple enzymes and proteins Enzyme cofactor 'zinc fingers' in DNA involved in the regulation of gene expression	Estimated average requirement
Macrominerals			
Calcium	Component of bones and teeth Involved in blood coagulation and intracellular communication	Excitability and contractility of striated muscles by transmission of nerve conduction Maintains the permeability of cell membranes	Estimated average requirement
Chlorine	With sodium, maintains fluid volume outside of cells and thus normal cell function Food digestion	Control of acid–base, water and salt balance	Adequate intake
Magnesium	Component of bones and teeth Acts in muscle relaxation and in control of acid–base, water and salt balance Cofactor for enzyme systems	Transmission of nerve conduction Involved in the enzymatic process for energy production	Estimated average requirement
Phosphorus	Component of bones and teeth Component of some lipids Maintenance of pH Storage and transfer of energy and nucleotide synthesis	Energy reserve as ATP and CP	Estimated average requirement
Potassium	Maintains fluid volume inside/outside of cells and thus normal cell function Acts to blunt the rise in blood pressure in response to excess sodium intake Decreases markers of bone turnover and recurrence of kidney stones	Generation and transmission of nerve impulse	Adequate intake
Sodium	Maintains fluid volume outside of cells and thus normal cell function Muscle contractility	Control of acid–base, water and salt balance Generation and transmission of nerve impulse	Adequate intake
Sulfur (as inorganic sulfate)	Provides sulfate when sulfur-containing compounds are needed such as chondroitin sulfate and cerebroside sulfate	Required for biosynthesis of 3′-phosphoadenosine-5′-phosphate	No recommendation as yet

It is important to highlight the fact that each mineral has a unique function, regardless of its categorization as either macromineral or micromineral. The microminerals, which are essential components of biological structures, can be toxic in amounts only slightly above the amounts required for their physiological action. In addition, this principle of toxicity can be extended to other elements which are not considered essential nutrients: by possessing similar atomic characteristics, they can imitate the reactivity of a micromineral [16].

The discovery of these elements was due to deficient states observed in different regions of the world. The deficiencies can be reversed with the use of supplements, primarily by consumption of specific foods which naturally contain the element, and subsequently by consumption of the isolated chemical component, since this can be obtained and purified as a result of advances in analytical methods [20]. Although the existence of nutritional requirements for other microminerals (e.g. B, Mo) has been confirmed (the case of fluorine being partly a question of semantics), the evidence for nutritional requirements of some of the 'essential' microminerals is tenuous or limited [21]. It is possible that in the not too distant future, B and Va may be included in the list of essential microminerals.

The biochemical investigations carried out with minerals in recent decades have led to a relatively good understanding of their mode of action, justifying their essential presence in the human diet. Initial studies identified a role for mineral nutrients in the activity of practically all enzymes, whether at the active site of the enzyme or as cofactors, it being apparent that adequate amounts are central to the ability to deal with the derived metabolic response. Other functions have been established for these nutrients in recent years, for example the capacity to modulate genetic transcription and the antioxidant capacity of metalloenzymes such as glutathione peroxidase (which contains Se) and superoxide dismutase (which contains Zn and Cu), with both catalysing reactions responsible for the removal of oxidizing species [20].

Of the macrominerals, Na, K and Cl are important intracellular components. They are responsible for creating the environment around almost all cells, across which metabolites and gases pass from one side of the membrane to the other. The electrolyte gradient across the cell membrane is a prerequisite for cell excitability, signal transport, cell transport and movement processes. The macromineral daily requirements may need adjustment according to the clinical situation. For example, the requirement could increase when there are excessive gastrointestinal losses or even decrease in cases of kidney failure [22].

1.3 Essentiality and toxicological aspects

An organism has the capacity to recognize a mineral nutrient and deal with the dichotomy between its essentiality and its toxicity by regulating the absorption and excretion of these essential nutrients and also by resorting to storage systems as a way of regulating the maintenance of levels compatible with life, while at the same time minimizing the likelihood that these mineral elements take part in toxic reactions. In this process, proteins are fundamental for the recognition and transport of mineral nutrients [16].

One definition of nutrient essentiality considers as essential an element that is indispensible to the maintenance of life [17]. However, even if a mineral is classified as essential for the functioning of the organism, macrominerals and microminerals can display toxicity in concentrations above those required for their biological functions. Such toxicity can also be displayed in elements not considered as nutrients but which mimic the functions of those that are due to the similarity of their chemical characteristics [16].

A mineral element is considered essential when deficient ingestion results in harm or suboptimal function and if supplementation with physiological levels of this specific element prevents or repairs this damage. The absence of an essential mineral can lead to death, particularly with those elements required in very low concentrations. Essentiality is considered to be present when demonstrated by more than one independent investigator in more than one animal model, it being easier to control exposure to macrominerals than to elements required in smaller concentrations. The criterion for essentiality is not related to the degree of debility resulting from nutritional deficit [17].

The animal model is among the methods employed to estimate nutritional requirements, and these have changed over time. Four approaches are among the principal methods used currently: the clinical approach, nutrient balance, functional indicators of nutritional

sufficiency (biochemical, physiological, molecular), and optimal nutrient intake. The dietary reference intakes (DRIs) for minerals are a set of recommendations for apparently healthy individuals (referring to the absence of disease based on clinical signs and symptoms and function) and are determined experimentally under controlled conditions, usually with regular or light physical activity [23–25].

The requirement is understood to be an intake level that will meet specified adequacy criteria, preventing any risk of deficit or excess, and including a gradient of biological effects related to the intake of the nutrient in question. The dose–response is assumed to have a Gaussian distribution unless known to be otherwise. A risk function (a probability of 0 to 1) of deficiency or excess can be derived [26]. Risk assessment was introduced for essential trace elements about 20 years ago, establishing that every essential trace element must have a range of intakes that are safe from toxicity but which are adequate to meet nutritional requirements [27]. The variation integrating the dose–response curve range, and also the lower and upper limits, are delineated as a function of the nutritional and toxicological data respectively, and are situated in a range in which there is low risk of deficiency [27].

The recommended value represents the recommended dietary allowance (RDA) or adequate intake (AI), whereas the tolerable upper intake level (UL) represents the limiting value above which the risk of toxicity is present. To establish a safe UL, the presence of a no-observed-adverse-effect level (NOAEL) in the intake response is assumed [24, 25]. Table 1.2 presents the DRIs of the nutrients essential for human life.

The minimum and maximum quantities necessary and compatible with the normal functions of the organism determine the RDA for safe daily intake. The AI range is situated in values that imply a low incidence of adverse health effects, avoiding both health risks resulting from inadequate ingestion and the risks of excessive ingestion (toxicity). There is an AI range for each element considered essential, although there can be great variation between the ranges for distinct elements [28].

For some essential nutrients, the interval between the maximum and minimum limits may be very small, some even showing overlapping values, which makes it difficult to define the safety margins for nutritional recommendations. For Se, a new approach method has been developed that models the relationships between intake and risks of either deficiency or excess, using an observed incidence for each effect and population distribution characteristics. Using this model it was possible to formulate advice for risk managers on the incidence of adverse effects due to either deficiency or excess at different intake levels [28]. Nevertheless, other elements such as Fe show wide variation in their bioavailability, and their biomarkers, such as ferritin, can be affected by infectious and/or inflammatory processes, resulting in confusion in the relationship between intake and nutritional status [29].

1.4 Diagnosis of mineral status

Optimal mineral status is essential for maintaining health and ensuring optimal body function. Since there are many minerals essential to metabolism, there are many ways to determine the nutritional status of the minerals [21]. The nutritional status can be understood as the body condition that results from the process of using the nutrients contained in foods, leading to an equilibrium between supply and assimilation of the nutrients, and the nutrient consumption of the organism [30], which is influenced by the sex and age of the individual. The nutritional mineral status can be diagnosed from diverse biomarkers which indicate a status that can be modified intentionally (i.e. dietary modifications) or non-intentionally (i.e. presence of pathology) (Figure 1.1).

For certain mineral elements, the concentration in human tissue is one of the current ways of determining nutritional status. The blood plasma can be collected and analysed for specific elements (i.e. Se, Mn) in clinical and population studies. However, the plasma concentration may show inadequate sensitivity due to the absence of reference values for many minerals, or even as a function of homeostatic adjustment mechanisms that maintain levels stable even where there is deficient intake (i.e. Ca, Zn). For Cu, Zn and Fe, a lack of specificity can also compromise the value determined in the plasma. For others, such as Mn, the plasma concentration does not represent adequate evaluation of the nutritional status of these elements [21, 31]. It was recently shown that dietary supplementation with Co produces an alteration in the blood level of this element and can be used to predict exposure to or intake of this metal [32].

Table 1.2 Nutrients essential to human life, showing dietary reference intakes (DRIs) for macronutrients and micronutrients.

	Males (9 to >70 years)		Female (9 to >70 years)			
	Min.	**Max.**	**Min.**	**Max.**	**Pregnancy**	**Lactation**
Macronutrients						
Carbohydrates (g)*	100	100	100	100	135	160
Lipids (% of energy)*	20	35	20	35	20–35	20–35
Linoleic acid* (% of energy)	5	10	5	10	5–10	5–10
Linolenic acid* (% of energy)	0.6	1.2	0.6	1.2	0.6–1.2	0.6–1.2
Protein (g)†	0.66	0.87	0.66	0.76	0.88	1.05
Histidine (mg/kg daily)	11	13	11	12	15	15
Isoleucine (mg/kg daily)	15	18	15	17	20	24
Leucine (mg/kg daily)	34	40	34	38	45	50
Lysine (mg/kg daily)	31	37	31	35	41	42
Methionine + cysteine (mg/kg daily)	15	18	15	17	20	21
Phenylalanine + tyrosine (mg/kg daily)	27	33	27	31	36	41
Threonine (mg/kg daily)	16	19	16	18	21	24
Tryptophan (mg/kg daily)	4	5	4	5	5	7
Valine (mg/kg daily)	19	23	19	22	25	28
Water (L/day)‡	2.4	3.7	2.1	2.7	3.0	3.8
Micronutrients						
Macroelements						
Calcium (Ca) (mg/day)†	800	1100	800	1100	800–1000	800–1000
Magnesium (Mg) (mg/day)†	200	350	200	300	290–335	255–300
Phosphorus (P) (mg/day)†	580	1055	580	1055	580–1055	580–1055
Sodium (Na) (g/day)‡	1.2	1.5	1.2	1.5	1.5	1.5
Potassium (K) (g/day)‡	4.5	4.7	4.5	4.7	4.7	5.1
Chloride (Cl) (g/day)‡	1.8	2.3	1.8	2.3	2.3	2.3
Microelements						
Iron (Fe) (mg/day)†	5.9	6.0	5.0	8.1	22–23	6.5–7.0
Zinc (Zn) (mg/day)†	7.0	9.4	6.8	7.3	9.5–10.5	10.4–10.9
Iodine (I) (μg/day)†	73	95	73	95	785–800	985–1000
Copper (Cu) (μg/day)†	540	700	540	700	785–800	985–1000
Selenium (Se) (μg/day)†	35	45	35	45	49	59
Molybdenum (Mo) (μg/day)†	26	34	26	34	40	35–36
Chromium (Cr) (μg/day)‡	25	35	21	25	29–30	44–45
Fluoride (F) (mg/day)‡	2	4	2	3	3	3
Manganese (Mn) (mg/day)‡	1.9	2.3	1.6	1.8	2.0	2.6
Vitamins and Choline						
Vitamin A (μg/day)†	445	630	420	500	530	885–900
Vitamin E (mg/day)†	9	12	9	12	12	16
Vitamin C (mg/day)†	39	75	39	60	66–70	96–100
Thiamine (mg/day)†	0.7	1.0	0.7	1.0	1.2	1.2
Riboflavin (mg/day)†	0.8	1.1	0.8	0.9	1.2	1.3
Vitamin B_6 (mg/day)†	0.8	1.4	0.8	1.3	1.6	1.7
Niacin (mg/day)†	9	12	9	11	14	13
Folate (μg/day)†	250	320	250	320	520	450
Vitamin B_{12} (μg/day)†	1.5	2.0	1.5	2.0	2.2	2.4
Vitamin D (μg/day)†	10	10	10	10	10	10
Vitamin K (μg/day)‡	60	120	60	90	75–90	75–90
Pantothenic acid (mg/day)‡	4	5	4	5	6	7
Biotin (μg/day)‡	20	30	20	30	30	35
Choline (mg/day)‡	375	550	375	425	450	550

*AMDR, acceptable macronutrient distribution ranges.

†EAR, estimated average requirement.

‡AI, adequate intake.

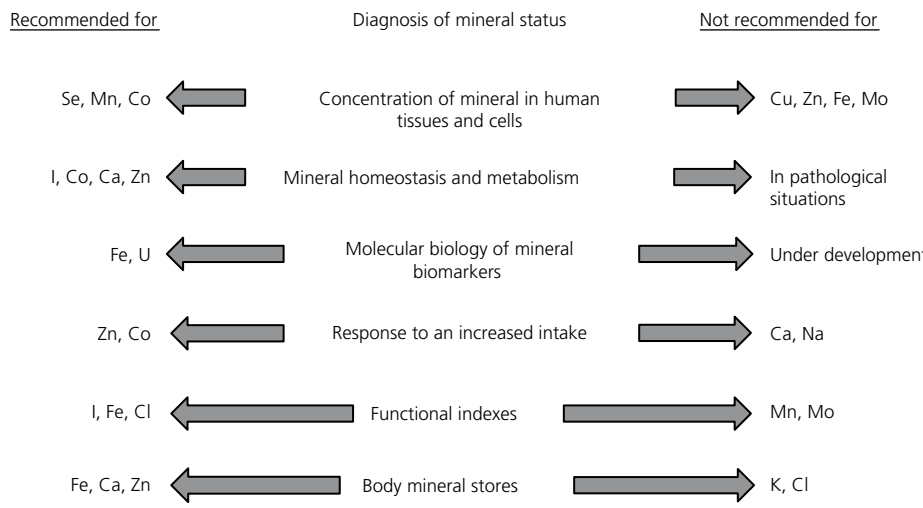


Figure 1.1 Methods for diagnosing individual mineral status.

The cellular components of the blood can also be used as indicators of the mineral nutritional status, although this type of analysis is more usual in research studies. The method requires sample preparation and has limited application for a variety of elements, so its use is restricted. A study involving analysis of the nutritional status of Mn in women undergoing menopause, after adjustment for the various covariants, showed that both plasma ferritin and the menopause can be used to predict Mn content in the blood, and that the hormonal state (before and after menopause) acts as a modifier of Mn concentration in the blood [32].

Use of the enterocyte membrane in determining the nutritional status of Zn and Co could be feasible in experimental nutrition, since this membrane contains these elements and has an association with the mineral content administered [33]. The mineral contents of hair and nails represent interesting alternatives for the determination of the prior nutritional status of certain minerals. However, the improper use of this method commercially has reduced the credibility of these tissues as indicators, and it is frequently difficult to infer a mineral nutritional deficiency from mere observation of alterations in the hair and nails. Prolonged mineral deficit leads to anaemia or severe hypocalcaemia, indicated by organic Fe or Ca deficits respectively, both of which impact nail structure. It should be mentioned that Ca does not contribute to nail hardness and represents only 0.2% of nail plate weight, a region which also contains other minerals such as Mg, Fe, Zn, Na and Cu [34].

The determination of mineral homeostasis and metabolism is another way to estimate body mineral status. Mineral homeostasis can be evaluated using daily values for mineral intake and excretion as the parameter, the renal system having fundamental importance in the maintenance of homeostasis of the majority of minerals and the intestine being the primary site of homeostasis for Ca. In pathological situations, alterations in excretion can occur, altering the homeostasis of a mineral or group of minerals. Urinary excretion only represents a useful biomarker for minerals where the renal system has an active role, such as with I and Co [21].

In cases where endogenous mineral excretion is regulated by the gastrointestinal tract, including its accessory organs the liver and pancreas, theoretically the quantification of minerals in the faeces is a biomarker of dietary intake and the nutritional status of these minerals, Zn being the main target of this type of investigation [35]. No less important is the fact that the mineral absorption process is, to a great extent, regulated by the amount in the digestive tract, this relationship being inversely proportional. Considering that there could be excretion of endogenous mineral concomitant with excretion of mineral intake, marking techniques of mineral intake and metabolic collection help in estimating homeostasis. Although marking techniques are useful

in determining the organic turnover of minerals such as Ca, I and Zn, their applicability is limited outside clinical trials or with restricted samples [21].

Advances in molecular biology have been promising in the area of mineral biomarkers. A recent study indicated that not only was it possible to determine the nutritional status of iron via transferrin receptors but that this was also useful in differentiating types of anaemia, whether due to iron deficiency or inflammation [36]. With respect to toxicological questions, osteopontin is considered a biomarker for exposure to U, suggesting kidney damage when its content is reduced in the urine [37].

The response to an increased intake can be used as a biomarker for some essential minerals, although insufficient in many cases (i.e. Ca, Na). A current detailed determination of the nutritional status of the mineral in question, for example Zn and Co, is required for evaluation of the physiological response, it being necessary to confirm the pre-existing dietary deficiency or nutritional status deficit for a randomized intervention of the mineral under investigation to be carried out [21, 32]. As a positive point, the usefulness of the analysis of the response to increased intake of the mineral resides in the fact that this combines the mineral deficit data with non-specific disturbances in normal physiology and also with non-specific morbidity [21].

The functional indices present a differentiated value, because they indicate when ingestion and nutritional status are so highly compromised as to cause measurable changes in the normal biochemical and physiological processes of the organism. The specificity of these alterations should be exclusive for a determined deficient element. Thyroid diseases due to iodine deficiency are an example, and have been recognized for decades and effectively prevented and treated [38].

Body mineral stores can be useful in determining nutritional status. Alterations in the store can represent an increase in intake, bioavailability or even a reduction in excretion. This situation can be noted classically in relation to Fe, whose organic values are rigidly controlled by the ferritin content, the storage protein of this element, since Fe is an oxidizing element. Another element whose storage level can be measured is Ca, by measuring the amount contained in the bones. More recently, such an evaluation has been studied for Zn, an element that occurs in hundreds of enzymes and millions of protein domains. It was found that Zn possessed a complex protein homeostatic system that regulated the amount of cellular Zn, coordinating the exportation, importation and detection of the organic status of the metal [39].

1.5 Food culture and mineral diet content

Nutritional recommendations define mineral consumption values that are not easily achieved with the contemporary Western diet [40, 41]. If current nutritional needs are not easily met in a situation that provides food diversity, improved access to foods, improved processing and supplementation, access to foods from various geographical regions, the application of improved agricultural technologies and availability of foods with high nutrient availability, one might wonder how our ancestors survived. In the past, diets were more susceptible to food shortages, seasonal variations in food supply, geographic restrictions of human groups, unpredictability of access to food, and other factors that denote the precarious food conditions of the past. These factors raise the interesting question of what were the ideal characteristics of our ancestors' diet [42]. The diversity of diets spread across the continents over different historical periods and time indicate that biological adaptive processes were fundamental conditions for nutritional needs.

Milton [43] suggests that studies on human food practices should use the reconstituted diets of our ancestors, with the argument that changes in diet are comparatively recent in relation to the human evolutionary process. Conversely, the forms of cooking, the domestication of grains, ultra-processed foods, and the increased consumption of sugar and saturated fat are recent events. The human gut, for example, derived from ancestral lines associated with a given range of possible food sources. For a better understanding of the nutritional requirements and nutrient intake patterns of other active components present in the diet, Milton investigated the diet of primates and the composition of wild foods, in comparison with the composition of cultivated foods. The study was conducted by comparing the mineral content of vegetarian food sources eaten by groups of non-human primates. The concentration of minerals was higher in wild specimens and it was concluded that the mixture of chemical constituents in the

plant, consumed simultaneously, seems to explain the advantages of this type of diet compared with the Western diet [43].

Based on the same premise, some studies have shown that the diet of Palaeolithic humans, comprising fruits, roots, meat and seafood, was dense in micronutrients, especially I, Fe, Zn, Cu and Se, although Ca and vitamin D were scare [44, 45]. Another study found a decrease in concentration of Mg in wheat, with a decline in the range of 15–23% since 1850, which coincides with the increase in cardiovascular disease and mortality [46]. These findings lead us to believe that a higher concentration of micronutrients in the diet of our ancestors ensured the conditions for their survival, and the modifications of these conditions may be associated with current diseases.

However, malnutrition and food shortages are factors that have contributed to the evolution and health of humankind. The diversity of human groups spread across different parts of the world, with differing climatic characteristics and geographies, have resulted in revised eating patterns, and possibly adaptive mechanisms to suit these factors.

Lazenby [47] suggests that the accelerated bone loss among the Inuit and Inupiat populations of the Arctic regions is due to the higher production and use of thyroid hormones (T_3 and T_4) as a mechanism for adaptation to cold rather than as a result of a high-protein diet, also called the 'acid-ash' diet. Studies with different ethnicities illustrate nutritional differences and adaptive mechanisms that add to the complexity of establishing nutritional recommendations for minerals [47].

1.6 Health consequences of human mineral malnutrition or excessive intake

Two distinct aspects need to be considered in order to determine the optimum interval of values for the RDA of a determined mineral nutrient: one refers to the minimum content necessary for the human organism to maintain its normal functions and the other to the maximum content considered compatible with normal functioning (risk). The interval between the upper and lower limits may be highly restricted, and can even show overlapping values, principally due to the fact that the upper limit is a derived value, with different uncertainty values being used in the toxicological evaluation of the risk of nutrient deficiency [29].

Thus the DRI – the nutrition recommendations of the Institute of Medicine (IOM) of the National Academy of Sciences of the USA – establishes for mineral nutrients the maximum tolerable UL that is safe and with negligible toxicity risk, and a minimum value that guarantees a low risk of nutritional deficiency [24, 25]. The health risk arising from inadequate intake, and the health risk caused by excessive intake, are both considered in the range of acceptable doses for the daily intake [48].

The estimated average requirement (EAR) values, the data on which the RDA values are based, require the existence of a dose–response relationship between one or more status biomarkers (which are predictive of optimal health) and dietary intake. However, for some elements (e.g. Cu) there are no sensitive and specific biomarkers of the status [29]. In part this difficulty is due to the complex network of transport routes available for mineral nutrients to arrive at the cells. The route chosen by the organism is determined by the physiological needs for each element [49], which is dynamic and not static. Processes such as pregnancy, breast-feeding or even intense exercise can alter the daily requirements for minerals [23].

The AI value should offer a low incidence of adverse health effects due to very low intake (deficiency or absence of a health benefit) or very high intake (toxicity) [48]. Information is required on the impact of both excessive and minimal intake on health and also on the dietary consumption of different population groups in order to carry out a risk–benefit analysis of the mineral nutrients, together with measures of variation, including:

1. determination of the risks and benefits for health on a short- and long-term basis (identifiable/measurable end points, evidence of causality, dietary reference values);
2. consumption data, with respective variation between population groups and the dose–response relationship;
3. information on the appropriate type of risk–benefit analysis (qualitative or quantitative), including phenotype and genotype data [29].

Insufficient and excessive mineral nutrition represent opposite states, both deleterious to adequate functioning of the organism. The degree of effect varies as a function of the mineral in question, the level of deficit

or excess, and the requirements of the individual, which vary as a function of age, sex and physiological state. The amount of mineral stored in the organism could be relevant in the case of dietary deficiency, when the organism depends on the organic reserve to supply its daily needs. An initially low mineral store could represent a smaller toxicological risk compared with an excessive intake, which, if it becomes chronic, can have negative repercussions on the individual.

It is important to point out that the organism needs to be efficient to deal, on a daily basis, with elements that are simultaneously essential and toxic, depending on the quantity consumed and the amount stored. Considering the existence of an adequate mineral store, a chronic intake deficit or increase in metabolic demand would imply an initial diminishing of the amount stored in the organism. For minerals such as Ca, the result of a chronic deficit will be observed in the long term by the induction of osteoporosis. For other elements such as Fe, deficiency will result in iron-deficiency anaemia, which usually develops after a few months of low dietary intake or increase in daily requirements. An increase in the metabolic demand due to sporting activities has been proposed for Fe, Ca and Na, although there is a need for greater scientific evidence in the case of Ca [23].

With respect to mineral toxicity, this goes beyond the UL parameter, and can be expanded to include other non-essential elements with atomic characteristics similar to those of mineral nutrients, thus mimicking their reactivity. The use of proteins, isolated or combined with other biomolecules, that have the ability to specifically recognize the target mineral enables the organism to manage this situation, capturing the element of nutritional interest and leading it to the target tissue at the same time as preventing the mineral nutrient from taking part in undesirable prejudicial reactions [16]. Absorption of the vitamin cyanocobalamin by mammals can be cited as an example of this, since this vitamin contains Co in its structure [50]. However, the use of protein transporters is not always sufficiently capable of separating the minerals of nutritional interest from non-nutrient elements, such as in the case of Fe and Al, which can compete with each other for transport by transferrin [51], a situation that can favour iron-deficiency anaemia.

The minimum and maximum values necessary to produce adverse effects can vary widely between distinct mineral nutrients. In deficiency, it is important to note that the adverse effects observed are the result of continued low intake, and that the severity and/or incidence would be attenuated by an increase in dietary intake [48]. The adverse effects resulting from excessive consumption will be aggravated if there is an increase in intake [48]. However, research has shown that to reach or surpass the UL for essential mineral nutrients by way of a varied diet is not commonly encountered among human population groups [52, 53], with the exception of Na, for which Western feeding is above the UL established, in contrast to the K content, which is below the nutritional recommendation and therefore favourable for high blood pressure [54]. This has been observed even in oral therapeutic diets served to hospital patients [55].

With respect to dietary deficient elements, apart from K, the most frequent involve Ca, Fe, Se, Zn and I, the latter being present in specific regions. Considering that minerals such as Se have an antioxidant role and that gender and age are individual characteristics that can affect the capacity to prevent oxidative stress [56], the proposed daily intake levels should be adjusted to supply the individual organic requirements.

An understanding of the consequences to human health of a deficient or excessive mineral intake, isolated or as a group, is also necessary for food production and even for the production of nutritional supplements. Fortification of food could be an alternative, to be implemented with a view to attending to the nutritional demands of the target population.

1.7 Minerals, health and ageing

To convert organic needs into dietary requirements, adjustments are important, taking into account diet- and host-related factors. The diet-related factors depend on the nature of the usual diet and may consider the chemical form of the nutrient in question, the nature of the dietary matrix and the interactions between nutrients and/or other components, food preparation and processing practices. The most important host-related factors are gastric secretion and intestinal motility, since both will change with age, ethnicity, genotype and sex [57].

Ageing is characterized by the progressive deterioration of physiological functions, including weakening of innate and acquired immunity, even in the 'healthy' elderly. The current idea is that improvements in the

nutritional status of elderly individuals will enhance their immune system, which could enhance their nutritional status by mechanisms preventing the consequences of infectious diseases, such as nutrient malabsorption, loss of nutrient and energy storage capacity, and reduced appetite [58].

However, the reduction in food intake that occurs between the ages of 20 and 80 years can be attributed to physiological actions [59], which can partly be explained by the age-related decline in olfaction and taste capacities. Chronic diseases and/or drug use can also have a negative effect on food consumption, resulting in the elderly showing a greater risk for micronutrient deficiency than healthy adults [60].

Micronutrient depletion leads to specific and well-known clinical symptoms in younger adults, which can be diagnosed and treated, whereas in the elderly mild micronutrient deficiencies and their consequences are difficult to assess [60]. For example, deficiencies of trace elements such as Zn, Fe and Cu can affect the organic response of the host and could confuse the diagnosis or the interpretation of the symptoms in older individuals [61].

Using Ca as an example, it is known that the daily Ca intake tends to decline with advancing age, and that the intestinal absorption process is reduced in older women compared with younger women as a function of oestrogen deficiency, a condition aggravated in the presence of a vitamin D deficiency, and which is favourable to an increased risk of osteoporosis. Many factors, including various disease conditions and medications, can also affect Ca absorption and become more relevant with increasing age [62].

Researchers working on the European Survey on Nutrition and the Elderly, a Concerted Action (SENECA) study examined 1005 elderly people from eight countries in relation to the adequacy of their intake of energy, some vitamins and Ca and Fe. The prevalence of an inadequate intake of micronutrients was high at all energy intake levels, especially in women. Fe was the most prevalent with respect to inadequate intake, Portugal being the country with more individuals showing inadequate intake of Fe [63]. It is important to note that the body requirements for Fe in postmenopausal women are reduced, since there are no menstrual blood losses [64].

The minerals Zn and Mg and some other trace elements may be present in less than optimal concentrations in the diet of older people [65]. It is known that Zn deficiency, common in the elderly, is linked to impaired immune function and an increased risk of acquiring infection, which can be rectified by zinc supplementation [66]. However, the administration of higher than recommended ULs for zinc may adversely affect immune function, so much more work is needed before recommendations can be made about Zn intake [65, 66].

With respect to Mg, it seems likely that Mg inadequacy interferes with cell metabolism, which could accelerate the senescence of human endothelial cells and fibroblasts, negatively affecting cell division. The Western diet is relatively deficient in Mg and correcting the intake might contribute to healthier ageing and the prevention of age-related diseases [9]. For most minerals, such as Mg, Ca, P, Fe, Zn, I, Cr, Mb and Se, body requirements do not seem to alter with age, except for Fe, which is reduced in older as compared to younger women. Dietary Na and its role in hypertension has stimulated concern and controversy [65].

However, the provision of more minerals in the form of a nutritional supplement to older subjects can offer some health benefits. The effects of 2 years of daily supplementation with trace elements (zinc sulfate and selenium sulfide) on immunity and the incidence of infections in 725 institutionalized elderly patients (>65 years) showed that the number of patients without respiratory tract infections during the study was higher in the groups that received the elements. The authors concluded that low-dose supplementation of Zn and Se provides significant improvement in elderly patients by increasing the humoral response after vaccination, and could have considerable public health importance by reducing morbidity from respiratory tract infections [67].

A recent randomized, blind, placebo-controlled trial carried out with 910 non-institutionalized men and women, aged 65, who received a daily placebo or multivitamin and multimineral supplement for 1 year [14 mg Fe (fumurate), 150 μg I (potassium iodide), 0.75 mg Cu (gluconate), 15 mg Zn (oxide) and 1 mg Mn (sulfate)] showed that supplementation did not alter the life quality of those who received it or contacts with primary care and days of infection. The study concluded that the routine multivitamin and multimineral supplementation of older people living at home does not affect self-reported infection-related morbidity [68].

Important differences among the study interventions include the use of institutionalized or non-institutionalized

subjects and supplements with just minerals or a mixture of minerals and vitamins, factors that can explain some of the discrepancies in the findings of the studies. However, a systematic review of the use of multivitamin and multimineral supplementation to reduce infections among the elderly showed no significant effect of the micronutrient mixture supplements. The review also showed that the elderly, aged 65 years or over, undernourished at the baseline, could benefit from 6 months of supplementation as compared with the other elderly individuals [69].

1.8 Foods or supplements as a source of minerals

Several epidemiological studies, observational studies and interventional trials have shown a significant inverse relationship between the intake of foods rich in mineral antioxidants, particularity Se, or intake of the mineral itself and a reduction in the risk of several age-related chronic diseases, including some types of cancer, eye and neurodegenerative diseases, and ischaemic cardiovascular diseases [57, 70]. The link between health and mineral nutrients is responsible, in part, for the increase in their use that has occurred since the 1970s. The multivitamin–multimineral is the most frequently reported dietary supplement used among North Americans, and use increases with advancing age [71].

In the USA, nutrient and dietary supplements are regulated as a food subcategory by the Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition. The Dietary Supplement Health and Education Act's safety and labelling requirements define dietary supplements as, in part, products intended to supplement the diet, containing any of the following dietary ingredients: a vitamin; a mineral; a herb or other botanical item; an amino acid; a dietary substance for use by humans to supplement the diet by increasing the total dietary intake; or a concentrate, metabolite, a constituent, extract, or combination of any of the above ingredients. Dietary supplements are intended to be taken orally and can be presented in many forms, including pills, capsules, tablets, liquids, powders or other forms, so long as they are not presented for use as a conventional food or as the sole item of a meal or diet [72].

In the European Union (EU), the European Commission has established rules to help ensure that food supplements are safe and properly labelled. In the EU, food supplements are regulated as foods and the legislation focuses on the vitamins and minerals used as ingredients in food supplements. The main EU legislation is Directive 2002/46/EC, which is related to food supplements containing vitamins and minerals [73]. The European Commission defined food supplements as a concentrated source of nutrients or other substances with a nutritional or physiological effect, whose purpose is to supplement the normal diet. Food supplements are marketed in 'dose' form, for example as pills, tablets, capsules or liquids in measured doses. Supplements may be used to correct nutritional deficiencies or maintain an adequate intake of certain nutrients [73].

A recent analysis of the data from the National Center for Health Statistics about National Health and the Nutrition Examination Survey (NHANES 2003–2006) on the use of mineral supplements showed that North American individuals are taking Fe, Se, Cr, Zn and Mg supplements. The results revealed that about 18–19% were using Fe, Se and Cr, while 26–27% were using Zn and Mg supplements. Overall analysis revealed that half of the North American population and 70% of adults aged 71 years or older use dietary supplements, and that one-third use multivitamin–multimineral dietary supplements [71].

However, recently published randomized placebo-controlled primary prevention trials have been unable to demonstrate the same beneficial effects when individual exposure occurs by ingestion of natural food mineral sources. The apparently contradictory results between the observational studies and the randomized trials could be explained by the fact that the doses used in clinical trials were much higher than the highest levels achieved by usual dietary intake [57].

In China, a 6-year prospective intervention study including supplementation with 14 vitamins and 12 minerals or a placebo among adults with precancerous lesions of the oesophagus showed that the cumulative cancer incidence rates were nearly the same. The authors concluded that there was no substantial short-term beneficial effect on the incidence or mortality for oesophageal/gastric cancer following daily supplementation [74].

Another randomized, double-blind, placebo-controlled primary prevention trial (SU.VI.MAX), involving a total of 13,017 French adults of both genders, tested the efficacy of single daily dose of a nutritional supplement

(120 mg ascorbic acid, 30 mg vitamin E, 6 mg β-carotene, 100 μg Se and 20 mg Zn) or a placebo on the incidence of cancer and ischaemic cardiovascular disease. The median follow-up time was 7.5 years. No significant differences were detected between the groups in total cancer incidence (4.1% for the study group vs. 4.5% for the placebo group), ischaemic cardiovascular disease incidence (2.1% vs. 2.1%), or all-cause mortality (1.2% vs. 1.5%). An ex-stratified analysis showed that a low-dose antioxidant supplementation lowered the total cancer incidence and all-cause mortality in men but not in women [75], and at the same time exhibited a fourfold higher melanoma risk in women but not men [76]. The authors suggested that the effectiveness of supplementation in men could be attributed to their lower baseline status of certain nutrient antioxidants [75].

Concerning the incidence of melanoma, another population-based prospective study of 69,671 men and women who self-reported the intake of multivitamins and supplemental antioxidants, including Se and β-carotene, over 10 years detected no significant association between multivitamin use and melanoma risk in women or in men, and no increased melanoma risk was noted with the use of supplemental β-carotene or Se at doses comparable with those used in the SU.VI.MAX study [77].

In India, a double-blind, matched-pair, cluster randomization study enrolled 214 pre-menarche girls, with the aim of investigating the effect of a 1-year nutritional supplement containing Ca, a multivitamin with Zn plus vitamin D on bone mass. Three groups were established: Ca group (500 mg/day Ca); Ca + MZ group (500 mg/day Ca + multivitamin tablet containing 15 mg/day Zn); control group (multivitamin tablet with no minerals). All subjects received vitamin D supplementation. The mean percent increase in total body bone mineral content was higher in the two Ca-supplemented groups (13.6–22.0%) compared with the control group, which showed no improvement [78].

Recently, a re-examination of the literature concerning the effect of antioxidant supplementation on mortality and health in randomized trials was carried out, using the data from 66 randomized clinical trials, and found that 36% showed a positive outcome, 60% had no outcome and 4% had a negative outcome [70]. In one of these studies, a randomized controlled trial, a supportive epidemiological and preclinical examination was carried out to determine whether Se, vitamin E or both could prevent prostate cancer and other diseases with little or no toxicity in relatively healthy men. The results showed that neither Se nor vitamin E, alone or in combination, at the doses and formulations used, could prevent prostate cancer in this population of relatively healthy men [79].

In the Biesalski group meta-analysis, the interventions in the studies analysed were categorized as primary prevention (risk reduction in healthy populations), secondary prevention (slow pathogenesis or prevention of recurrent events and/or cause-specific mortality) or therapeutic (treatment to improve quality of life, limit complications, and/or provide rehabilitation). The authors found positive outcomes in 8 of 20 primary prevention studies, 10 of 34 secondary prevention studies, and 6 of 16 therapeutic studies. In addition, seven of the eight randomized clinical trials with a positive outcome in primary prevention included participants in a population where malnutrition is frequently described. The authors suggested that the analyses of potential risks from antioxidant supplementation should be placed in the context of a benefit–risk ratio [70].

The use of dietary supplements may not provide minerals in a soluble and metabolically available form [80]. On the other hand, given the widespread use of supplements, data should include nutrient intakes from foods to correctly determine the total nutrient exposure, since the use of nutrient supplements can have a substantial impact on total nutrient intake [81, 82]. The European Commission considered that in some cases the excessive intake of vitamins and minerals could be harmful or cause unwanted side effects, and therefore maximum levels were necessary to ensure the safe use of supplements [73]. Mineral supplements should be better formulated to reduce the prevalence of inadequacy and to reduce the risk of excessive intakes, since they could increase intakes to above the UL.

Scientific evidence suggests that nutrient supplements cannot replace a healthful diet and that the positive health outcomes are related more to dietary patterns and the types and amounts of foods consumed than to intakes of individual nutrients. A wise selection of nutrient-rich foods is generally the best strategy for meeting all nutrient requirements. The consumption of a wide variety of nutritious foods is the best way to maintain health and prevent chronic disease. Foods, particularly plant foods such as fruits, vegetables, whole grains, beans, nuts,

seeds and teas, provide an array of other health-promoting substances beyond the mineral nutrients, as proposed by the American Dietetic Association [82].

1.9 The effect of dietetic interventions on mineral status

Many studies on dietetic intervention have been carried out, involving a variety of possibilities. The dietary resources available are highly varied, for example the inclusion or exclusion of a determined food, changes in meal timing and frequency, changes in the material of the containers used to prepare the food, and a particular combination of foods in the same meal, among others.

Dietetic intervention can be applied in the treatment of chronic or acute diseases, the prevention of specific diseases, or simply to improve the nutritional status. Research on dietetic intervention can involve testing whether a specific dietary change can affect the end point of a disease or disease risk. Dietetic intervention can be applied to individuals, groups or entire communities. The efficacy of the dietetic intervention can be evaluated using parameters based on the mineral, design/time and interest, and usually measures biological indicators to evaluate the outcome of the responses, focusing on the evaluation of quantitative outcomes and, in particular, on whether an intervention is effective in achieving changes in dietary behaviour. These indicators can be classified broadly into exposure, effect and susceptibility markers and have numerous applications in nutrition. They can be used to assess dietary intakes, biochemical or physiological responses to dietary behaviour or intervention, a clinical end point or disease outcome, and predisposition to a particular disease or response to treatment (Figure 1.2).

A clinical trial can be more useful when aiming to establish a causal relationship that observational studies can only suggest. For example, dietary clinical trials designed to examine the effect of increased Ca intake on blood pressure regulation cannot be applied to all studies, the results sometimes exhibiting no or negative effects [83]. However, in a meta-analysis of 33 appropriately designed, randomized, controlled clinical trials, researchers reported that increased Ca intake resulted in average blood pressure reductions of 1.3 mmHg for systolic pressure and 0.2 mmHg for diastolic pressure in the general population, and 4.3 mmHg and 1.5 mmHg respectively in hypertensive persons [84]. These results were confirmed in another meta-analysis, reaffirming the beneficial effect of dietetic Ca on blood pressure, and adding that there was a greater blood pressure-lowering effect when the increased Ca was derived from foods, primarily dairy products, rather than from supplements [85].

While observational studies are limited to identifying associations between factors, clinical trials are required to determine the actual cause and effect. A dietetic

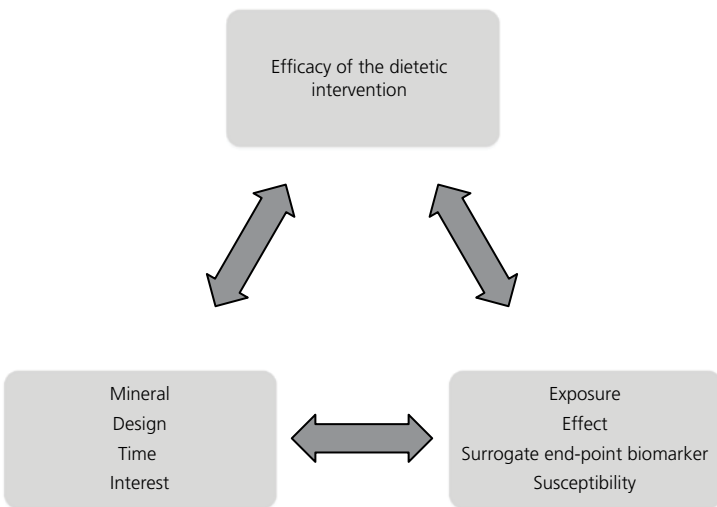


Figure 1.2 Factors associated with the efficacy of a dietetic intervention.

intervention which results in alteration of the mineral status can be seen in the longitudinal study carried out to determine the effects of a low-sodium diet (2 g/day) on human bones. The subjects were postmenopausal African–American and white women subjected to this low-sodium diet for 6 months. Na and Ca excretion, bone turnover and calcitropic hormones were measured before and after the intervention period. In women who had baseline Na excretion equal to or greater than the average Na intake in the USA (≥3.4 g/day), the low-sodium diet resulted in significant decreases in Na and Ca excretion and in that of the bone turnover biomarker type I collagen amino-terminal propeptide, with no significant changes in the calcitropic hormones. The results suggest that in postmenopausal women with Na intakes of 3.4 g/day or greater, a low-sodium diet may have benefits for skeletal health [86].

Other dietetic interventions on mineral status have focused on Fe status. Iron deficiency occurs when iron requirements cannot be met by absorption from the diet, such as during periods of rapid growth (infancy, adolescence), in pregnancy, and as a result of menstrual or pathological blood loss. Monotonous plant-based diets can limit Fe absorption as a function of the high level of phytate and polyphenols, and diets with little animal tissue (or vegetarian ones) may also result in an imbalance between iron requirements and absorption, and can result in Fe deficiency or even in Fe anaemia. Such diet compositions are characteristic of developing countries [87].

A recent study combined an increase in the dietary and bioavailable iron intakes of pre-school children (1–6 years old) from Burkina Faso by increasing the amounts of meat and citrus fruits in the diet and adding iron-rich condiments. The bioavailable Fe intake was increased from 0.4 to 0.9 mg/day, at an estimated cost of US$0.59 per month. After 18 weeks significant outcomes were found: the haemoglobin concentration increased (from 9.87 to 10.38 g/dL), the total iron-binding capacity decreased (from 107 to 91 μmol/L) and there was a marginal increase in transferrin saturation (from 13 to 17%), with no significant improvement in serum ferritin concentration. The intervention promoted a decrease in anaemia (from 64 to 30%) and iron-deficiency anaemia (from 61 to 30%) [88].

Another low-cost dietetic intervention on Fe status includes the introduction of iron cookware for food preparation. This kind of intervention has been shown to be a good alternative for treatment, since it does not require changing the food habits and can be applied to subjects such as children and vegetarians, the latter without changing their food principles [89, 90]. Food prepared in iron cooking pots functions as an intervention for reducing iron-deficiency anaemia in developing countries [91].

1.10 Current research and development

Over the last few decades, advances in analytical methods have provided an avalanche of information that has led to a tremendous increase in knowledge of the importance of minerals in the human diet. Currently, the use of molecular biology techniques appears to offer the possibility of understanding the chemical and physiological mechanisms of the mineral nutrients, especially the microminerals, and maybe even the discovery of new biological roles for already known minerals. One should not ignore the fact that metabolic differences between the sexes exist, such that study of the molecular mechanisms of food components in cell systems from a sex-gender perspective may help clarify the potential impact of diet on human health.

Initially the mineral elements were categorized in a simplistic way into macromineral and micromineral nutrients. However, currently we know that each of these elements is of unique importance to the organism, in the same way as vitamins. Because of the large number of these elements and their interrelationships, achieving a complete understanding is an enormously difficult task for researchers and professionals working in this area to obtain.

Also, in addition to those minerals already classified as essential, there are indications that others, such as F and Sr, could also be classified as essential mineral nutrients for humans: F stimulates bone formation while Sr is a bone seeker, and both have stimulatory actions on osteoblasts and inhibitory actions on osteoclasts. Further studies are warranted to determine whether there are optimal amounts (deficiency and toxicity) to provide a dietary guideline for their adequate intake, and also to discuss whether these effects are sufficient to classify these elements as essential. More research aimed at characterizing the role of these minerals in the prevention and treatment of osteoporosis is required [92].

The interrelationships between the various minerals also need to be better understood. Recent research has suggested that children (1–6 years old) with iron-deficiency anaemia could be subject to a greater risk of Mn toxicity (whole blood Mn >20 μg/L), which could result in neurological problems. Thus in these cases it is important to treat the anaemia not only to normalize the nutritional status of the iron but also to reduce the risk of Mn toxicity [93]. It is not known if this situation also occurs in adults. Thus additional studies on the role of minerals in the human diet and their impact on human health still represent a vast field to be explored, with numerous possibilities.

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Abbreviations

ADA: American Dietetic Association
AI: Adequate intake
DRI: Dietary references intake
DSHEA: Dietary Supplement Health and Education Act
EAR: Estimated average requirement
EU: European Union
FDA: Food and Drug Administration
IOM: Institute of Medicine
NHANES: National Health and the Nutrition Examination Survey
NOAEL: No-observed-adverse-effect level
RDA: Recommended dietary allowance
SR-XRF: Synchrotron radiation X-ray fluorescence
UL: Tolerable upper intake level

References

1 Garcia RWD. Notas sobre a origem da culinária: uma abordagem evolutiva. *Rev Nutr* 1995;**8**:231–244.
2 Carvalho ML, Marquesa AF, Lima MT, Reus U. Trace elements distribution and post-mortem intake in human bones from Middle Age by total reflection X-ray fluorescence. *Spectrochim Acta B* 2004;**58**:1251–1257.
3 Maurer AF, Gérard M, Person A *et al*. Intra-skeletal variability in trace elemental content of Precolumbian Chupicuaro human bones: the record of post-mortem alteration and a tool for palaeodietary reconstruction. *J Archaeol Sci* 2011;**38**:1784–1797.
4 Bumsted MP. Past human behavior from bone chemical analysis: respects and prospects. *J Hum Evol* 1985;**14**: 539–551.
5 Quattropani L, Charlet L, Lumley H, Menu M. Early palaeolithic bone diagenesis in the Arago cave at Tautavel, France. *Mineral Mag* 1999;**63**;801–812.
6 Tandon L, Iyengar GV, Parr RM. A review of radiologically important trace elements in human bones. *Appl Radiat Isot* 1998;**49**:903–910.
7 Jaworowski Z, Barbalat F, Blain C, Pyere, E. Heavy metals in human and animal bones from ancient and contemporary France. *Sci Total Environ* 1985;**34**:106–123.
8 Swanston T, Varney T, Coulthard I *et al*. Element localization in archaeological bone using synchrotron radiation X-ray fluorescence: identification of biogenic uptake. *J Archaeol Sci* 2012;**39**:2409–2413.
9 Killilea DW, Maier JAM. A connection between magnesium deficiency and aging: new insights from cellular studies. *Magnesium Res* 2008;**21**:77–82.
10 Penafiel D, Lachat C, Espinel R, Van Damme, P, Kolsteren P. A systematic review on the contributions of edible plant and animal biodiversity to human diets. *Ecohealth* 2011;**8**: 381–399.
11 Woolgar CM. Gifts of food in late medieval England. *J. Medieval History* 2010;**37**:6–18.
12 Martínez-García MJ, Moreno JM, Moreno-Clavel J *et al*. Heavy metals in human bones in different historical epochs. *Sci Total Environ* 2005;**348**:51–72.
13 Fan MS, Zhao F-J, Fairweather-Tait SJ, Poulton PR, Dunham SJ, McGrath SP. Evidence of decreasing mineral density in wheat grain over the last 160 years. *J Trace Elem Med Biol* 2008;**22**:315–324.
14 dos Santos KMO. *O desenvolvimento histórico da ciência da nutrição em relação ao de outras ciências*. Dissertation, Coleção CLE, UNICAMP, Campinas, SP, Brasil, 1989.
15 Welch RM, Graham RD. Breeding for micronutrients in staple food crops from a human nutrition perspective. *J Exp Bot* 2004;**55**:353–364.
16 Fraga CG. Relevance, essentiality and toxicity of trace elements in human health. *Mol Aspects Med* 2005;**26**: 235–244.
17 Mertz W. The essential trace elements. *Science* 1981; **213**:1332–1338.
18 Ryan E. *Trace element nutrition: revitalising health and agriculture*. Nuffield Australia, 2010. Available at http://www.nuffieldinternational.org/rep_pdf/1283473559Evan_Ryan_Final_%282%29.pdf
19 Verdú JM, Marín EC. *Nutricion Para Educadores*. Díaz de Santos, Madrid, España, 1995.
20 Shenkin A. The key role of micronutrients. *Clin Nutr* 2006;**25**:1–13.
21 Hambidge M. Biomarkers of trace mineral intake and status. *J Nutr* 2003;**133**:948S–955S.

22 Sobotka L, Allison S, Stanga Z. Basics in clinical nutrition: water and electrolytes in health and disease. *e-SPEN, the European e-Journal of Clinical Nutrition and Metabolism* 2008;**3**:e259–e266.
23 Whiting SJ, Barabash WA. Dietary Reference Intakes for the micronutrients: considerations for physical activity. *Appl Physiol Nutr Metab* 2006;**31**:80–85.
24 Institute of Medicine. Dietary reference intakes: elements (2004). Available at http://www.iom.edu/Global/News%20Announcements/~/media/48FAAA2FD9E74D95BBDA2236E7387B49.ashx (accessed 23 April 2013).
25 Institute of Medicine. Dietary reference intakes: electrolytes and water (2004). Available at http://www.iom.edu/~/media/Files/Activity%20Files/Nutrition/DRIs/DRI_Electrolytes_Water.pdf (accessed 23 April 2013).
26 World Health Organization. Human vitamin and mineral requirement. Report of a joint FAO/WHO expert consultation, 2001. Available at ftp://ftp.fao.org/docrep/fao/004/y2809e/y2809e00.pdf (accessed 29 April 2013).
27 Mertz W. Risk assessment of essential trace elements: new approaches to setting recommended dietary allowances and safety limits. *Nutr Rev* 1995;**53**:179–185.
28 Renwick AG, Dragsted LO, Fletcher RJ *et al*. Minimising the population risk of micronutrient deficiency and overconsumption: a new approach using selenium as an example. *Eur J Nutr* 2008;**47**:17–25.
29 Fairweather-Tait SJ, Harvey LJ, Collings R. Risk–benefit analysis of mineral intakes: case studies on copper and iron. *Proc Nutr Soc* 2011;**70**:1–9.
30 Shenkin A. Impact of disease on markers of macronutrient status. *Proc Nutr Soc* 1997;**56**:433–441.
31 Lee B, Kim Y. Effects of menopause on blood manganese levels in women: analysis of 2008–2009 Korean National Health and Nutrition Examination Survey data. *Neurotoxicology* 2012;**33**:401–405.
32 Unice KM, Monnot AD, Gaffney SH *et al*. Inorganic cobalt supplementation: prediction of cobalt levels in whole blood and urine using a biokinetic model. *Food Chem Toxicol* 2012;**50**:2456–2461.
33 Isfaoun F, Bureau M, Mouly-Boudey M, Drosdowsky P, Arhan P, Bouglá, D. Relationships between iron and zinc metabolism: predictive value of digestive absorption on tissue storage. *J Trace Elem Med Biol* 1997;**11**:23–27.
34 Cashman MW, Sloan SB. Nutrition and nail disease. *Clin Dermatol* 2010;**28**:420–425.
35 Oberleas D. Mechanism of zinc homeostasis. *J Inorg Biochem* 1996;**62**:231–241.
36 Domanski D, Freue GVC, Sojo L *et al*. The use of multiplexed MRM for the discovery of biomarkers to differentiate iron-deficiency anemia from anemia of inflammation. *J Proteomics* 2012;**75**:3514–3528.
37 Prat O, Ansoborlo E, Sage N *et al*. From cell to man: evaluation of osteopontin as a possible biomarker of uranium exposure. *Environ Int* 2011;**37**:657–662.
38 Laurberg P, Cerqueira C, Ovesen L *et al*. Iodine intake as a determinant of thyroid disorders in populations. *Best Pract Res Clin Endocrinol Metab* 2010;**24**:13–27.
39 Maret W, Sandstead HH. Zinc requirements and the risks and benefits of zinc supplementation. *J Trace Elem Med Biol* 2006;**20**:3–18.
40 Murphy SP, Beaton GH, Calloway DH. Estimated mineral intakes of toddlers: predicted prevalence of inadequacy in village populations in Egypt, Kenya, and Mexico. *Am J Clin Nutr* 1992;**56**:565–572.
41 Vaquero MP. Magnesium and trace elements in the elderly: intake, status and recommendations. *J Nutr Health Aging* 2002;**6**:147–153.
42 Lipski E. Traditional non-Western diets. *Nutr Clin Pract* 2010;**25**:585–593.
43 Milton K. Nutritional characteristics of wild primate foods: do the diets of our closest living relatives have lessons for us? *Nutrition* 1999;**15**:488–498.
44 Lindeberg S. Palaeolithic diet ('stone age' diet). *Scand J Food Nutr* 2005;**49**:75–77.
45 Lindeberg S, Cordain L, Eaton SB. Biological and clinical potential of a Palaeolithic diet. *J Nutr Environ Med* 2003;**13**:149–160.
46 Rosanoff A. Changing crop magnesium concentrations: impact on human health. *Plant Soil* 2013;**368**:139–153.
47 Lazenby RA. Bone loss, traditional diet, and cold adaptation in Arctic populations. *Am J Hum Biol* 1997;**9**:329–341.
48 Renwick AG, Flynn A, Fletcher RJ, Müller DJ, Tuijtelaars S, Verhagen H. Risk–benefit analysis of micronutrients. *Food Chem Toxicol* 2004;**42**:1903–1922.
49 Luk E, Jensen LT, Culotta VC. The many highways for intracellular trafficking of metals. *J Biol Inorg Chem* 2003;**8**:803–809.
50 Kozyraki R, Cases O. Vitamin B12 absorption: mammalian physiology and acquired and inherited disorders. *Biochimie* 2013;**95**:1002–1007.
51 El Hage Chahine JM, Hémadi M, Ha-Duong NT. Uptake and release of metal ions by transferrin and interaction with receptor 1. *Biochim Biophys Acta* **2012**;**1820**:334–347.
52 Nasreddine L, Nashalian O, Naja F *et al*. Dietary exposure to essential and toxic trace elements from a total diet study in an adult Lebanese urban population. *Food Chem Toxicol* 2010;**48**:1262–1269.
53 Becker W, Jorhem L, Sundström B, Grawé KP. Contents of mineral elements in Swedish market basket diets. *J Food Comp Anal* 2011;**24**:279–287.
54 Tanase CM, Griffin P, Koski KG, Cooper MJ, Cockell KA. Sodium and potassium in composite food samples from the Canadian Total Diet Study. *J Food Comp Anal* 2011;**24**:237–243.
55 Moreira DCF, Manzoli de Sá JS, Cerqueira IB *et al*. Mineral inadequacy of oral diets offered to patients in a Brazilian hospital. *Nutr Hosp* 2012;**27**:288–297.

56 Malorni W, Campesi I, Straface E, Vella S, Franconi F. Redox features of the cell: a gender perspective. *Antioxid Redox Signal* 2007;**9**:1779–1801.

57 Marino M, Masella R, Bulzomi P, Campesi I, Malorni W, Franconi F. Nutrition and human health from a sex-gender perspective. *Mol Aspects Med* 2011;**32**:1–70.

58 Dao MC, Meydani SN. Micronutrient status, immune response and infectious disease in elderly of less developed countries. *Sight Live Mag* 2009;**3**:6–15.

59 Wakimoto P, Block G. Dietary intake, dietary patterns, and changes with age: an epidemiological perspective. *J Gerontol A Biol Sci Med Sci* 2001;**56**:65–80.

60 Raynaud-Simon A. Virtual Clinical Nutrition University: malnutrition in the elderly, epidemiology and consequences. e-SPEN J, the European e-Journal of Clinical *Nutrition and Metabolism* 2009;**4**:e86–e89.

61 Failla ML. Trace elements and host defense: recent advances and continuing challenges. *J Nutr* 2003;**133**: 1443S–1447S.

62 Emkey RD, Emkey GR. Calcium metabolism and correcting calcium deficiencies. *Endocrinol Metab Clin North Am* 2012;**41**:527–556.

63 de Groot CP, van den Broek T, van Staveren W. Energy intake and micronutrient intake in elderly Europeans: seeking the minimum requirement in the SENECA study. *Age Ageing* 1999;**28**:469–474.

64 Schümann K, Ettle T, Szegner B, Elsenhans B, Solomons NW. On risks and benefits of iron supplementation: recommendations for iron intake revisited. *J Trace Elem Med Biol* 2007;**21**:147–168.

65 Stanga Z. Basics in clinical nutrition: nutrition in the elderly. e-SPEN J, the European e-Journal of Clinical *Nutrition and Metabolism* 2009;**4**:e289–e299.

66 Pae M, Meydani SN, Wu D. The role of nutrition in enhancing immunity in aging. *Aging Dis* 2012;**3**:91–129.

67 Girodon F, Galan P, Monget AL *et al*. Impact of trace elements and vitamin supplementation on immunity and infections in institutionalized elderly patients: a randomized controlled trial. MIN. VIT. AOX. geriatric network. *Arch Intern Med* 1999;**159**:748–754.

68 Avenell A, Campbell MK, Cook JA *et al*. Effect of multivitamin and multimineral supplements on morbidity from infections in older people (MAVIS trial): pragmatic, randomised, double blind, placebo controlled trial. *BMJ* 2005;**331**:1–6.

69 Stephen AI, Avenell A. A systematic review of multivitamin and multimineral supplementation for infection. *J Hum Nutr Diet* 2006;**19**:179–190.

70 Biesalski HK, Grune T, Tinz J, Zöllner I, Blumberg JB. Reexamination of a meta-analysis of the effect of antioxidant supplementation on mortality and health in randomized trials. *Nutrients* 2010;**2**:929–949.

71 Bailey LR, Gahche JJ, Lentino CV *et al*. Dietary supplement use in the United States, 2003–2006. *J Nutr* 2011;**141**:261–266.

72 Dietary Supplement Health and Education Act of 1994. Public L No. 103–417 (codified at 42 USC 287C-11).

73 European Food Safety Authority (EFSA). Available at http://www.efsa.europa.eu/en/topics/topic/supplements.htm (accessed 30 April 2013).

74 Blot WJ, Li J-Y, Taylor PR *et al*. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J Natl Cancer Inst* 1993;**85**:1483–1491.

75 Hercberg S, Galan P, Preziosi P *et al*. The SU.VI. MAX study: a randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. *Arch Intern Med* 2004;**164**:2335–2342.

76 Hercberg S, Ezzedine K, Guinot C *et al*. Antioxidant supplementation increases the risk of skin cancers in women but not in men. *J Nutr* 2007;**137**:2098–2105.

77 Asgari MM, Maruti SS, Kushi LH, White E. Antioxidant supplementation and risk of incident melanomas: results of a large prospective cohort study. *Arch Dermatol* 2009;**145**: 879–882.

78 Khadilkar A, Kadam N, Chiplonkar S, Fischer PR, Khadilkar V. School-based calcium–vitamin D with micronutrient supplementation enhances bone mass in underprivileged Indian premenarchal girls. *Bone* 2012;**51**:1–7.

79 Lippman SM, Klein EA, Goodman PJ *et al*. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA* 2009;**301**:39–51.

80 Fairweather-Tait SJ. Bioavailability of dietary minerals. *Biochem Soc Trans* 1996;**24**:775–780.

81 Murphy SP, White KK, Park S-Y, Sharma S. Multivitamin-multimineral supplements' effect on total nutrient intake. *Am J Clin Nutr* 2007;**85**:280S–284S.

82 Marra MV, Boyar AP. Position of the American Dietetic Association: nutrient supplementation. *J Am Diet Assoc* 2009;**109**:2073–2085.

83 McCarron DA, Reusser ME. Are low intakes of calcium and potassium important causes of cardiovascular disease? *Am J Hypertens* 2001;**14**:206S–212S.

84 Bucher HC, Cook RJ, Guyatt GH *et al*. Effects of dietary calcium supplementation on blood pressure: a meta-analysis of randomized controlled trials. *JAMA* 1996;**275**:1016–1022.

85 Griffith L, Guyatt GH, Cook RJ, Bucher HC, Cook DJ. The influence of dietary and nondietary calcium supplementation on blood pressure: an updated metaanalysis of randomized clinical trials. *Am J Hypertens* 1999;**12**:84–92.

86 Carbone LD, Barrow KD, Bush AJ, Boatright DM, Michelson JA, Pitts KA. Effects of a low sodium diet on bone metabolism. *J Bone Miner Metab* 2005;**23**:506–513.

87 Hurrell R, Ranum P, de Pee S *et al*. Revised recommendations for iron fortification of wheat flour and an evaluation of the expected impact of current national wheat flour fortification programs. *Food Nutr Bull* 2010;**31**:S7–S21.
88 Sanou D, Turgeon-O'Brien H, Desrosiers T. Nutrition intervention and adequate hygiene practices to improve iron status of vulnerable preschool Burkinabe children. *Nutrition* 2010;**26**:68–74.
89 Adish AA, Esrey SA, Gyorkos TW, Jean-Baptiste J, Rojhani A. Effect of consumption of food cooked in iron pots on iron status and growth of young children: a randomised trial. *Lancet* 1999;**353**:712–716.
90 Quintaes KD, Amaya-Farfan J, Tomazini FM, Morgano MA, Haj-Isa NMA, Trezza Netto J. Mineral migration and influence of meal preparation in iron cookware on the iron nutritional status of vegetarian students. *Ecology Food Nutr* 2007;**46**:125–141.
91 Geerligs PDP, Brabin BJ, Omari AAA. Food prepared in iron cooking pots as an intervention for reducing iron deficiency anaemia in developing countries: a systematic review. *J Hum Nutr Diet* 2003;**16**:275–281.
92 Aaseth J, Boivin G, Ole Andersen O. Osteoporosis and trace elements: an overview. *J Trace Elem Med Biol* 2012;**26**: 149–152.
93 Smith EA, Newland P, Bestwick KG, Nessar Ahmed N. Increased whole blood manganese concentrations observed in children with iron deficiency anaemia. *J Trace Elem Med Biol* 2013;**27**:65–69.

CHAPTER 2
Dietary intake of minerals

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Abstract: Essential elements are crucial for the interaction between genetic and physiological factors. If a dietary deficiency of these elements exists, it will lead to physiological and structural abnormalities that are preventable and which may be reversed by administration of the element. However, there are two aspects to the nutritional imbalance spectrum: (i) low intake resulting in deficiencies and (ii) high intake resulting in potential toxicity. This chapter focuses on the role of selected essential and trace- element components of foods and the recommended daily intake for different groups of age and sex, as well as the bioavailability and nutrient interactions in food.

Keywords: bioavailability, diet, essential, trace and toxic elements, infant, nutrient interaction

2.1 Essential, trace and toxic elements in foods

Dietary minerals are inorganic compounds including essential and essential trace elements. Essential elements are those that occur in the body in milligram per kilogram quantities. They include calcium, magnesium, sodium, potassium, iron and zinc as well as chloride and fluoride ions. Essential trace elements, however, are required in milligram and sub-milligram quantities, and include copper, manganese, cobalt, chromium, selenium, molybdenum, nickel and iodine (Table 2.1) [1].

Essential elements are crucial for the interaction between genetic and physiological factors and if a dietary deficiency of these elements exists it will lead to physiological and structural abnormalities which are preventable and which may be reversed by administration of the element [2, 3]. However, there are two aspects to the nutritional imbalance spectrum: (i) low intake resulting in deficiencies and (ii) high intake resulting in potential toxicity [4].

The non-essential elements, such as arsenic, barium, nickel, cadmium, antimony, lead, mercury and aluminium, are food contaminants with cumulative properties and are thus considered potentially dangerous (toxic) for the consumer. The presence of non-essential elements in food is usually attributed to (i) naturally occurring sources such as raw materials or (ii) contamination during processing of food throughout the supply chain. It should be noted that infants have a relatively higher absorption (intake/body size) and less effective excretion of several elements compared with adults [5]. The contamination of food by these non-essential elements must therefore be kept at an absolute minimum – ideally they should not be present – especially if the food is intended for consumption by vulnerable groups such as infants and young children, pregnant women and the elderly. In the study by Ljung *et al.* [6], the arsenic concentration of rice-based infant powdered food was found to be in excess of that considered safe. The situation is further hindered by the fact that the data required for science-based risk assessment of infant exposure to non-essential elements is currently lacking [7].

2.1.1 Iron

Iron is an essential nutrient for oxygen transport and cellular energy generation, and is a functional component/cofactor in over 200 metalloenzymes. However, excess iron is toxic because of Fenton chemistry-derived free radicals; consequently iron homeostasis is highly regulated.

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Table 2.1 Examples of the major essential and trace elements and their function in the body.

Nutrients	RNI*/day	Functions	Deficiency
Calcium (mg)	525	Healthy bones and teeth, regulation of muscle contraction and nerve conduction, blood clotting, enzyme activation and hormone secretion	Rickets, irritability, jitteriness, tremors and convulsions in newborn babies
Magnesium (mg)	75	Involved in glycolysis, replication of DNA and synthesis of RNA , regulatory effect on Ca and K level, parathyroid hormones secretion, vitamin D metabolism and subsequently bone function, normal neuromuscular function, steady heart rate	Muscle spasm and weakness, sleep disorders, irritability, poor nail growth, neuromuscular excitability
Iron (mg)	7.8	Major part of haemoglobin and role in cognitive development	Anaemia, pale skin, tiredness, motor and mental problems
Zinc (μg)	5	Growth and immune function, site-specific antioxidant, synthesis and activation of enzymes and proteins such as insulin, vitamin A, nucleic acids	Growth retardation, immune deficiency, loss of appetite, night blindness
Copper (mg)	0.3	Catalyst in mobilization of iron, connective tissue synthesis	Skeletal demineralization, decreased skin tone, decreased plasma iron
Selenium (μg)	10	Antioxidant, maintenance of healthy immune system, interaction with heavy metals	Asthma, vulnerability to infection

*UK recommended nutrient intake for an infant 6–9 months of age.
Source: Department of Health [23].

The World Health Organization (WHO) estimates that some 2 billion people suffer from anaemia, and in approximately half of these patients the condition is secondary to iron-deficiency anaemia (IDA) [8]. The majority of these cases occur in the developing world, but IDA also exists in the developed world; for example, in the UK 3% of men and 8% of women have haemoglobin levels consistent with IDA and in certain vulnerable subgroups the estimated incidence is considerably higher [9]. Populations at high risk for IDA include toddlers, adolescents, menstruating women and the elderly.

IDA causes significant morbidity. Adults with IDA suffer from fatigue and impaired work performance and immune responses; pregnant women are at increased risk for premature birth and small-for-gestational-age infants [10]. In toddlers and young children, lack of iron is a particular problem as it can cause delayed psychomotor and cognitive development that does not reverse even with adequate intake later in life [11]. IDA is caused by both low absolute amounts of iron relative to need and poorly bioavailable iron.

Excess iron in the body is also deleterious. However, because of tight homeostatic regulation of iron absorption, iron overload primarily as a result of disorders of iron metabolism, such as the genetic condition hereditary haemochromatosis, or conditions of ineffective erythropoiesis especially those requiring multiple blood transfusions. However, accidental overdose of iron supplements, particularly in children, can overwhelm regulatory systems and be lethal.

2.1.2 Calcium

Calcium is essential for the maintenance of skeletal growth and structure, muscular function, nerve conduction, and normal blood clotting; in addition, calcium is integral to multiple intracellular signalling pathways and serves as a cofactor in enzyme reactions. It is one of the major minerals of the body, accounting for approximately 2% of adult body weight; 99% of body calcium is contained in bone, the rest being found in teeth, connective tissue, extracellular fluid and blood (0.1%). It is plasma calcium that is critical for normal neuromuscular function throughout the body and in particular for the heart and nervous system; thus blood calcium levels are very tightly regulated and defended within a narrow concentration range of 2.3–2.6 mmol/L.

Calcium is mainly absorbed in the jejunum, although a small amount is taken up in the ileum and colon; it is excreted via the gut and kidney, and insensible losses occur from hair and skin and via nail loss. Gut absorption

and kidney reabsorption are primarily under the control of vitamin D, which induces the expression of several calcium transport proteins that enhance active uptake of the mineral; there is also a passive calcium absorption pathway in the gut but this has not been well characterized [12]. Gut absorption and kidney reabsorption are inversely correlated with dietary calcium intake; at low intakes absorption may go up to 45%, whereas with intakes above 2000 mg/day absorption decreases to 15% of intake [13].

If dietary calcium intake is inadequate to maintain plasma calcium, vitamin D in concert with parathyroid hormone (PTH), a peptide hormone released in response to hypocalcaemia, induces bone resorption and release of calcium. Blood calcium homeostasis takes precedence over skeletal mineralization and maintenance, and chronic inadequate calcium intake causes bone loss or suboptimal skeletal growth.

The WHO states that calcium deficiency is a significant public health problem in the developing world, especially for high-risk groups including young children up to 2 years old, prepubescent children and adolescents, and pregnant women [12]. In all these groups bone development and growth require a positive calcium balance. Within the developed world there are also vulnerable populations such as pregnant adolescents and the elderly. However, care is required when considering reports of inadequate intake, as there is considerable lack of agreement among countries regarding recommended calcium intakes. Discrepancies arise for several reasons.

1 Estimates of dietary calcium bioavailability differ between countries, ranging from 15–75% (USA) to 30–40% (UK and EU) [14]; requirements are partly based on assumptions about how much of the consumed nutrient is available for absorption.
2 It is difficult to assess 'adequate' calcium status as blood calcium levels are highly defended and may be normal even if intake is inadequate for optimal bone health; there are no other direct biochemical markers of adequate status.
3 Although correlating optimal skeletal health with measured calcium intake may be used to determine requirements, calcium intake varies widely across the world, ranging from 200 to 800 mg/day, and intakes do not always correlate with bone health [12].

Interestingly, some parts of the world with the highest calcium intakes (such as Scandinavia) suffer from higher rates of osteoporosis (bone loss) compared with areas with much lower dietary calcium. Current recommended intakes are derived primarily from calcium balance studies carried out in northern latitude populations with high average dietary calcium from dairy foods, and it is unclear how generalizable these results are to other populations. There is evidence of adaptation in populations with low intakes; it is suggested that absorption is increased and urinary excretion decreased. The importance of such adaptations, and also the need for population-specific recommended intakes, is illustrated by several recent studies carried out by Prentice *et al*. [15] in the Gambia where calcium intakes are among the lowest in the world. Calcium supplementation of a cohort of Gambian prepubescent boys actually resulted in shorter adult height compared with controls, and calcium-supplemented pregnant women had subsequent greater maternal bone loss during lactation compared with controls [15, 16]. Further studies are needed in populations from different geographic regions, and with different genetic, dietary and lifestyle factors, in order to be able to make valid worldwide calcium intake recommendations.

Calcium toxicity from excess dietary intake is rare and is primarily seen in people consuming large amounts of calcium or vitamin D supplements, or in populations with impaired renal excretion of the mineral. Toxicity may cause cardiac arrhythmias, constipation, nephrolithiasis, delirium and in extreme cases death.

2.1.3 Zinc

Zinc is an important trace element. It acts as a cofactor for a number of enzymes and has a structural role in zinc finger proteins in transcription factors. Zinc acts as an antioxidant and has anti-inflammatory actions [17].

In its role as an antioxidant, zinc is an inhibitor of the NADPH oxidase enzyme which produces reactive oxygen species. The enzyme superoxide dismutase (SOD), which contains zinc, catalyses the dismutation of superoxide into hydrogen peroxide [17].

Zinc is vital for the normal development and function of cells involved in innate immunity, neutrophils and natural killer cells. Zinc is needed for DNA synthesis, RNA transcription, call division and activation. Zinc deficiency will affect macrophages, phagocytosis, intracellular killing and cytokine production [17]. Zinc also has a major role in the immune system. Deficiency of zinc has been shown to increase susceptibility to

numerous pathogens and has effects on specific and non-specific immunity. Deficiency can cause depression of T and B lymphocyte function, and decrease proliferative responses to mitogens, rescues thymulin activity and production of numerous cytokines, along with an imbalance of Th1 and Th2 cells. In infants, zinc deficiency causes immunological impairments due to reduced antibody production [18].

Zinc is essential for reproduction, for the synthesis and secretion of luteinizing hormone and follicle stimulating hormone, gonadal differentiation, testicle growth, formation and maturation of spermatozoa and testicular steroidogeneis and fertilization. Underlying mechanisms involve numerous zinc-dependent enzymes [18]. Zinc is also important during pregnancy as all the hormones and many of the enzymes involved in reproduction and development are sensitive to zinc status [18].

Absorption of zinc occurs in the small intestine by both active and passive mechanisms. Absorption activators include picolinic acid (which is secreted by the pancreas), vitamin B_6 (which enhances picolinic acid secretion), citrate and certain amino acids. Absorption inhibitors include phytic and oxalic acids, tannins, fibre and selenium. Absorption efficiency is only 15–40% and zinc that is not absorbed is excreted via faeces. Transport of zinc in the blood is mainly by albumin, where most of it reaches the liver and is stored bound to metallothionein, which can bind seven zinc atoms per molecule. However, they bind to copper with higher affinity [18].

Zinc deficiency is a worldwide nutritional problem affecting both developed and developing countries. Newborns, children, pregnant women and the elderly are considered the most at risk of zinc deficiency. Zinc can interfere with copper metabolism [18]. Zinc deficiency can cause growth retardation, male hypogonadism, rough skin, poor appetite, delayed wound healing, cell-mediated immune dysfunction, and abnormal neurosensory changes [17].

2.1.4 Selenium

Selenium is a trace mineral that is an essential component for many metabolic pathways, including thyroid hormone metabolism, immune function and antioxidant defence systems. There have been over 35 selenoproteins identified, although many of their roles have not been fully elucidated [19, 20]. There has been a decline in the concentration of blood selenium in the UK and Europe over the last two decades, probably caused by reduced selenium content in the soil [21].

Selenium is incorporated as selenocysteine into the active site of numerous selenoproteins, such as glutathione peroxidase enzymes, thioredoxin reductase and iodothyronine deiodinase enzymes. In addition, selenoproteins in the sperm capsule can stabilize the integrity of the sperm flagella [21]. Glutathione peroxidase enzymes function as antioxidants by reducing hydrogen peroxide and phospholipase A_2-cleaved lipid hydroperoxides to harmless products, which helps maintain membrane integrity and protects against oxidative damage [20]. There are four types of glutathione peroxidase enzymes: classical, gastrointestinal, plasma and phospholipid hydroperoxide. Thioredoxin reductase enzymes catalyse the NADPH-dependent reduction of thioredoxin. Iodothyronine deiodinase enzymes catalyse the 5′-monodeiodination of the prohormone thyroxine to the active thyroid hormone 3,5,3′-triiodothyronine. Thyroid hormones play an essential regulatory role in hepatic enzyme expression and neutrophil function [21].

There has been evidence to suggest that selenium has a protective effect against certain forms of cancer, can enhance fertility, decrease cardiovascular disease and regulate inflammatory mediators [21].

Overall absorption of selenium is relatively high, ranging from 70 to 95%; however this is dependent on the source of selenium and status of the individual [22]. Around 60% of the selenium found in plasma is incorporated into selenoprotein P; this contains 10 selenium atoms in the form of selenocysteine and serves as a transport protein for selenium [21]. Selenophosphate synthetase is required for the biosynthesis of selenophosphate, the precursor of selenocysteine. The main route of selenium excretion is via the urine and it is mainly stored in skeletal muscle [23].

Clear clinical signs of selenium deficiency are rare, although areas in north-east China where soil selenium is extremely low have caused an endemic of fatal cardiomyopathy known as Keshan disease. Another deficiency of selenium is Kashin–Beck disease, which is a form of osteoarthritis [23]. In countries such as New Zealand, Finland and the eastern USA, governments have begun mandatory selenium supplementation schemes, such as enriched food products, soil fertilizers or direct supplementation regimens [21].

There is strong evidence to suggest that selenium can be protective against certain forms of cancer, including

colon, prostate and lung. Low blood selenium concentrations have also been linked to increased cardiovascular disease mortality [21]. Selenium deficiency can induce reduced T-cell counts and impair lymphocyte proliferation and responses [20]. Insufficient selenium can impair sperm mitochondrial capsule synthesis, which can lead to sterility and decreased sperm motility. Functional selenium and vitamin E status has been suggested to influence leukotriene metabolism, which is important in relation to chronic inflammatory diseases, especially asthma [21].

Although selenium deficiency may contribute to the aetiology of many diseases, in certain cases such as HIV lower selenium status is a consequence of the disease itself. Although selenium is essential for human health it is also extremely toxic. Excessive selenium in the blood can cause selenosis, which causes hair loss, nausea, fatigue and mild nerve damage.

2.1.5 Copper

Copper is an essential trace element present in all tissues [24]. Copper is a vital component of numerous enzymes, including cytochrome c oxidase and SOD. Copper enzymes are also involved in the synthesis of a number of neuroactive amines and peptides such as catecholamines and enkephalins. Copper helps make red blood cells, keeps nerve cells and the immune system healthy, helps form collagen, helps the body absorb iron and is required for energy production [25].

Copper is present throughout the brain. Numerous enzymes in the central nervous system are copper dependent, including tyrosinase, peptidylglycine α- amidating monooxygenase, copper/zinc SOD, ceruloplasmin, hephaestin, dopamine β-hydroxylase and cytochrome c oxidase. Dopamine β-monooxygenase catalyses the conversion of dopamine into norepinephrine (noradrenaline). Tyrosinase is needed for the formation of melanin [26]. Cytochrome c oxidase is important for energy production within cells; it catalyses the reduction of oxygen to water within the mitochondria by creating an electrical gradient to produce ATP. Lysyl oxidase is needed for the cross-linking of collagen and elastin, which are required for the formation of strong and flexible connective tissue of heart and blood vessels. Cytochrome c oxidase is also involved in myelin sheath synthesis. Ceruloplasmin and ferroxidase II are both copper-containing enzymes that can oxidize ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}). Ferric iron is the form of iron that can be loaded onto the protein transferrrin for transportation to the site of red blood cell formation [26].

There are two forms of SOD which contain copper. Copper/zinc SOD is located within most cells of the body, while extracellular SOD is found in high levels within the lung. SOD is an antioxidant that catalyses the conversion of superoxide radicals to hydrogen peroxide [26]. Certain transcription factors are also copper dependent, including genes for copper/zinc SOD, catalase and proteins associated with cellular storage of copper [26].

Copper appears to be important for normal iron metabolism. High zinc intakes may result in copper deficiency, because zinc increases the synthesis of metallothionein which binds certain metals and prevents absorption in the intestine.

In infants and children with copper deficiency, symptoms include leucopenia, skeletal fragility and increased susceptibility to respiratory tract and other infections. If deficiency is prolonged, anaemia and osteoporosis can develop. Neutropenia, an abnormally low number of neutrophils, is a clinical sign of copper deficiency.

Wilson's disease is an inherited genetic disorder where copper deposits in the tissues of the brain, liver and other organs leads to hepatitis, brain disorders and kidney problems. Menkes disease is another genetic disorder that causes severe copper deficiency, where sufferers have frequent and severe infections [26].

2.1.6 Magnesium

Magnesium is an essential mineral, the second intracellular cation after sodium and fourth most abundant cation in the human body [27]. Magnesium plays a major role in skeletal development and maintains electrical potential in both nerve and muscle membranes. It can also act as a cofactor for numerous enzymes that require ATP. Therefore magnesium is essential for membrane stabilization, energy production, oxidative phosphorylation, glycolysis, DNA transcription and protein synthesis. Magnesium is essential for bone health and plays a role in skeletal homeostasis by influencing the secretion of hormones such as PTH and the active form of vitamin D. Bone cell function can be directly affected by magnesium, as well as hydroxyapatite formation and growth [28]. Calcium can inhibit many of the magnesium-activated enzymes; in some cases manganese can be replaced by magnesium. Magnesium is also involved in

the active transport of calcium and potassium across cell membranes, which is essential for nerve impulse conduction, muscle contraction and heart rhythm [29].

Nearly two-thirds (60%) of magnesium is stored in the skeleton and the rest in soft tissues. Only 1% is present in blood serum, which is tightly regulated between 0.75 and 0.95 mmol/L [30, 31]. Approximately 30–40% of dietary magnesium that is consumed will be absorbed by the body [32, 33].

Absorption of magnesium is both by passive diffusion and active transport along the intestinal tract, major sites of absorption being the ileum and distal jejunum [28]. Excretion mainly occurs through the kidneys. In states of excessive intake magnesium can pass through the intestine unabsorbed. On the other hand, when intakes decrease the kidneys conserve magnesium and the skeleton acts as a store [23].

Hypomagnesaemia (serum magnesium below 0.75 mmol/kg) can be induced by a number of factors, including starvation, malabsorption syndromes, alcoholism, acute pancreatitis, and prolonged vomiting or diarrhea, but is uncommonly due to low dietary intakes. In addition hypomagnesaemia is almost always accompanied by hypocalcaemia. Characteristics of magnesium deficiency include progressive muscle weakness, neuromuscular dysfunction, tachycardia, ventricular fibrillation, coma and even death [23].

2.2 Recommended daily intake

Different sources are available for daily requirements of micronutrients for infants and young children (Table 2.2). The dietary requirements frequently used for infants and young children are the recommended nutrient intake (RNI) from the dietary reference values (DRVs) recommended by the Department of Health [23]. RNIs are based on the estimated average requirement (EAR) plus 2SD and reflect the average daily intake of a nutrient sufficient to meet the needs of almost all members (97.5%) of a healthy population. Intakes above this amount are thought to almost certainly be adequate. Values set may vary according to age, gender and physiological state (e.g. breast-fed or formula fed).

DRVs are based on the amount of nutrients given to a wholly breast-fed infant. However, there are a few limitations associated with how these values have been set, including uncertainty concerning the actual intake of infant foods, changes in composition of milk during the course of lactation between women, variability in the volume of milk supply, and because of difficulties in measuring these factors as well as ethical issues [34]. Another major limitation is the variation in efficacy of nutrient absorption in breast-fed infants, which is higher than for formula-fed infants. Examples of such differences are energy, protein and iron requirements between the two feeding regimens. Breast-fed infants absorb 50% of the iron in milk, whereas formula-fed infants absorb only 10% of the iron in the formula milk. The higher rate of weight gain in formula-fed infants may be related to the higher protein and energy content of formula than of human milk [35]. In addition, estimates of nutrient requirements usually match the amounts required to correct or avoid deficiency and therefore will supply the nutrients at a marginally adequate level.

2.2.1 Dietary recommendations for iron

There is no active form of iron excretion, but daily iron losses occur from desquamation of epithelial cells of the gut and urinary system, and menstrual blood loss in women. Growth increases iron requirements, as iron is needed for expanded tissue mass and increased blood volume. Losses and increased needs are met by iron from the diet. Recommended iron intakes differ depending on age, gender, pregnancy, lactation and iron bioavailability of the general diet. Recommendations also vary between countries because of differing evidence bases and selection criteria of evidence used to determine needs [36]. The WHO has developed the most extensive recommendations taking into account not only life stage but also dietary bioavailability for different stages and populations [12]. Recommended intakes for a Western diet with an estimated iron bioavailability of 15% is approximately 6 mg/day for infants and children (0.5–10 years), about 9–12.5 mg/day for adolescent males (11–18 years) and about 9–22 mg/day for adolescent females. Recommended intakes for adult women are 20 mg/day; postmenopausal women and men over the age of 18 years are recommended 7.5 mg/day [12].

2.2.2 Dietary recommendations for calcium

The basis for recommended calcium intakes has been discussed in section 2.1.2. Calcium requirements depend on life stage and are primarily related to skeletal growth

Table 2.2 Comparison of recommended nutrient intake (RNI) of infants (WHO*, DoH†) and dietary reference intake (DRI) by Institute of Medicine (IOM)‡.

	6–8 months			9–11 months			12–23 months		
	WHO 2004	RNI, DoH	DRI§, IOM	WHO 2004	RNI, DoH	DRI§ IOM	WHO 2004	RNI, DoH	DRI, IOM
Protein (g/day)	NA	12.7	NA	NA	13.7	NA	NA	14.9	NA
Vitamin A (RE μg/day)	400	350	500	400	350	500	400	400	300
Folate (μg/day)	80	50	80	80	50	80	160	70	150
Niacin (mg/day)	4	4	4	4	5	4	6	8	6
Riboflavin (mg/day)	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.6	0.5
Thiamine (mg/day)	0.3	0.2	0.3	0.3	0.3	0.3	0.5	0.5	0.5
Vitamin B_6 (mg/day)	0.3	0.3	0.3	0.3	0.4	0.3	0.5	0.7	0.5
Vitamin B_{12} (μg/day)	0.5	0.4	0.5	0.5	0.4	0.5	0.9	0.5	0.9
Vitamin C (mg/day)	30	25	50	30	25	50	30	30	15
Vitamin D (μg/day)	5	7	5	5	7	5	5	7	5§
Vitamin K (μg/day)	10	NA	2.5	10	NA	2.5	15	NA	30§
Calcium (mg/day)	400	525	270	400	525	270	500	350	500§
Chloride (mg/day)	NA	500	NA	NA	500	NA	NA	800	NA
Copper (mg/day)	NA	0.3	0.2	NA	0.3	0.2	NA	0.4	0.3
Fluoride (μg/day)	NA	60	0.5	Na	60	0.5	NA	70	0.7
Iodine (μg/day)	90	NA	130	90	NA	130	90	NA	90
Iron (mg/day)¶	9.3	7.8	11	9.3	7.8	11	5.8	6.9	7§
Magnesium (mg/day)	54	75	75	54	80	75	60	85	80
Manganese (mg/day)	NA	NA	0.6	NA	NA	0.6	NA	NA	1.2§
Phosphorus (mg/day)	NA	400	275	NA	400	275	NA	270	460
Potassium (mg/day)	NA	700	NA	NA	700	NA	NA	800	NA
Selenium (μg/day)	10	10	20	10	10	20	17	15	20
Sodium (mg/day)	NA	320	NA	NA	350	NA	NA	500	NA
Zinc (mg/day)	4.1‖	5	3	4.1‖	5	3	4.1‖	5	3

*World Health Organization, 2004 [12].

†Department of Health, 1991 [23].

‡Institute of Medicine, 1997 [30].

§Based on adequate intake (AI) estimates, apart from zinc.

¶Assuming medium bioavailability (10%).

‖Assuming moderate bioavailability (30%).

Table 2.3 RNI and EAR for zinc intakes.

Age	EAR (mg/day)	RNI (mg/day)
0–6 months	3.3	4.0
7 months to 3 years	3.8	5.0
4–6 years	5.0	6.5
7–10 years	5.4	7.0
11–14 years (males)	7.0	9.0
15+ years (males)	7.3	9.5
11–14 years (females)	7.0	9.0
15+ years (females)	5.5	7.0

Source: Department of Health [23].

in relation to dietary calcium levels, bioavailability, absorption and excretion. The WHO recommendations specify intakes of 300 mg/day for infants aged 0–6 months and 700 mg/day for children aged 7–9 years; requirements increase to 1000 mg/day for adolescents (10–18 years) undergoing the pubertal growth spurt [12]. Recommended intakes for adults (19–65 years) are 750 mg/day for both men and women, but increase to 800 mg/day for pregnant and postmenopausal women; recommended intakes for elderly men (65+ years) are 800 mg/day [12].

2.2.3 Dietary recommendations for zinc

According to the WHO, the standard for zinc intake is based on the sum of total endogenous zinc losses (without considering the influence of variation in absorbed zinc). However, due to variation in the bioavailability of zinc, the WHO sets standards for high, moderate and low availability in diets in which 50, 30 and 15% of the dietary zinc is absorbable, respectively. Zinc requirements in adults have been based on determining basal loss during metabolic studies of deprivation, turnover time of radiolabelled endogenous zinc pools and factorial studies. However, none are ideal but all indicate systemic needs of 2–3 mg/day; assuming 30% absorptive efficiency, these figures translate to RNIs of 7.0–9.5 mg/day (Table 2.3) [23].

2.2.4 Dietary recommendations for selenium

RNIs for selenium are based on blood glutathione peroxidase activity [19]. Glutathione peroxidase activity is used to assess intake as enzyme activity is proportional to dietary intake (Table 2.4).

Table 2.4 RNI of selenium for different age groups.

Age	RNI (μg/day)
0–3 months	10
4–6 months	13
7–12 months	10
1–3 years	15
4–6 years	20
7–10 years	30
11–14 years	45
15+ years (males)	70
15+ years (females)	60

Source: Department of Health [23].

Table 2.5 RNI of copper for different age groups.

Age	RNI (mg/day)
0–12 months	0.3
1–3 years	0.4
4–6 years	0.6
7–10 years	0.7
11–14 years	0.8
15–16 years	1.0
18+ years	1.2
Lactation	+0.3

Source: Department of Health [23].

Table 2.6 RNI and EAR of magnesium for different age groups.

Age	EAR (mg/day)	RNI (mg/day)
0–3 months	40	55
4–6 months	50	60
7–9 months	60	75
10–12 months	60	80
1–3 years	65	85
4–6 years	90	120
7–10 years	150	200
11–14 years	230	280
15–18 years	250	300
19–50 years (males)	250	300
19–50 years (females)	200	270
50+ years (males)	250	300
50+ years (females)	200	270
Lactation		+50

Source: Department of Health [23].

2.2.5 Dietary recommendations for copper

There are no adequate data on human copper requirements; however, biochemical changes in adults associated with varying copper intakes have been used to base RNI (Table 2.5).

2.2.6 Dietary recommendations for magnesium

The assessment of magnesium status is difficult as the majority of magnesium is present within the bones or cells. The most commonly used method of assessing magnesium status is by measuring serum concentrations [37]. The RNI is calculated from the EAR per kilogram of body weight (Table 2.6).

2.3 The presence of minerals in diets

2.3.1 Dietary iron

Iron in the diet is found in two distinct biochemical forms: haem iron and non-haem iron. Haem iron is iron complexed with porphyrin (a naturally occurring aromatic compound consisting of four modified pyrrole rings) and is derived predominantly from the metalloproteins haemoglobin and myoglobin; non-haem iron consists of inorganic ferric or ferrous iron, bound to peptides, salts or organic acids [38]. Non-haem iron is the predominant dietary form, and accounts for 70–90% of dietary iron intake throughout the world, higher in the developing and less in the developed world [39]. Non-haem iron has low bioavailability; in contrast, haem iron is highly bioavailable and although it contributes a relatively low absolute amount of dietary iron may be responsible for up to 50% of absorbed iron [39].

Meat, poultry and fish (MPF) are major sources of both haem and non-haem iron, and iron bioavailability in diets with significant amounts of MPF is approximately 15% because not only is haem iron very available but an as-yet unidentified 'meat factor' in MPF increases non-haem iron bioavailability.

Fortified cereals and cereal products such as bread and breakfast cereals provide approximately 50% of iron intake in the developed world because of both fortification and the frequency with which they are eaten. Other dietary sources of non-haem iron include legumes, nuts, vegetables and eggs, although bioavailability from these sources is moderate to low.

In general, non-haem iron bioavailability is low because many dietary factors interact with the metal and render it unavailable for absorption. Non-haem iron must be soluble and in the reduced state (Fe^{2+}) to be absorbed; however, most dietary iron is in the oxidized form (Fe^{3+}). Factors that alter non-haem iron speciation thus alter iron bioavailability. The two major inhibitors of non-haem iron absorption are phytates (plant energy storage molecules consisting of inositol phosphate), and polyphenols such as tannic acid and caffeine found in red wine, and coffee and tea respectively. Polyphenols are stronger inhibitors, but phytates, because of their ubiquitous presence in diets, have the biggest effect on iron availability. Both inhibitors form insoluble oxidized complexes with the mineral. The most significant enhancer of iron bioavailability is ascorbic acid (vitamin C); it both reduces and chelates iron, rendering it soluble and available for absorption in the gut. Low stomach acidity secondary to medications or disease also decreases iron bioavailability.

Food processing can change iron bioavailability. Cooking alters levels of haem iron: prolonged cooking at high temperatures breaks down the iron porphyrin ring and essentially partially converts haem to non-haem iron [40]. Besides the loss of a bioavailable iron form, processing may change bioavailability by causing degradation of enhancers such as vitamin C, or alternatively destruction of inhibitors, for example the breakdown of phytate by yeast phytase in leavened bread. Fortification of foods with multiple micronutrients may also change iron bioavailability; single meal studies demonstrate decreased iron absorption when iron is co-ingested with either calcium or zinc [41, 42], although the overall effects of these interactions in a free-living diet is unclear. A recent review concluded that, over the long term, calcium intake does not negatively affect iron status [43], suggesting that homeostatic mechanisms adjust to the short-term effects of calcium on iron absorption.

2.3.2 Dietary calcium

Calcium in the diet occurs in many forms, for example calcium–phosphate complexes, calcium phytate salt complexes and milk proteins (caseins). The main sources of dietary calcium in the Western developed world are dairy products, followed by fortified cereal products, while in the developing world cereals like millet and dried fishmeal including bones are also important food sources [44]. Low-oxalate green leafy

vegetables may be an alternative source of calcium for vegetarians [45]. Oxalate and phytates (inositol phosphate complexes found in plants) are the major dietary factors that decrease calcium bioavailability by forming insoluble complexes with the mineral; because of their prevalence in the diet, phytates are the most important inhibitors. Low stomach acidity secondary to age, disease or medications decreases calcium available for absorption. Low vitamin D status also decreases calcium absorption as previously discussed. Two other dietary factors can significantly alter calcium status. Meat protein and sodium intake both lead to increased urinary calcium levels; the mechanism underlying protein effects on calcium are unknown but it is proposed that sodium and calcium compete for kidney reabsorption by the same transporter [46, 47]. Lower dietary levels of sodium and meat protein in the developing world may mean that calcium requirements are also lower compared with populations in the developed world with its high meat protein and salt diets.

Food processing and cooking both affect calcium bioavailability. Processes that break down binding ligands such as fibre and phytates improve calcium bioavailability; conversely, milling of cereals strips the grains of the mineral-rich germ and bran. In certain countries, such as the UK, fortification of milled (white) flour with calcium to the levels present in wholemeal flours is mandatory.

2.3.3 Dietary zinc

Zinc compounds commonly used in supplements and food fortification include zinc oxide and zinc sulfate. Zinc sulfate has adequate bioavailability but it interacts with the food matrix causing poor food flavour. Zinc oxide can only be used in solid foods as it precipitates in liquids [18]. The zinc content of foods is low even in foods classed as zinc-rich foods, such as red meats [23]. Unrefined cereals have a high zinc content, but due to other components in the cereals bioavailability is low [18].

2.3.4 Dietary selenium

Selenium is present in the soil and enters the food chain via plants. The majority of dietary selenium comes from bread, cereal, meat, fish and poultry [21]. Geographical differences exist due to the availability of selenium in the soil. Selenium exists in two forms: the organic form includes selenocysteine (animal sources) or selenomethionine (plant and animal sources and supplements) [19]; the inorganic forms include selenate and selenite (supplements) [22].

The bioavailability of selenium is low and has recently decreased due to acid rain and excessive fertilization [21]. Bioavailability and distribution within tissues is dependent on the form that selenium is ingested [19]. High-protein diets and ascorbic acid enhance the bioavailability of selenium. Selenium is destroyed during food processing and refinement [48].

2.3.5 Dietary copper

Most nuts (brazils and cashews), seeds (poppy and sunflower), chickpeas, liver and oysters are rich in copper [25]. Copper supplements are available in the form of cupric oxide, copper gluconate, copper sulfate and copper amino acid chelates [26].

2.3.6 Dietary magnesium

Magnesium is present in the chloroplast of green plants, so the main sources of magnesium in the diet are cereals and green vegetables such as spinach, legumes, nuts, seeds and whole grains [29, 30]. Meat and animal products are rich in magnesium; however, because of the presence of calcium, phosphate and protein in these products, the bioavailability of magnesium is reduced [23]. Magnesium has been added to some cereals and can be used to fortify foods. Certain processing methods can lower the magnesium content of foods [23]. Magnesium supplements are available in the form of magnesium oxide, citrate and chloride [32, 49].

2.4 Total content in complete diets

Providing an appropriate diet is critical in terms of growth and development, as low intake or reduced bioavailability of nutrients may lead to deficiencies and cause impairment of body function [50, 51]. For example, vitamins and minerals have a crucial influence on the interaction between genetic and biological factors [2, 3]. They are involved in many important functions in the body, for example bone mineralization, enzymatic reactions, secretion of hormones, as well as protection of cells and lipids in biological membranes [52, 53].

To meet the body's mineral requirements a variety of micronutrient-rich foods should be offered, as their nutritional bioavailability is directly influenced by the

interaction between specific nutrients or groups of nutrients [54]. For example, consumption of foods of animal origin rich in iron, as well as fruit and vegetables rich in vitamins C and A, is highly recommended. Vitamin C-rich foods are important not only as a source of vitamin C but also because they enhance non-haem iron absorption.

Calcium and vitamin D is another example of an interaction between a vital nutrient for linear growth and bone health in an infant diet. The accumulation of calcium for bone mineralization is positive during the first 18 months of life, with the highest rate of accretion taking place during the first year [55]. Many nutritionists believe that early bone mass accumulation is important in the prevention of adult osteoporosis [55, 56]. Vitamin D is one of the major factors affecting intestinal calcium absorption and it is required for the secretion of PTH. Infant vitamin D stores are usually adequate unless there is insufficient maternal dietary intake or lack of exposure to sunlight. Children with vitamin D deficiency develop rickets and often show bowed limbs [57, 58].

However, the nutritional composition of complementary foods will differ widely due to socioeconomic factors. For instance, the diets of infants from certain ethnic backgrounds as well as vegetarian infants are particularly poor in vitamin B_{12}, iron and zinc, as well as being high in carbohydrates and phytic acid [59]. In most developing countries, complementary foods do not provide sufficient iron, zinc and vitamin B_6 [60]. Even in the USA, iron and zinc were identified as problem nutrients in the first year of life, despite the availability of iron-fortified products [60].

Currently, nutritional iron deficiency continues to be the most commonly diagnosed nutritional disorder of early childhood worldwide, and the UK is no exception [61, 62]. The evidence is that iron deficiency is directly linked to bad weaning practices, especially among ethnic minorities and socially disadvantaged inner city families in the UK [63–65]. A large survey carried out in 1992 has suggested that the median iron intake of full-term babies 6–12 months of age is below the RNI and that the mean zinc intake for this age group is only 90% of the RNI for zinc [66]. It is worth mentioning that a high intake of cheese and milk powder in the diet of young children in the UK has been identified as one of the factors contributing to low iron bioavailability [62, 67]. With iron being a vital component of haemoglobin and an integral part of various enzymes, poor weight gain, increased vulnerability to infection and impaired intellectual development are all worrying clinical consequences of iron deficiency. However, it is important to note that excessive iron and zinc intake may be detrimental in respect of other minerals, for example they can counter the effect on absorption of copper [62, 67]. In general, zinc and iron are known as problematic micronutrients in terms of their bioavailability as a result of poor utilization and the effect of inhibitors (calcium, phytate and tannin) [68–70].

The concept of bioavailability, natural inhibitors and enhancers is further discussed in section 2.5.1. The bioavailability of nutrients is mostly influenced by the presence of dietary components that interfere with digestion and therefore potentially inhibit the absorption of nutrients.

2.5 New challenges: speciation

2.5.1 Micronutrient interactions in food and bioavailability

A nutrient-dense food (amount of nutrient per 100 kcal of food) that provides substantial amounts of micronutrients (especially iron, zinc, calcium, vitamin A, vitamin C and folates), with relatively fewer calories [71] is an essential part of a healthy diet.

The index of nutrient quality (INQ) in food is a good indicator of nutrient density and relates to the amount of a nutrient per 1000 kcal in comparison to the recommended intake of that particular nutrient [72]. A food with an INQ substantially greater than 1 is generally considered to be a good source for that specific nutrient in excess of calories (lipids are an exception to the foregoing premise; in excess they are known to be detrimental).

$$\text{INQ} = \frac{\text{Amount of nutrient (g) per 1000 kcal of sample food}}{\text{Allowed nutrient (g) in 1000 kcal of food}}$$

where allowed nutrient in 1000 kcal = (RNI for each age group/estimated energy requirement for that age group) × 1000.

The micronutrient density of complementary food is of particular importance due to the nutrient need per unit body weight and the limitations associated with ingestion, digestion and storage of nutrients during infancy. It is estimated that the complementary food

target for a 9-month-old infant should provide about 97% of the intake of iron, 86% of zinc, 76% of magnesium, 73% of sodium and 72% of calcium [64, 73–75].

Appropriate food should also include a low content of anti-nutrients (e.g. phytate and polyphenols) as only a portion of ingested nutrients are biologically available [76]. The bioavailability of nutrients is most influenced by the presence of a dietary component that interferes with digestion and inhibits absorption. For instance, non-haem iron is affected by the many compounds in meals which impact on solubility, oxidation state (ferric), and amounts of 'free iron' and also alter the iron available for uptake by specific transporters on the surface of enterocytes in the upper intestine [77].

The natural inhibitors of iron include tannin, non-starch polysaccharides, phytic acid, phosphates, oxalates and polyphenols. The promoters of non-haem iron in the diet include vitamin C (keeps iron in its ferric state) and animal protein [67]. On the other hand, there are also concerns that high dietary intake of calcium may interfere with iron absorption in food, although one study reported an inhibitory effect on haem iron only [77]. In a study of the risk factors associated with poor iron status in British toddlers, Thane *et al*. [78] identified an over-dependence on milk and cream consumption as one of the risk factors associated with a high prevalence of poor iron status.

Another example of nutrient interaction in food is the counter-effect of iron and zinc on the absorption of copper where amino acids, in particular histidine, will act as an enhancer [79]. Conversely, copper is believed to have a regulatory effect on the homeostasis of iron via participation in ferroxidase activity as a catalyst. This suggests that anaemia due to copper deficiency is not caused by iron limitation but rather impairment in iron utilization [80].

In relation to the bioavailability of calcium, there have been a number of studies on formula milk in relation to their Ca^{2+} content, its bioavailability and interrelationship with other nutrients which affect its absorption and retention in the body [55]. These studies have shown that calcium bioavailability is slightly increased by carbohydrates such as lactose; however, fibrous non-digestible carbohydrates (e.g. guar gum and locust bean gum) are known to decrease the availability of calcium. Green leafy vegetables are also a good source of calcium, but in those foods that are also high in oxalates (such as spinach), the bioavailability of calcium becomes poor [60].

While casein has been reported to improve (accelerate) the passive absorption of calcium, phytate from soy protein in soy-based formulas was found to bind with Ca^{2+} and inhibit its absorption. Thus, soya-based formulas have been associated with inadequate growth and bone mineralization in term and preterm infants. The rate of calcium absorption is also influenced by the type of fat that interacts (binds) to Ca^{2+}. Calcium absorption increases when medium-chain triacylglycerols are used as the fat source, whereas long-chain fatty acids will form fatty acid calcium soaps that reduce absorption [55]. Neville *et al*. [81] and Koo *et al*. [82] reported that infants fed on formulas containing vegetable palm oil, which is different to the palmitate in human milk, developed reduced total body bone mineral content compared with infants fed on formula free from palm oil.

As outlined above, much attention has been placed on the nutritional quality and bioavailability of food supplements. Conversely, very few data are available on the nutritional quality and bioavailability of foods in general. The exceptions are a few studies on the influence of phytic acid on trace element and mineral bioavailability with specific focus on iron and zinc [61, 67, 73, 75, 78]. Based on these limited studies, it is evident that phytic acid prevalent in vegetable protein sources is a potent inhibitor of iron but its influence on bioavailability of zinc is thought to be modest and may be more important in children recovering from infection [83]. A high concentration of calcium in a diet comprising a high level of dairy products is also believed to aggravate the inhibitory effect of phytic acid on zinc absorption. These findings could be a cause for concern in the formulation of products with a high level of calcium, phytic acid and non-starch polysaccharides, due to their inhibitory effects on iron and zinc absorption.

2.5.2 Current methods of processing: nutritional consequences

As far as nutritional quality is concerned, there are a number of areas of concern. These include lack of knowledge of nutritional requirements, traditional recipe development and natural inhibitors, low nutrient density and high energy content, poor nutrient retention, poor diversity of ingredients in relation to cultural differences, and cost [59, 84].

Based on the results of elemental analysis of commercially available complementary food in the UK market, there was also some evidence of a lack of attention to

Table 2.7 Application, conditions and advantages/disadvantages of common thermal processes applied to food.

Treatment	Temperature	Application	Advantages	Disadvantages
Blanching	75–95 °C for 1–10 min	Fruit and vegetables for canning and freezing	Inhibition of damaging enzymes, air removal (canning)	Loss of water-soluble vitamins and minerals
Pasteurization	LTLT: 63 °C for 30 min HTST: 72 °C for 10 s	Milk	Killing of pathogens Lipase, reduced spoilage organisms	Refrigeration required, loss of volatile compounds
UHT	135 °C for <10 s	Milk, juices, desserts	Shelf-stable, good flavour	Nutrient losses, damages texture
Canning	115–125 °C for 10–120 min	Fruit, vegetables, canned food	Shelf-stable	Nutrient losses, damages texture
Baking	175–260 °C for 4–30 min	Bread and cookies	Flavour development and preservation	Loss of vitamin from browning reaction
Extrusion	115–200 °C for 10–90 s	Expanded snacks	Rapid processing, texture	High cost

LTLT, low temperature long time; HTST, high temperature short time; UHT, ultrahigh temperature.
Source: Shils *et al*. [90].

micronutrient interactions in food. With reference to the guidelines (the RNI values for 6- to 9-month old infants), all samples provided less than 20% of RNI values except for potassium (20%). In terms of the risk of exposure to toxicity, the concentration of non-essential elements in ready-to-feed products were not considered to be of concern [85, 86].

The demand by consumers for high-quality foods having 'fresh' or natural characteristics with an extended shelf-life has led to the application of combined processing techniques in order to maximize the post-process integrity and quality of the food, as first developed by Leistner and others in the so-called 'hurdle' concept [87, 88]. In combined processes, an understanding of the interaction between temperature, water activity (a_w), pH, chemical preservation and modified atmosphere packaging is used to design a series of hurdles to ensure the safety of processed foods.

By combining hurdle technology [88] and hazard analysis critical control point (HACCP) [89] in process design, the intensity of preservation techniques are kept comparatively low to minimize the loss of product quality, while overall there is a high impact on controlling microbial growth. For example, in the case of fruit juice the combination of minimal processing and high pH (<4.5) can result in the destruction of most pathogenic organisms, reduction of spoilage organisms and enzyme inactivation (e.g. polyphenol oxidase and pectinases) via pasteurization [88]. Thermal processes also enhance the digestibility of protein and complex carbohydrates and improve the bioavailability of indigenous micronutrients such as vitamin B_6, folic acid, niacin and carotenoids via destruction of antagonistic ligands, for example phytate–zinc antagonism [90].

A summary of the applications, conditions, and advantages and disadvantages of common thermal processes applied to food are presented in Table 2.7.

2.5.3 Assesment of nutritional quality: optimization and food design

To assess and improve the nutritional quality of composite food, it is important to consider the effect of the different stages of processing on the nutritional constituents of the food.

As outlined above, thermal treatment in particular leads to significant changes in the structure of food constituents, for example hydrolysis of polymeric carbohydrates, hydrolysis and coagulation of protein [91]. As a result, essential nutrients present in the raw materials are exposed to different processing conditions that can reduce the nutritional and sensory values of the final product [92]. The extent of any change depends on many factors, such as the kind of ingredients, cooking method, equipment, temperature and time. The damaging pathways indicated for thermal treatment concerning nutritional quality include heat degradation of nutrients, oxidation of fat-soluble vitamins and lipids, and leaching of water-soluble vitamins and minerals

[93, 94]. Most of the research that has been carried out to date with respect to the effect of processing on foods has been undertaken by examining a particular ingredient in isolation, for example the influence of dehydration on the composition of spinach rather than spinach as part of a 'meal' [92].

Very few studies have been undertaken to determine the effect of varying conditions of thermal processing on the nutritional quality of the final food composite [94–96]. Recent research shows that different constituents in food may have a protective effect on vulnerable nutrients and thereby promote the organoleptic properties of food, for example the retention of the naturally occurring antioxidants in fish [91, 92, 97]. Such studies show that when accurate time and temperature conditions are employed during thermal treatments, most of the nutrients are retained at an acceptable level, providing that high-quality raw materials are used in the recipe.

While consumer interest lies in natural, healthy and nutritious food products, there is scope and opportunity for optimization and product development. Ingredient selection for a food formulation is one of the critical aspects. In formulating an optimized product, target population, safety, nutrient content and molecular interactions, food processing functionality and legislative requirements as well as consumer acceptance need to be considered. While improving food formulation is an effective approach, the use of encapsulation in design and fortification is an emerging approach in development of functional foods [98–100].

2.5.4 A new paradigm for meeting human needs

Welch and Graham [101] suggest a new paradigm for world agriculture which must be productive, sustainable and nutritious. Micronutrient malnutrition ('hidden hunger') is currently affecting over 2 billion people worldwide, resulting in poor health, low worker productivity, high rates of mortality and morbidity, increased rates of chronic diseases (coronary heart disease, cancer, stroke and diabetes), and permanent impairment of cognitive abilities of infants born to micronutrient-deficient mothers. Agriculture is partly responsible because it has never held nutrient output as an explicit goal of its production systems. Indeed, many agricultural policies have fostered a decline in nutrition and diet diversity for the poor in many countries. Plant foods can serve as dietary sources of all essential minerals required by humans. Unfortunately, mineral concentrations are low in some plants, especially many staple food crops; thus, efforts are underway to increase the mineral content of these foods as a means to ensure adequate attainment of dietary minerals in all individuals. While in the past these efforts have included classical breeding approaches, it is clear that future progress can be made by utilizing the tools of biotechnology to effect directed changes in plant mineral status. Nutrition and health communities are also partly responsible because they have never considered using agriculture as a primary tool in their programmes to alleviate poor nutrition and ill health globally. Now is the time to consider ways in which agriculture can contribute to finding sustainable solutions to food system failures through holistic food-based system approaches, thereby closely linking agricultural production to improving human health, livelihood and well-being [101].

In the search for new functional food ingredients from algae for instance, the brown alga *Undaria pinnatifida* and the red alga *Chondrus crispus* could be used as food supplements to help meet the recommended daily intake of some minerals, macroelements (Na, K, Ca, Mg, ranging from 8.1 to 17.9 mg/100 g), and trace elements (Fe, Zn, Mn, Cu ranging from 5.1 to 15.2 mg/100 g) according to [102].

Abbreviations

DRV: Dietary reference value
EAR: Estimated average requirements
HACCP: Hazard analysis critical control point
IDA: iron-deficiency anaemia
INQ: Index of nutrient quality
MPF: Meat, poultry and fish
PTH: Parathyroid hormone
RNI: Recommended nutrient intake
SOD: Superoxide dismutase
WHO: World Health Organization

References

1 Mann J, Truswell AS. *Essentials of Human Nutrition*, 3rd edn. Oxford University Press, New York, 2007.
2 Gibson RS, Hotz C. The adequacy of micronutrients in complementary foods. *J Pediatr* 2000;**106**:1298–1299.

3 Melo R, Gellein K, Evje L, Syversen T. Minerals and trace elements in commercial infant food. *Food Chem Toxicol* 2008; **46**:3339–3342.

4 Goldhaber BS. Trace element risk assessment: essentiality vs toxicity. *Regul Toxicol Pharmacol* 2003;**38**:232–242.

5 Koletzko B, Akerblom H, Dodds P, Ashwell M (eds) Early Nutrition and Its Later Consequences: New Opportunities. *Adv Exp Med Biol* 2005;**569**:1–237.

6 Ljung K, Palm B, Grandér M, Vahter M. High concentrations of essential and toxic elements in infant formula and infant foods: a matter of concern. *Food Chem* 2011;**127**: 943–951.

7 Codex Alimentarius Commission. Standard for infant formula and formulas for special medical purposes intended for infants. Codex Stan 72–1981, 2007. Available at CXS_072e.pdf.

8 de Benoist B, McLean E, Egli I, Cogswell M. Worldwide prevalence of anaemia 1993–2005. World Health Organization, Geneva, 2008. Available at http://whqlibdoc.who.int/publications/2008/9789241596657_eng.pdf.

9 Hoare J, Henderson L, Bates CJ *et al*. *The National Diet and Nutrition Survey: Adults Aged 19–64 Years, Vol. 5: Summary Report*. HMSO, London, 2004. Available at http://multimedia.food.gov.uk/multimedia/pdfs/ndns5full.pdf.

10 Lieberman E, Ryan KJ, Monson RR, Schoenbaum SC. Association of maternal hematocrit with premature labor. *Am J Obstet Gynecol* 1988;**159**:107–114.

11 Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. *J Nutr* 2001;**131**:649S–668S.

12 Joint FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirement. *Vitamin and Mineral Requirements in Human Nutrition*, 2nd edn. World Health Organization and Food and Agricultural Organization of the United Nations, Geneva, 2004.

13 Fairweather-Tait SJ, Teucher B. Iron and calcium bioavailability of fortified foods and dietary supplements. *Nutr Rev* 2002;**60**:360–367.

14 Fairweather-Tait SJ. The importance of trace element speciation in nutritional sciences. *Fresenius J Anal Chem* 1999;**363**: 563–540.

15 Prentice A, Dibba B, Sawo Y, Cole TJ. The effect of prepubertal calcium carbonate supplementation on the age of peak height velocity in Gambian adolescents. *Am J Clin Nutr* 2012;**96**:1042–1050.

16 Jarjou LM, Laskey MA, Sawo Y *et al*. Effect of calcium supplementation in pregnancy on maternal bone outcomes in women with a low calcium intake. *Am J Clin Nutr* 2010;**92**: 450–457.

17 Prasad AS. Clinical, immunological, anti-inflammatory and antioxidant roles of zinc. *Exp Gerontol* 2008;**43**:370–377.

18 Salgueiro MJ, Zubillaga M, Lysionek A *et al*. Zinc as an essential micronutrient: a review. *Nutr Res* 2000;**20**:737–755.

19 Brown KM, Arthur JR. Selenium, selenoproteins and human health: a review. *Public Health Nutr* 2001;**4**(2B): 593–599.

20 Arthur JR, Mckenzie RC, Beckett GJ. Selenium in the immune system. *J Nutr* 2003;**133**:1457S–1459S.

21 Rayman MP. The importance of selenium to human health. *Lancet* 2000;**356**:233–241.

22 Finley JW. Bioavailability of selenium from foods. *Nutr Rev* 2006;**64**:146–151.

23 Department of Health. *Dietary References Values for Food Energy and Nutrients for the United Kingdom*, 15th edn. HMSO, London, 1991.

24 Collins JF, Klevay LM. Copper. *Adv Nutr* 2011;**2**:520–522.

25 University of Maryland Medical Center. Copper (updated 7 May 2013). Available at http://umm.edu/health/medical/altmed/supplement/copper (accessed 4 November 2013).

26 Linus Pauling Institute. Copper (updated January 2014). Available at http://lpi.oregonstate.edu/infocenter/minerals/copper/ (accessed 4 November 2013).

27 Allen LH, Prentice A, Caballero B (eds) *Encyclopedia of Human Nutrition*, 3rd edn. Academic Press, Oxford, 2013.

28 Rude RK. Magnesium homeostasis. In: Bilezikian JP, Raisz LG, Martin TJ (eds) *Principles of Bone Physiology*, 3rd edn. Academic Press, San Diego, CA, 2008, pp. 487–514.

29 Rude RK. Magnesium. In: Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR (eds) *Modern Nutrition in Health and Disease*, 11th edn. Lippincott Williams & Wilkins, Baltimore, 2012, pp. 159–175.

30 Institute of Medicine (IOM), Food and Nutrition Board. *Dietary Reference Intakes: Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride*. National Academy Press, Washington, DC, 1997.

31 Elin RJ. Assessment of magnesium status for diagnosis and therapy. *Magnesium Res* 2010;**23**:1–5.

32 Rude RK. Magnesium. In: Coates PM, Betz JM, Blackman MR, Cragg GM, Levine M, Moss J, White JD (eds) *Encyclopedia of Dietary Supplements*, 2nd edn. Informa Healthcare, New York, 2010, pp. 527–537.

33 Fine KD, Santa Ana CA, Porter JL, Fordtran JS. Intestinal absorption of magnesium from food and supplements. *J Clin Invest* 1991;**88**:396–402.

34 Aggett PJ, Bresson J, Haschke F *et al*. Recommended Dietary Allowances (RDAs), Recommended Dietary Intakes (RDIs), Recommended Nutrient Intakes (RNIs), and Population Reference Intakes (PRIs) are not 'recommended intakes'. *J Pediatr Gastroenterol Nutr* 1997;**25**:236–241.

35 Dupont C. Protein requirements during the first year of life. *Am J Clin Nutr* 2003;**77**:1544S–1549S.

36 Doets EL, de Wit LS, Dhonukshe-Rutten RA *et al*. Current micronutrient recommendations in Europe: towards understanding their differences and similarities. *Eur J Nutr* 2008; **47**(Suppl. 1):17–40.

37 Gibson RS. *Principles of Nutritional Assessment*, 2nd edn. Oxford University Press, New York, 2005.

38 Miret S, Simpson RJ, McKie AT. Physiology and molecular biology of dietary iron absorption. *Annu Rev Nutr* 2003;**23**: 283–301.

39 Carpenter CE, Mahoney AW. Contributions of heme and nonheme iron to human nutrition. *Crit Rev Food Sci Nutr* 1992;**31**:333–367.

40 Buchowski MS, Mahoney AW, Carpenter CE, Cornforth DP. Heating and the distribution of total and heme iron between meat and broth. *J Food Sci* 1988;**53**:43–45.

41 Hallberg L, Brune M, Erlandsson M, Sandberg AS, Rossander-Hulten L. Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. *Am J Clin Nutr* 1991;**53**:112–119.

42 Olivares M, Pizarro F, Ruz M. Zinc inhibits nonheme iron bioavailability in humans. *Biol Trace Elem Res* 2007; **117**:7–14.

43 Lönnerdal B. Calcium and iron absorption: mechanisms and public health relevance. *Int J Vitam Nutr Res* 2010;**80**: 293–299.

44 Miller DD. Calcium in the diet: food sources, recommended intakes, and nutritional bioavailability. *Adv Food Nutr Res* 1989;**33**:103–156.

45 Heaney RP, Weaver CM. Newer perspectives on calcium nutrition and bone quality. *J Am Coll Nutr* 2005;**24**: 574S–581S.

46 Heaney RP. Protein intake and the calcium economy. *J Am Diet Assoc* 1993;**93**:1259–1260.

47 Sabto J, Powell MJ, Breidahl MJ, Gurr FW. Influence of urinary sodium on calcium excretion in normal individuals. A redefinition of hypercalciuria. *Med J Aust* 1984;**140**: 354–356.

48 Sager M. Selenium in agriculture, food and nutrition. *Pure Appl Chem* 2006;**78**:111–133.

49 Firoz M, Graber M. Bioavailability of US commercial magnesium preparations. *Magnesium Res* 2001;**14**:257–262.

50 Gokhale R, Kirschner BS. Assessment of growth and nutrition. *Best Pract Res Clin Gastroenterol* 2003;**17**:153–162.

51 Hulzebos C, Sauer PJJ. Energy requirements. *Semin Fetal Neonatal Med* 2007;**12**:2–10.

52 Williams SR, Schlenker ED. *Essentials of Nutrition and Diet Therapy*. Elsevier, Amsterdam, 2003.

53 Taylor LJ, Gallagher M, McCullough FSW. The role of parental influence and additional factors in the determination of food choices for pre-school children. *Int J Consumer Stud* 2004;**28**:337–346.

54 Lönnerdal B. Trace element nutrition of infants: molecular approaches. *J Trace Elem Med Biol* 2005;**19**:3–6.

55 Bass JK, Chan GM. Calcium nutrition and metabolism during infancy. *Nutrition* 2006;**22**:1057–1066.

56 Cole ZA, Gale CR, Kassim Javaid M *et al*. Maternal dietary patterns during pregnancy and childhood bone mass: a longitudinal study. *J Bone Miner Res* 2009;**24**:663–668.

57 Crocombe S, Mughal MZ, Berry JL. Symptomatic vitamin D deficiency among non-Caucasian adolescents living in the United Kingdom. *Arch Dis Child* 2004;**89**:197–199.

58 Cooper C, Harvey N, Javaid K, Hanson M, Dennison E. Growth and bone development. In: Barker DJP, Bergmann RL, Ogra PL (eds) *The Window of Opportunity: Pre-Pregnancy to 24 Months of Age*. Nestlé Nutrition Institute Workshop Series, Vol. **61**. Karger, Basel, 2008, pp. 53–68.

59 Davies DP, O'Hare B. Weaning: a worry as old as time. *Curr Paediatr* 2004;**14**:83–96.

60 World Health Organization. Complementary feeding: report of the global consultation. Summary of guiding principles for complementary feeding of the breastfed child. WHO, Geneva, 2003. Available at http://whqlibdoc.who.int/publications/2002/924154614X.pdf?ua=1.

61 Yeung DL. Iron and micronutrients: complementary food fortification. *Food Nutr Bull* 1998;**19**:159–163.

62 Aggett PJ, Agostoni C, Axelsson I *et al*. Iron metabolism and requirements in early childhood: do we know enough? A commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2002;**34**:337–345.

63 Daly AM, Booth IW. Diet and disadvantage: observations on infant feeding from an inner city. *J Hum Nutr Diet* 1998;**11**:381–389.

64 Kimmons JE, Dewey KG, Haque E *et al*. Proposed nutrient composition for fortified complementary foods. *J Nutr* 2003;**133**:3011S–3020S.

65 Köster EP. Diversity in the determinants of food choice: a psychological perspective. *Food Qual Prefer* 2009;**20**:70–82.

66 Foote KD, Marriott LD. Weaning of infants. *Arch Dis Child* 2003;**88**:488–492.

67 Singh M, Sanderson P, Hurrell RF *et al*. Iron bioavailability: UK Food Standards Agency workshop report. *Br J Nutr* 2006;**96**:985–990.

68 Wellinghausen N. Immunobiology of gestational zinc deficiency. *Br J Nutr* 2001;**85**:S81–S86.

69 Dewey KG, Brown KH. Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs. *Food Nutr Bull* 2003;**24**:5–29.

70 da Costa GA, Marreiro DD, Eulalio JML *et al*. Erythrocytary zinc and the infant growth profile in Northeast Brazil. *Biol Trace Elem Res* 2008;**126**:S15–S20.

71 Monte CMG, Giugliani ERJ. Recommendation for the complementary feeding of the breastfed child. *J Pediatria* 2004;**80**:S131–S141.

72 Lee RD, Nieman DC. *Nutritional Assessment*, 6th edn. McGraw-Hill, New York, 2013.

73 Bhutta ZA. Iron and zinc intake from complementary foods: some issues from Pakistan. *J Pediatr* 2000;**106**:1295–1297.

74 Dewey KG. Nutrition, growth and complementary feeding of the breastfed infant. *Pediatr Clin North Am* 2001;**48**: 87–104.

75 Noble S, Emmett P. Differences in weaning practice, food and nutrient intake between breast- and formula-fed 4-month-old infants in England. *J Hum Nutr Diet* 2006;**19**: 303–313.

76 Barclay A, Weaver L. Feeding the normal infant, child and adolescent. *Medicine* 2003;**34**:551–556.

77 Roughead ZK, Zito CA, Hunt JR. Inhibitory effects of dietary calcium on the initial uptake and subsequent retention of heme and non-heme iron in humans: comparisons using an intestinal lavage method. *Am J Clin Nutr* 2005;**82**:589–597.
78 Thane CW, Walmsley CM, Bates CJ, Prentice A, Cole TJ. Risk factors for poor iron status in British toddlers: further analysis of data from the National Diet and Nutrition Survey of children aged 1.5–4.5 years. *Public Health Nutr* 2000;**3**: 433–440.
79 Rosado JL. Zinc and copper: proposed fortification levels and recommended zinc compounds. *J Nutr* 2003;**133**: 2985S–2989S.
80 Prohaska JR. Impact of copper limitation on expression and function of multicopper oxidases (ferroxidases). *Adv Nutr* 2011;**2**:89–95.
81 Neville M, Allen J, Archer P *et al*. Studies in human lactation: milk volume and nutrient composition during weaning and lactogenesis. *Am J Clin Nutr* 1991;**54**:81–92.
82 Koo WWK, Hammami M, Margeson DP, Nwaesei C, Montalto MB, Lasekan JB. Reduced bone mineralization in infants fed palm olein-containing formula: a randomized, double-blinded, prospective trial. *Pediatrics* 2003;**111**: 1017–1023.
83 Hurrell RF. Influence of vegetable protein sources on trace element and mineral bioavailability. *J Nutr* 2003;**133**: 2973S–2977S.
84 Michaelsen KF, Friis H. Complementary feeding: a global perspective. *Nutrition* 1998;**14**:763–766.
85 Zand N, Chowdhry BZ, Zotor FB, Wray DS, Amuna P, Pullen FS. Essential and trace element content of commercial infant foods in the UK. *Food Chem* 2011;**128**:123–128.
86 Zand N, Chowdhry BZ, Wray DS, Pullen FS, Snowden MJ. Elemental content of commercial 'ready to-feed' poultry and fish based infant foods in the UK. *Food Chem* 2012; **135**:2796–2801.
87 Leistner L. Basic aspects of food preservation by hurdle technology. *Int J Food Microbiol* 2000;**55**:181–186.
88 Fellows PJ. *Food Processing Technology: Principles and Practice*, 3rd edn. Woodhead Publishing, Cambridge, 2009.
89 Mortimore S. How to make HACCP really work in practice. *Food Control* 2001;**12**:209–215.
90 Shils ME, Shike M, Ross AC, Caballero B, Cousins R (eds) *Modern Nutrition in Health and Disease*, 10th edn. Lippincott Williams & Wilkins, Baltimore, 2006.
91 Sun JP, Hou CY, Zhang SY. Effect of protein on the rheological properties of rice flour. *J Food Process Preserv* 2008;**32**: 987–1001.
92 Kaur A, Kaur D, Oberoi DPS, Gill BS, Sogi DS. Effect of dehydration on physicochemical properties of mustard, mint and spinach. *J Food Process Preserv* 2008;**32**:103–116.
93 Thiel RJ. Natural vitamins may be superior to synthetic ones. *Med Hypotheses* 2000;**55**:461–469.
94 Morris A, Barnett A, Burrows O-J. Effect of processing on nutrient content of foods. *Cajanus* 2004;**37**:160–164.
95 Holdsworth SD. Optimising the safety and quality of thermally processed packaged foods. In: Richardson P (ed.) *Improving the Thermal Processing of Foods*. Woodhead Publishing, Cambridge, 2004, pp. 3–31.
96 Meade SJ, Rieid EA, Gerrard JA. The impact of processing on the nutritional quality of food properties. *J AOAC Int* 2005;**88**:904–922.
97 Aubourg SP. Review: Loss of quality during the manufacture of canned fish products. *Food Sci Technol Int* 2001;**7**: 199–215.
98 Song Y-B, Chang P-S, Lee HG. Microencapsulation of alpha-tocopherol using sodium alginate and its controlled release properties. *Int J Biol Macromol* 2006;**38**:25–30.
99 Mozafari MR, Johnson C, Hatziantoniou S, Demetzos C. Nanoliposomes and their applications in food nanotechnology. *J Liposome Res* 2008;**18**:309–327.
100 Given PS. Encapsulation of flavours in emulsions for beverages. *Curr Opin Colloid Interface Sci* 2009;**14**:43–47.
101 Welch RM, Graham RD. A new paradigm for world agriculture: meeting human needs. Productive, sustainable, nutritious. *Field Crops Res* 1999;**60**:1–10.
102 Plaza M, Cifuentes A, Ibáñez E. In the search of new functional food ingredients from algae. *Trends Food Sci Technol* 2008;**19**:31–39.

CHAPTER 3
Bioavailability of minerals in foods

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Abstract: The bioavailability of ingested components is an extremely important area of food and pharmaceutical research. The main issue is to determine which fraction of ingested nutrients or food pollutants can be truly used by the body and exert its healthy or deleterious effects. In this sense, knowledge of bioavailability is essential to estimate the quantity of a mineral that must be supplied to satisfy the requirements, since low intake from diet and deficiencies (with negative consequences for health) are found worldwide, and also to evaluate the toxicological risk of contaminant minerals. Physiological and dietetic factors, as well as food processing, can have an impact on mineral bioavailability. Accordingly, this chapter reviews the bioavailability of minerals of nutritional interest (Ca, Fe, Zn and Se) and with toxicological risk (As, Hg, Cd and Pb).

Keywords: minerals, bioavailability, nutritional interest, toxicological risk

3.1 Bioavailability: concept, bioaccessibility and bioactivity

Nowadays, the objectives of nutrition have moved from merely supplying enough food to meet the energy and nutrient requirements of the population while avoiding nutritional deficiencies ('essentiality' of nutrients), to providing nutrients in such a quantity and quality as to improve health and well-being and to prevent the risk of diseases ('optimal' nutrition).

Knowledge of bioavailability is essential to estimate the quantity of a mineral that must be supplied to satisfy the requirements, since low intake from diet and deficiencies (with negative consequences for health) are found worldwide, and also to evaluate the toxicological risk of contaminant minerals. In addition, complex interactions between minerals and other compounds in the diet can occur, leading to positive or negative effects on bioavailability. Furthermore, factors affecting bioavailability (intrinsic or extrinsic), as well as the effect of processing on the food matrix, can potentially modulate mineral bioavailability.

3.1.1 Definitions

The term 'bioavailability' has several working applications, and there is no universally accepted definition. From a nutritional standpoint, it is defined as the fraction of ingested component available for utilization in normal physiological functions, and is determined from *in vivo* assays [1]. Absorption and/or retention of mineral nutrients are often used as indicators of bioavailability, though they are not synonymous and include terms such as apparent absorption (intake – total faecal excretion), net retention [intake – (total faecal excretion + total urinary excretion)] and true absorption [intake – (total faecal excretion – endogenous faecal excretion) – (total urinary excretion – endogenous urinary excretion)] [2]. In addition, bioavailability includes two additional terms: bioaccessibility and bioactivity [3].

Bioaccessibility has been defined as the fraction of a compound that is released from its food matrix in the gastrointestinal tract and thus becomes available for intestinal absorption (typically established from *in vitro* procedures). Bioaccessibility includes the sequence of events that take place during food digestion for transformation into potentially bioaccessible material, absorption/assimilation through epithelial tissue and presystemic metabolism (both intestinal and hepatic) [3].

Bioactivity, in turn, includes events linked to how the nutrient/bioactive compound is transported and reaches the target tissue, how it interacts with biomolecules, the metabolism or biotransformation it may experience,

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and the generation of biomarkers and the physiological responses caused [3].

3.1.2 Factors influencing bioavailability

The mineral bioavailability process is the result of three main steps [4]: (i) digestibility and solubility of the element in the gastrointestinal tract; (ii) absorption of the element and transport into the circulation; and (iii) incorporation from the circulation to the functional entity or target. Two kinds of factors may influence mineral bioavailability: physiological (or intrinsic) and dietetic (or extrinsic).

In the first step listed above (i), dietetic factors (element supply; chemical form; solubility and interaction with other dietary components acting as enhancers, such as ascorbate, organic acids, sugars, inulin and oligofructose, amino acids, peptides and fatty acids; inhibitors such as phosphates, phytates, tannins, oxalates and carbonates; or competitors, i.e. minerals with similar binding characteristics for the absorption site) are the main determinants of bioavailability due to their influence on absorption.

On the other hand, in the last two steps listed above (ii and iii), physiological factors (age, gastric acidity, luminal redox state, intestinal secretion, gut motility, possible genetic defects, pathological states, physiological states such as pregnancy and lactation, gut microflora and the individual capacity of adaptation to nutrient supply) are the parameters that exert most influence on the absorption and metabolism of minerals [5, 6].

3.1.3 Effect of processing on mineral bioavailability

The impact of food processing on the bioavailability of minerals has been reported as not a simple cause–effect relationship. In fact, processing can have a positive impact through separation, partitioning or the destruction of inhibitors and the transformation of food components into complex ligands for metal ions, thereby enhancing their availability. However, the impact can also be negative, by deactivating enzymes that degrade inhibitors or by generating insoluble metal compounds (e.g. oxidation, precipitation) [5].

Some clear examples of the negative impact of processing on mineral bioavailability are soaking, milling, drying, freezing, extrusion, and some heat treatments (sterilization and frying), which can cause mineral losses due to leaching, bran separation or the Maillard reaction [5]. Conversely, an example of the positive effect of processing on mineral bioavailability is dephytinization by adding exogenous phytase or by activating naturally occurring plant phytases (through fermentation or germination), as described in infant cereals for iron and zinc bioavailability in Caco-2 cells [7].

Conflicting and opposite results have been reported for cooking and high-pressure processing (HPP). Cooking has been shown to exert a positive or negative impact on the bioavailability of Ca, Fe and Zn from legumes [8] and of Fe and Zn from cereals and pulses [9], in Caco-2 cells. Differences can be attributed to different mineral chemical forms, differences in their association with other food matrix constituents and/or differences in their localization within the food.

A benefit of cooking, with an impact on public health, is the fact that the bioaccessibility of contaminants such as Hg, Cd and As can be reduced in cooked fish [10, 11]. In the case of HPP, an increase in Ca and P bioavailability in Caco-2 cells from milk-based fruit beverages has been reported [12]. However, in apples and carob seeds, Fe and Zn bioaccessibility is seen to increase, while Ca bioaccessibility is seen to decrease [13, 14].

3.2 Methods for evaluating mineral bioavailability

Many *in vivo* and *in vitro* techniques have been developed for determining mineral bioavailability in foods. For most elements, it is not possible to measure trace element bioavailability directly. Therefore, various methods are used to assess trace element bioavailability. However, none of the techniques are without limitations [15].

3.2.1 *In vivo* bioavailability methods

In vivo methods are used in humans and animals, and allow us to estimate the influence of physiological factors on the bioavailability of minerals of nutritional and toxicological interest. Human studies are the method of choice, but are expensive, time-consuming, difficult to carry out, and the results obtained are not always generalizable due to important variability between and even within individuals. Studies in animals are subject to fewer limitations, though the main problem in investigations of this kind is that the results are not always extrapolated, due to the metabolic differences between humans

and other species. Therefore, they should only be used for mechanistic and/or invasive investigations [15].

Balance techniques

Chemical balance [2]

The first method used for determining trace element absorption, before radioisotopic techniques were introduced, was chemical balance. This method evaluates a positive or negative balance from the difference between the amount of mineral ingested and excreted (faecal and urinary excretion) over a period of time. In the case of iron, zinc and copper, urinary excretion is often insignificant, so apparent absorption (intake – total faecal excretion) is evaluated in balance studies. However, in the case of other trace elements such as Se and I, which are absorbed as anions, urinary excretion is considered in these studies, and net retention (intake – total faecal and urinary excretion) is calculated.

The main advantages of this method are its easy application to animals and human experiments, without exposing subjects to ionizing radiation. However, errors in estimates of mineral contents in diets, faecal material and urine can occur, because trace levels are involved, and their determination can be affected by method sensitivity and the presence of contaminants, producing errors in the absorption estimates. Moreover, if endogenous faecal excretion is not different or the element not absorbed, an error can result in the mineral absorption value determined, which is often very low.

Radioisotope and stable isotope balance

The most widely used methods for studying mineral metabolism/bioavailability employ isotopes to label dietary sources of trace elements [15]. The basic principle for isotope studies is that an added isotope of the element exchanges with or behaves in an identical way to the native element. The added isotopes could be β- or γ-emitting radioactive isotopes, or an enriched naturally occurring isotope. Most elements in their native form are a mixture of isotopes with slightly different masses [16]. Foods are labelled intrinsically and extrinsically with an isotope. In the former case, the isotope in question is incorporated into food via biosynthetic routes, or into the growing plant or animal by injection or feeding; the isotope is therefore assumed to behave in exactly the same way as the endogenous element in the food. Intrinsic labelling does not allow the determination of mineral bioavailability in the entire meal. Extrinsic labelling is easier and less expensive than intrinsic labelling, and is characterized by mixing the radioisotope into the food or meal, followed by equilibration for a few hours before consumption. The isotope behaves in the same way as all the forms of the element in the food [2, 15, 17]. Knowledge of the quantity of isotope labelling given with foods or meals, and of the quantity recovered in stools, suffices to calculate apparent absorption. If the mineral is prone to large endogenous losses, a second label can be given intravenously at the same time as the oral label, and both are traced in the stools. Endogenous losses can then be accounted for, and true mineral absorption can be calculated. This is known as a dual-tracer experiment, and is valid if the oral and intravenous labels exhibit the same kinetic behaviour [18].

Early human experiments (e.g. the study of Fe metabolism in anaemia) used radioactive isotopes as tracers because they are easy to measure and inexpensive in comparison with stable isotopes [17]. Endogenous excretion can be estimated because two radioactive isotopes (double isotope technique) of the same element are administered: one via the oral route and the other via the parenteral route (this being information that cannot be obtained by the aforementioned chemical balance techniques). The main advantages of radioisotopes are the ease and precision of the measurements, and the fact that the radioactive element that is absorbed or retained is not affected by contamination and is relatively cheap. The principal disadvantage is the potential risk posed by the ionizing radiation. In this context, although the doses used in the assays show no evidence of harmful effects in humans, understandable caution is applied when using radioactive isotopes in target groups such as infants, young children or pregnant women [2, 16, 17]. The observation that the groups vulnerable to ionizing radiation are also the groups at highest risk of mineral deficiencies has resulted in a rapid decline in radioisotope use for nutritional studies in human volunteers [17]. Moreover, radioisotopes are less suitable for multi-element studies and multiple administrations. Apart from the cumulative radiation dose, there are also measurement difficulties due to overlapping energy spectra [16].

The use of stable isotopes of minerals has been viewed as an alternative to radioisotopes for studying mineral metabolism, based mostly on previously described radioisotope techniques. A stable isotope, unlike a

radioisotope, is present in the body tissues; therefore, in order to follow the metabolism of a mineral it is necessary to ingest it in an altered form by enriching one of the low-abundance isotopes. The basic requirement for using such isotopes is therefore to achieve sufficient isotope enrichment in the body tissue/fluid or food of interest so that the added tracers can be quantified with the necessary degree of precision. The amount of isotope added depends on four factors: the amount absorbed and retained, the relative distribution between different tissues and fluids, the natural abundance of the isotope administered, and the degree of isotope enrichment required [16].

The main advantage of these isotopes is their safety of application in humans, with little or no risk to volunteers, and with the possibility of use in vulnerable population groups, provided their purity is guaranteed and there are no losses due to radioactive decay. Accordingly, the labelled samples can be stored indefinitely and without cost increments associated with the elimination of radioactive residues. The main disadvantages of stable isotopes are their high cost, the need for sophisticated analytical equipment for analysis, the fact that stable isotope addition is not always able to produce the necessary doses to afford suitable enrichment, and the fact that stable isotopes do not exist for all elements [2, 16, 17, 19].

Several stable isotope techniques have been used to study mineral metabolism and trace element availability, and the concepts, difficulties and limitations of these methodologies have been reviewed by a number of authors [15, 16, 19]. These techniques range from simple measurements of absorption and endogenous faecal excretion to complex studies using multi-compartmental modelling. In the isotope balance technique, faecal and/or urinary excretion is monitored, and influences homeostasis of the trace element under study. For example, iron homeostasis is maintained by variations in absorption, and Fe losses are small and unregulated. However, for other minerals such as Cu or Zn, losses are more significant and may influence their homeostasis [19]. Other techniques include plasma appearance kinetic studies, with measurement of the appearance of oral doses of an isotopically enriched source of the element in plasma, or urine appearance kinetic studies (double isotope technique). Compartmental modelling/mathematical modelling is a non-invasive approach for predicting both the size of inaccessible mineral stores and the rate at which the mineral is moved from the stores to the tissues [18, 19]. Tissue retention has been used exclusively to measure the incorporation of Fe into haemoglobin (direct measure of Fe bioavailability).

Tissue concentrations or indirect indicators [2]

Increases in plasma or serum mineral concentration have been used as an indicator of the bioavailability of several minerals such as Fe, Cu, Zn and Se. These techniques consist of the oral administration of a dose of the element, followed by measurements of the concentrations in plasma at different times, and this is used as an estimation of absorption. High oral doses are needed in order to appreciate a mineral increase in plasma that would be considered pharmacological rather than physiological (e.g. 25 mg or more of Fe). For Se, since absorption is high in the anion form (>50%), this method is considered a good indicator of bioavailability. In contrast, these techniques do not afford quantitative information on mineral absorption, since the increase in plasma concentration depends on both absorption and the renal clearance rate.

These procedures have been used for evaluating iron bioavailability referred to haemoglobin repletion (in anaemic animals or humans), haemoglobin incorporation, and changes in the serum and/or plasma concentration of ferritin (storage protein). The latter application may be limited to long-term studies where the Fe sources are moderately to widely available, and it should not be used in rapidly growing infants or children, or in individuals with inflammatory disorders, because serum ferritin levels are affected. The serum transferrin receptor is a reliable measure of tissue Fe deficiency, and may be used even in the last of the aforementioned situations.

In animals, the concentrations of minerals in different tissues (as reflected by weight gain; changes in bone concentration for Zn; or heart, liver, bone and bile levels for Cu), metal-binding proteins (e.g. metallothionein-Zn) and metal-containing enzymes in tissue or plasma (e.g. alkaline phosphatase and Zn; cytochrome c oxidase and ceruloplasmin and Cu; Cu-Zn superoxide dismutase; Se-glutathione peroxidase) have all been used as indicators of their bioavailability.

Whole-gut lavage technique

The whole-gut lavage technique has been used in humans to assess the intestinal absorption of mineral elements such as Ca, P, Mg and Zn present in foods. The

gastrointestinal tract is cleansed with an iso-osmotic lavage solution, after which food is administered and a time interval is allowed for intestinal absorption to occur. The non-absorbed fraction of the food is collected by means of a second intestinal cleansing step. Mineral absorption is calculated from the difference between the amount administered and the amount recovered from the liquids of the second cleansing step, correcting for the amount of mineral excreted into the intestinal lumen under basal conditions (assessed by administering water only). According to Zheng *et al*. [20], this method does not alter the efficiency of intestinal absorption, is rapid (24 hours), requires no stable or radioactive isotopes, and can be used to simultaneously evaluate the absorption of several elements contained in food. The main inconvenience is the lack of tolerance of the technique observed in some individuals.

3.2.2 *In vitro* bioavailability methods

In vitro methods have been proposed as an alternative to *in vivo* methods for estimating mineral bioavailability. Most of the *in vitro* methods have been developed to simulate the physiological conditions and the sequence of events that occur during digestion in the human gasstrointestinal tract.

Solubility/dialysability methods

These methods involve conditions (temperature, agitation, pH, and enzyme and chemical composition) similar to those found in the human body during digestion. Although digestion begins in the mouth in the presence of saliva, the entire process in the mouth lasts from a few seconds to minutes, and since the salivary pH value is close to neutral, significant compound dissolution from food samples is not expected in this stage. This is why most gastrointestinal simulation methods only include the gastric and intestinal phases of digestion [21].

The gastric phase is performed with HCl or HCl-pepsin under fixed pH and temperature conditions, for a set period (pH 1–2 at 37 °C for 1–3 hours), followed by an intestinal phase, subsequent neutralization using a base (usually NaOH or $NaHCO_3$), and incubation with pancreatic enzymes with or without bile salts (pH 7 at 37 °C for 1–5 hours). This is followed by determination of the amount of soluble element present in the supernatant obtained by centrifugation or filtration of the gastrointestinal digest of the food (solubility methods). The amount of solubilized element can be used as a measure of mineral bioaccessibility.

Early attempts to develop an improved *in vitro* method for Fe availability include those of Naransiga Rao and Prabhavathi [22]. These authors developed a procedure in which a food was incubated with pepsin-HCl at pH 1.35, followed by centrifugation and filtration. An aliquot was then adjusted to pH 7.5, with the addition of pancreatin, and the ionizable iron was subsequently determined.

A new approach to the *in vitro* estimation of Fe solubility was introduced by Miller *et al*. [23]. These authors digested a food sample with pepsin and HCl, after which a dialysis bag containing $NaHCO_3$ was added. This allows for a slow increase in pH before and during pancreatic digestion. The method closely parallels the events occurring when the food leaves the stomach and enters the duodenum. After incubation, pancreatin–bile addition to the digest, and another incubation period, the Fe in the dialysate was analysed. An important methodological difference with previous systems is the introduction of equilibrium dialysis. Instead of the Fe fraction present in the supernatant after centrifugation, dialysable Fe is measured. This eliminates the problems encountered when using centrifugation to separate soluble and insoluble components resulting from the digestion of complex meals. The use of a dialysis bag of a specific pore size also permits discrimination between high and low molecular weight soluble Fe complexes. Enhancing and inhibiting factors of Fe absorption such as ascorbic acid, coffee, wheat bread, tea and eggs were shown to respond *in vitro* as they affect Fe absorption *in vivo* [23]. This method has been frequently used to estimate the bioavailability of essential and toxic minerals.

Simulated gastrointestinal digestion can be performed with static models where the products of digestion remain largely immobile and do not mimic physical processes such as shear, mixing and hydration. Static models are the most widespread digestive systems. However, none of these static models reproduce the dynamic processes occurring during human digestion, such as gastric emptying or continuous changes in pH and secretion flow rates. To overcome these limitations, several dynamic gastric models (computer-controlled systems) haven been developed. These systems allow gradual modifications in pH and enzymes, and the removal of the dialysed components, thereby better simulating the actual *in vivo* situation. To date, the TIM-1

(TNO gastrointestinal Model 1) is the system that allows the closest simulation of *in vivo* conditions. It is composed of the stomach, duodenum, jejunum and ileum [24]. The system integrates key parameters of human digestion: temperature, kinetics of gastric and intestinal pH, gastric and ileal deliveries, transit time, peristaltic mixing and transport, sequential addition of digestive secretions, and passive absorption of water and small molecules through a dialysis system [1]. The principal requirement for successfully conducting *in vitro* bioavailability studies is to achieve conditions which are similar to the *in vivo* conditions. Temperature, shaking or agitation, and the chemical and enzymatic composition of saliva and gastric, duodenal and bile juice are all relevant aspects in these studies. Interactions with other food components must also be taken into account, since they can influence the efficiency of digestion [25]. Different factors affecting the *in vitro* bioaccessibility of trace elements in food samples have been investigated [26].

All these systems evaluate 'bioaccessibility', and can be used to establish trends in relative bioaccessibility. However, it is generally recognized that not all soluble or dialysable minerals are absorbable. The usefulness of *in vitro* solubility methods for the prediction of iron bioavailability has been revised by Miller and Berner [27]. These authors indicated that iron solubility following *in vitro* gastrointestinal digestion is a reliable predictor of ascorbic acid effects on bioavailability, but not of protein effects, and that the solubility of low molecular weight iron is better than simple solubility in predicting Fe bioavailability.

Cell cultures

In vitro mineral bioavailability assays based on *in vitro* digestion and on measurements of the soluble or dialysable element have been improved by incorporating cell cultures, the most widely used being the Caco-2 human colon carcinoma cell line. These cells, under appropriate conditions, differentiate into polarized enterocyte-like monolayers. They develop microvilli, and in many ways act similarly to small intestinal epithelial cells [2].

In vitro models combining solubility or dialysability and uptake and/or transport by Caco-2 cells have been used mostly to estimate Fe bioavailability in foods. Thus, Glahn *et al*. [28] have developed a model whereby foods undergo simulated peptic digestion followed by intestinal digestion (using a dialysis method) in the presence of Caco-2 cell monolayers. ^{59}Fe uptake by the cell line is used as an estimator of Fe bioavailability. Later, the authors adapted an *in vitro* digestion/Caco-2 cell model to assess Fe availability from foods through ferritin formation by the Caco-2 cells as an indicator of Fe uptake. This methodology circumvents the need to use radioactive Fe, and thus eliminates the costs and controversies associated with food radiolabelling. Ferritin formation by Caco-2 cell monolayers is highly sensitive, and accurately measures food Fe availability in this *in vitro* system [29]. These models have been well correlated qualitatively to human data [30].

On the other hand, Caco-2 cells grown on bicameral filters have been used to examine mineral transport. In such experiments, Caco-2 cells growing on a microporous membrane form a barrier between the apical and basolateral compartments. Digest from foods are added to the apical side, and it is possible to measure the mineral element retained by the cell culture and that transported to the basolateral side [31].

The Caco-2 model offers a relatively rapid, inexpensive, and versatile system for determining the effects of various aspects of the meal itself or of the digestion conditions on cell mineral uptake. The most serious disadvantage of the Caco-2 cell line is the transformed nature of the cells, since they are derived from colon carcinoma, and the question that arises is to what extent normal metabolic processes are maintained in these cells. On the other hand, the Caco-2 cell line lacks a mucin layer, which may play a significant role in iron intestinal absorption, and the transepithelial resistance is much higher than in the human small intestine and resembles that of the human colon. Furthermore, these cells have low carrier expression, resulting in very low transport rates [4]. The usefulness of *in vitro* methods (dialysability, Caco-2 cell systems and algorithms) to predict the bioavailability of Fe and Zn has been reviewed [32].

Different *in vitro* methods are currently available for measuring the bioaccessibility and/or bioavailability of minerals in foods, but the results are not generally comparable between methods. Differences in laboratory conditions and the lack of standardized methods are the main causes. Validation of *in vitro* methods is required. Moreover, there is clearly an urgent need for more research into *in vivo*/*in vitro* correlations using well-defined systems, so that more realistic *in vitro* models can be developed to screen the bioavailability and digestibility of foods [30].

Recent reviews of the different *in vitro* digestion models for food applications have been published by Hur *et al*. [30] and Guerra *et al*. [1], and the application of intestinal cell models in food and nutritional sciences has been described [33].

3.3 Bioavailability of minerals of nutritional interest: Ca, Fe, Zn, Se

3.3.1 Calcium

Calcium is an essential micronutrient required for biological functions in the body such as nerve conduction, muscle contraction, mitosis, blood coagulation, and structural support of the skeleton [34]. Adequate intake of Ca has been demonstrated to reduce the risk of chronic diseases such as osteoporosis, hypertension, colon cancer, breast cancer, kidney stones, polycystic ovary syndrome, ovarian cancer, and a number of other disorders [35]. Insufficient Ca uptake results in diseases such as rickets in children and osteoporosis in the elderly [36].

The main source of Ca in the diet is dairy products, which supply 75% of global intake, and vegetables, fruit and grain products which supply most of the rest. Meat, poultry and fish supply a very small amount [37].

Of the dietary factors that influence bioavailability, dietary fibre (mainly phytates) found in bran and most cereals and seeds can form insoluble complexes with calcium, thereby reducing its absorbability. This is only relevant in high-fibre strict vegetarian diets lacking dairy products. Fibres themselves (cellulose, hemicelluloses, lignins and non-cellulose polysaccharides) seem to have no direct effect on Ca absorbability. Others inhibitors of Ca bioavailability are oxalates in spinach, rhubarb, walnuts and sorrel, and tannins (tea). Lipids, especially milk fats, form insoluble soaps with calcium, reducing its bioavailability. However, the amount of fat in the diet does not affect calcium absorption [36].

Milk components such as proteins, phosphopeptides and lactose favour Ca bioavailability, keeping it in a soluble form until it reaches the distal intestine, where Ca is absorbed via routes independent of vitamin D. Phosphopeptides derived from the enzymatic hydrolysis of casein and whey proteins, such as α-lactalbumin and β-globulin, bind Ca and protect it from potential precipitating anions such as phosphates in the intestine. Different hypotheses have been proposed to explain the 'lactose effect'. It has been described that lactose competes with inhibitors such as phosphates, keeping Ca soluble in the lumen. However, it has also been reported that lactose does not act by increasing soluble Ca in the lumen. Other studies support the idea that lactose acts on the intestinal mucosa to increase its permeability, facilitating the passive diffusion of Ca when Ca intake is high, particularly in infants, and in the elderly, where solubility is a limiting factor and passive absorption is the predominant route [36].

Several commercial Ca salts have been used to fortify milk/beverages, which are the foods of choice for Ca addition. Calcium gluconate, lactate, sulfate, acetate, citrate and carbonate are approved for fortification, and their Ca absorbability is similar to that of Ca from milk [38].

Calcium carbonate is the least costly Ca source, and is often used in products where clarity or dry and chalky mouthfeel would likely not be noticeable, such as food bars, cereals and soya beverages. However, it functions best when stabilizers and ingredients to reduce interaction with proteins are employed to keep this insoluble Ca form suspended, and to minimize protein–Ca complexation and sedimentation. Calcium phosphate is a low-solubility Ca source used in foods and non-clear beverages. Calcium citrate, lactate, lactate gluconate, and gluconate are Ca salts that prove more soluble over a broad pH and concentration range, while other highly soluble forms of calcium, such as calcium chloride have undesirable taste acceptance when used at concentrations above 10%. Lastly, calcium fumarate would appear to be useful for clear beverages, due to its taste and solubility characteristics [39].

3.3.2 Iron

Iron has metabolic and enzymatic functions (e.g. synthesis of haemoglobin or myoglobin, and Fe-containing enzymes which participate in electron transfer and redox reactions) [40]. Fe deficiency is a major global public health problem, and is particularly common in developing countries, affecting more than 2 billion people worldwide. Fe deficiency is associated with diminished work productivity, lowered immunity, and impaired cognitive development [41].

Dietary Fe comprises two types: the non-haem form (inorganic Fe salts), which is present in plant foods and animal tissues; and haem Fe (of animal origin), which comes from haemoglobin and myoglobin present in

animal food sources. The latter contributes around 10–15% of the total Fe consumption in omnivorous individuals, and is absorbed by a separate pathway and more efficiently than non-haem Fe, with greater bioavailability (15–35%) than non-haem Fe (5–15%). Haem Fe is a component of haemoglobin and myoglobin, and is therefore found only in animal tissue. The haem Fe content of meat is estimated to afford 40% of total Fe, while less haem Fe is provided by fish and poultry. Factors such as phytates and fibre do not interfere with its absorption. Haem Fe absorption is only conditioned by meat presence, and to lesser degree by the presence of Ca [40].

The main dietary sources of non-haem Fe include cereals, pulses, vegetables and meat, and it is also present in eggs and dairy products for example. All non-haem Fe present in food forms a common Fe pool in the digestive tract, and is absorbed to the same extent, which depends on the balance between the absorption inhibitors and enhancer compounds, and on the Fe status of the individual [42]. Dietary factors that influence non-haem Fe bioavailability are the chemical form in the food (the ferrous state being absorbed much better than the ferric state), Fe intake, and the presence of enhancers and inhibitors. Meat factors and ascorbic acid are the main enhancers. The protein fraction of muscle tissue is most probably responsible for the meat factor effect, and good evidence to support this effect are the peptides liberated during digestion of proteins binding Fe and forming complexes in the intestinal lumen which would increase its solubility. Ascorbic acid reduces ferric Fe to ferrous Fe, but is also able to chelate Fe. Phytic acid (myoinositol hexaphosphate) found in vegetable foods (such as whole grains cereals, legumes, nuts and seeds) is an important inhibitor of non-haem Fe. Vitamin C, meat consumption and food processing to remove or degrade phytate can counteract this inhibitory effect. Other inhibitors are polyphenols, associated with the galloyl group of phenolic compounds, while ascorbic acid has been shown to counteract this inhibitory effect. Another inhibitor of Fe absorption is Ca. It was suggested that this inhibition takes place during the initial uptake of Fe into the enterocytes [40, 42, 43]. Other sources of non-haem Fe are fortification iron and contamination Fe from soils or foods cooked in cast-iron or steel cookware [43, 44].

Food fortification has been identified as one of the most cost-effective and sustainable approaches to control iron-deficiency anaemia. Cereals, dairy products, and to a lesser extent sugar, salt and condiments, are the products most commonly used for Fe enrichment [45].

Several forms of inorganic water-insoluble Fe with varying bioavailability and organoleptic effects are used as fortificants. Ferrous sulfate has the highest relative bioavailability among conventional iron compounds, but causes unacceptable colour and flavour changes, and promotes fat oxidation. Ferrous fumarate and succinate are less reactive forms, with comparable bioavailability. Ferric pyrophosphate causes no adverse colour or flavour changes in food vehicles. However, its absorption in humans is low. Recently, novel ferric pyrophosphate compounds have been developed, based on small particle size ferric pyrophosphate and encapsulation with a mixture of emulsifiers, with similar absorption [46, 47]. Reducing the particle size of low-solubility Fe compounds to the micrometer scale may improve their bioavailability from foods [48].

3.3.3 Zinc

Zinc plays an essential role in gene expression and in the regulation of cellular growth and differentiation, since it participates as a cofactor in over 200 enzymes involved in the metabolism of nucleic acids, carbohydrates and proteins. It also plays an important role in the immune system and in male reproductive function. Likewise, one of the most important functions of Zn is related to its antioxidant role and its participation in the antioxidant defence system [49]. Zn deficiency is a serious global issue, particularly in developing countries. The food supply of nearly 50% of the world population is low in absorbable Zn, due to limited availability of animal products [50].

The major problem in Zn fortification is finding a suitable Zn compound. The choice of compound to be added, considering Zn bioavailability, side effects and technological aspects, remains an unresolved issue. The Zn compounds most commonly used in food fortification are zinc sulfate and zinc oxide, but both have drawbacks. Zinc sulfate has adequate bioavailability, but affects food sensorial properties (mainly food flavour). Zinc oxide does not interact with the food matrix, but its solubility is low. Zinc gluconate stabilized with glycine is a new alternative source. Since Fe and Zn deficiencies frequently coexist, both Fe and Zn fortification is proposed. Fe-containing and atomically mixed Fe/Zn-containing nanostructured compounds have demonstrated

superior sensory qualities in reactive food matrices at solubilities equivalent to those of commercial ferrous sulfate and zinc sulfate [47].

Some compounds enhance Zn absorption, such as picolinic acid secreted by the pancreas, vitamin B_6 (which increases picolinic acid secretion), citrate and amino acids such as glycine, histidine, lysine, cysteine and methionine. The presence of animal proteins can improve Zn availability, in the same way as increasing the presence of Zn in the diet. Phytic acid is the main inhibitor of Zn absorption, other inhibitors being oxalic acid, tannins, Se, Fe and Ca, though the effect of the interaction of Zn with Fe and Ca is subject to debate [49, 51].

3.3.4 Selenium

Selenium is an essential element, although the margin between Se toxicity and deficiency is very narrow. Se is part of glutathione peroxidase (GPX), an enzyme involved in the protection of tissues against oxidative stress. Other selenoproteins, such as thioredoxin reductases that regulate the cellular redox balance, deiodinases involved in thyroid function, and selenoprotein P which probably acts as a transporter between the liver and other organs, have particularly important functions. The element has also been reported to possess anticancer properties, playing a pro-oxidative role in the induction of cancer cell apoptosis. Besides its antioxidative and anticancer effects, other beneficial influences of Se include the prevention of cardiovascular diseases, heavy metal detoxification, and participation in cerebral functions, reproduction and the immune system [52]. Se deficiency probably affects at least 1 billion individuals, and is associated with health disorders including oxidative stress-related conditions, reduced fertility and immune functions, and an increased risk of cancer [53].

Food sources of Se can be characterized as follows: good sources include Se-enriched yeast and wheat, moderate sources include most plant materials, and poor sources are most meat and fish products and soybean. Se is frequently included in food supplements (Se-enriched foods) such as broccoli, yeast and potatoes [54].

In foods, Se is present in different chemical forms as organic and inorganic compounds. Plant foods can be divided into two general groups: (i) high-Se plants that accumulate Se in response to the amount of Se available from the soil, the major species being selenate, selenomethionine (SeMet), with smaller amounts of selenocysteine (SeCys); and (ii) plants that actively accumulate Se well in excess of the amounts found in the soil (often called 'Se hyperaccumulators'), where methylated forms of Se, including Se-methylselenocysteine (SeMSC), γ-glutamylSeMSC, selenocystathione, selenohomocysteine, γ-glutamyl selenocystathione and methylselenol are the major Se species [55].

There are limited data on the forms of Se in animal foodstuffs, the major forms being SeMet and SeCys [56, 57]. Beef is the single greatest source of Se in the Western diet, and Se from beef is highly bioavailable for the restoration of GPX activity. The bioavailability of Se from veal has been reported to be similar to that of beef, but the bioavailability of Se from lamb was reported to be lower than that of beef. Seafoods have relatively high concentrations of Se. Some studies report the bioavailability of Se from seafood to be quite high, while others describe it as lower than from mammal and avian meats. Other animal products that contribute substantial amounts of Se to the diet include milk and eggs [55].

Se bioavailability in food depends on food sources associated with different forms of Se contained in them. Most dietary Se is highly bioavailable (about 80%). Selenate or selenite appears to be very well absorbed (about 100% of selenate is absorbed but a significant fraction is lost in urine, while <50% of selenite is absorbed and is better retained than selenate), but is less well retained in the body than SeMet or SeCys. Over 90% of SeMet is absorbed, and SeCys appears to be absorbed very well. Although most forms of Se are efficiently absorbed, SeMet, SeCys, selenate and selenite enter the selenide pool and from here the Se is used for selenoprotein synthesis or is excreted in urine as a selenosugar. A different pathway is followed by the organic compounds. Thus, γ-glutamylSeMSC is first converted to Se-methylselenocysteine and then transformed into methylselenol, followed by elimination in breath and urine, but it may also enter the selenide pool [57]. Species-dependent bioavailability of Se is related to the distinct metabolic pathways that different species follow in the body (consultation of a recent review published by Thiry *et al.* [52] is recommended in this respect).

It has been described that after simulation of human digestion with green onions and chives supplemented with Se(IV), organic Se undergoes degradation. Inorganic Se increases significantly, and selenoximethionine (SeOMet) can be detected. As an effect of the

significant pH change between the stomach and intestinal tract, two oxidation processes take place: selenite is oxidized to selenite, while SeMet is oxidized to SeOMet [58]. No modification of SeMet has been reported during digestion of fish [59].

Heavy metals and fibres reduce Se bioavailability as does high dietary sulfur (especially from methionine), probably because of competition between chemically similar sulfur and Se species [56, 57].

Some examples of studies using *in vitro* gastrointestinal digestion techniques to make preliminary assessments with respect to Ca, Fe, Zn and Se bioavailability are shown in Table 3.1.

3.4 Bioavailability of minerals with toxicological risk: As, Hg, Cd, Pb

The elements As, Hg, Cd and Pb are environmental contaminants that occur naturally and, to a greater extent, are generated from anthropogenic activities, followed by concentration in the food chain. As such, they constitute a public health concern. The main chemical forms, food sources and toxicities of these elements, as well as the maximum levels for As, Hg, Cd and Pb in foodstuffs [60], are shown in Tables 3.2 and 3.3.

3.4.1 Arsenic

Low levels of inorganic and organic As have been measured in most foodstuffs (<0.25 mg/kg). Food type (e.g. seafood vs. meat or dairy products), growing conditions (e.g. soil type, water, use of arsenic-containing pesticides), and food-processing techniques all influence the total concentration of As in food [61, 62]. The main food sources of As are shown in Table 3.2. Inorganic As is the predominant form found in meats, poultry, dairy products and cereals. Organic As predominates in seafood, fruit, and vegetables [62], and represents over 70% of the As found in white fish. It is followed, in order of prevalence, by dimethylarsenic (DMA) (~23% As) and a lesser percentage (<4% total As) of the rest of the arsenical species such as arsenobetaine, arsenocholine, and the methylated species monomethylarsenic (MMA) and trimethylarsenic (TMA) [63]. White fish contains more As than blue fish, though the latter has a greater inorganic As content and therefore a larger presence of biogenic amines and other amines from methylation-mediated inorganic As detoxification [64].

Regional differences are seen in the daily intake of total As through food, and are mainly attributable to variations in the quantity of seafood consumed. For example, the daily dietary intake of total As in Japan is higher than that in Europe and the USA (Table 3.2). Based on the limited data available, it is estimated that approximately 25% of daily dietary As intake is from inorganic sources [62]. Significant exposures have been documented in Argentina, Bangladesh, Chile, China, India (West Bengal), Mexico, Taiwan, China, and parts of the south-western USA [61]. In areas where soils and underground waters are contaminated with As, important amounts of As can find their way into the human diet through cereals and other plant foods (e.g. 123–300 ng/g in rice), as well as contaminated water used for cooking purposes. Such As is mostly inorganic, due to low methylation of the element from soils [65–67].

Further information on As exposure (environmental occurrence, human exposure and toxicological effects) can be found in the published reviews and evaluations/monographs of the International Agency for Research on Cancer [61, 62] and the report of the European Food Safety Authority (EFSA), which offers an evaluation of the risk associated with the intake of arsenic through the diet [68].

Each of the forms of As has different physicochemical properties and bioavailability. Several studies in rats, mice and humans indicate that arsenite and arsenate present in drinking water are rapidly and nearly completely (~95%) absorbed after ingestion [69]. However, the absorption of ingested inorganic As varies, depending on the solubility of the arsenical compounds (the more water soluble the compound, the greater its absorption), the presence of other food constituents and nutrients in the gastrointestinal tract, and on the food matrix itself. For example, Juhasz *et al.* [70] demonstrated that while the bioavailability of inorganic As present in mung beans and radish was 9% and 77%, respectively, in swine this percentage was only 52% and 50% for chard and lettuce, respectively, suggesting an influence of the non-digestible polysaccharide component of the vegetable on the gastrointestinal absorption of As. These authors, using the same animal model, showed that speciation played a major role in determining the amount of As absorbed after consuming As-contaminated rice [71]. For rice bought at the supermarket and cooked with As-contaminated water, As was present entirely in the inorganic form,

Table 3.1 Examples of recent studies of *in vitro* bioavailability of Ca, Fe, Zn and Se.

Element	Sample matrix	*In vitro* method	Analytical technique	Results	Reference
Fe	School menus	**Solubility** *Gastric digestion*: pepsin pH 2, 1 h, 37 °C *Intestinal digestion*: adjust pH 5. Pancreatin–bile salts 2 h, 37 °C, pH 7.2. Centrifugation: 3500 *g*, 1 h, 4 °C	Total Fe: AAS Ionic Fe [Fe(II) + Fe(III)]: bathophenantroline	Soluble Fe was mainly in ionic form (49–100%) A significant correlation was found between soluble ionic Fe and Fe(II) Fe(II)/Fe(III) ratio increased with increasing meat protein in the dish	Cámara *et al*. [98]
Fe	Iron fortified Breakfast cereals	**Dialysis/Caco-2 cells** *Gastric digestion*: pepsin pH 2, 110 oscillations/min, 1 h, 37 °C *Intestinal digestion*: pancreatin–bile mixture pH 7, 6 oscillations/min, 2 h, 37 °C in upper chamber created with insert ring with dialysis membrane (15,000 molecular weight cut-off) Difussion of soluble Fe to Caco-2 cells *Caco-2 cells*: cell culture plate incubated 37 °C, 5% CO_2, 22 h. Cells harvested for ferritin analysis	Radioimmunoassay	Fourteen Fe forms were evaluated: Na_2FeEDTA and Ferrochel are the forms more availables Milk increases and coffe decreases Fe bioavailability, the effect depending on the Fe compound	Wortley *et al*. [99]
Fe	Porcine meat (raw and heated)	***In vitro* protein digestion/dialysis** Pepsin pH 4.5, 1 h, 37 °C). Pancreatin and sodium taurocholate pH 6.5–7, 1 h, 37 °C Dialysis bag containing PIPES buffer (pH 6.1) and continuing until equilibrium 4 h at 37 °C with stirring	Ferrozine: Fe^{2+} and total Fe	Heat treatment enhances Fe availability *In vitro* Fe^{2+}-dialysability following pepsin digestion correlates well with human studies	Sorensen *et al*. [100]
Ca, Fe, Zn	Legumes, raw and cooked (traditional, microwave and industrial processing)	**Dialysis** *Gastric digestion*: pepsin pH 2, 140 strokes/min, 2 h, 37 °C *Intestinal digestion*: dialysis tubing (cut-off 12–14 kDa) containing $NaHCO_3$ is introduced and incubated for 90 min at 37 °C. Pancreatin–bile extract mixture for 2 h at 37 °C	AAS	Mineral dialysabilities can be classified as follows: Zn > Ca > Fe Mineral dialysability is highest in industrial processed legumes	Sebastiá *et al*. [101]
Ca, Fe, Zn	Fortified and non-fortified Ca cow milks	**Solubility/dialysis** *Gastric digestion*: demineralized pepsin pH 2, 120 strokes/min, 2 h, 37 °C *Intestinal digestion*: adjust pH 5. Demineralized pancreatin–bile salts, 2 h, 37 °C, pH 7.2. Centrifugation: 3500 *g*, 1 h, 4 °C	AAS	Increase in Ca solubility, dialysis, transport, and uptake by Caco-2 cells in Ca fortified milks vs. non-fortified milks	Perales *et al*. [102]

(Continued)

Table 3.1 (Continued)

Element	Sample matrix	*In vitro* method	Analytical technique	Results	Reference
		Dialysis: after gastric digestion a dialysis bag (containing $NaHCO_3$) is introduced and incubated for 30 min at 37 °C. Demineralized pancreatin–bile salts incubation continued up to 2 h **Solubility/Caco-2 cells** Gastrointestinal digests heated 4 min at 100 °C and then cooled by immersion in ice bath. Centrifugation: 3500 *g*, 1 h, 4 °C. Added to supernatants Glucose and HEPES to adjust osmolarity to 310 mosmol/kg before addition to cell culture Caco-2 cells: cell cultures were incubated with the soluble fraction 37 °C, 5% CO_2, 95% relative humidity, 2 h		Inhibitory effect of Ca from fortified milks on Fe absorption found. The effect of Ca from fortified milks on Zn bioavailability depends on the *in vitro* method used: Zn solubility and dialysis decrease in Ca fortified milks, and percentage Zn uptake remains unchanged	
Ca, Fe, Zn	Infant formulas	**Dialysis** *Gastric digestion*: pepsin pH 2, 3.5 or 4.5, 2 h, 37 °C in a shaking water bath *Intestinal digestion*: dialysis bags containing PIPES buffer were placed in the pesin digests and incubated 50 min at 37 °C. Pancreatin–bile mixture 2 h at 37 °C. Dialysate pH checked to be 6.5 ± 0.2	AAS	Influence of gastric digestion pH on mineral dialysability depends on the infant formula evaluated Effect of gastric pH is more evident on Fe and Zn dialysability than on Ca dialysability	Drago *et al*. [103]
Se	Radish	**Solubility** *Gastric digestion*: pepsin–NaCl pH 1.8, 4 h, 37 °C *Intestinal digestion*: adjust pH 6.8 with $NaHCO_3$. Pancreatin–α-amylase–NaCl for 4 h at 37 °C, shaking periodically. Centrifugation 7500 *g* for 30 min and filtration (0.22 μm)	HPLC-ICP-MS	90–100% of Se is bioaccessible In radish plants enriched with selenite SeMetSeCys was the highest specie (75%) In radish grown in selenate media 60% of the Se was present in inorganic forms	Pedrero *et al*. [104]

Se	Fish (swordfish, sardine and tuna)	**Solubility** *Gastric digestion*: pepsin–NaCl pH 1.8, 4 h, 37°C shaking periodically *Intestinal digestion*: adjust pH 6.8 with $NaHCO_3$. Pancreatin–α-amylase–bile salts for 4 h at 37°C shaking periodically. Centrifugation: 1575 *g*, 1 h and filtration (0.45 μm)	HPLC-ICP-MS	Bioaccessible percentages for total Se and SeMet were 50–81% and 14–19% respectively	Cabañero *et al*. [59]
Se	Selenized yeast	**Solubility** *Gastric digestion*: pepsin–NaCl pH 2, 4 h, 37°C shaking periodically *Intestinal digestion*: adjust pH 6.8 with $NaHCO_3$. Pancreatin–α-amylase–bile salts for 4 h at 37°C shaking periodically. Centrifugation: 6000 *g*, 1 h, 4°C	Total Se: ICP-MS Se species: HPLC-ICP-MS ESI Q-TOF MS and MALDI TOF MS	Bioaccessible percentages for total Se and SeM were 89 ± 3% and 34 ± 1% respectively	Hinojosa Reyes *et al*. [105]

AAS, atomic absorption spectrometry; HPLC-ICP-MS, high performance liquid chromatography inductively coupled plasma mass spectrometry; ESI Q-TOF MS, electrospray tandem mass spectrometry; MALDI TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Table 3.2 The main chemical forms present in food, dietetic sources and toxicity of As and Hg.

	Arsenic	**Mercury**
Inorganic forms	Arsenite or arsenous acid (III) and arsenate or arsenic acid (V): higher absorption and toxicity than organoarsenical compounds. Accumulation and metabolism as arsenobetaine in marine organisms	Elemental or metallic mercury (Hg), inorganic mercury, i.e. mercurous (Hg_2^{2+}) and mercuric (Hg^{2+}) cations [77]
Organic forms	Arsenobetaine (AB), methylated species (monomethyl-, dimethyl, trimethyl-arsenic), trimethylarsine oxide, tetramethylarsonium (TMA^+) ion, arsenocholine (AC), arsenosugars and arsenolipids [63, 68]	Methylmercury (MeHg) and dimethylmercury (DMeHg): inorganic forms are methylated by microorganisms. Higher toxicity than inorganic Hg [115]
Food sources	*Fish and fish products*: fish and seafood (2.4–16.7 mg/kg in marine fish, 3.5 mg/kg in mussels, and >100 mg/kg in certain crustaceans), products or supplements based on algae (especially *hijiki*), and cereal and cereal products, with particularly high concentrations in rice grains and rice-based products and bran and germ; drinking water and cooked water (contamined soils) [62, 68] *Water and natural mineral water*: maximum 10 μg/L [106, 107]	*Fish and fish products*: fish are the source of 75–95% of organic Hg [76]. In particular tuna, swordfish, cod, whiting and pike were major contributors to MeHg dietary exposure in adult age groups, while the same species, with the addition of hake, were the most important contributors in the child age groups [77] *Maximum levels*: fishery products and muscle meat of fish (including crustaceans but excluding the brown meat of crab and head and thorax meat of lobster and similar large crustaceans, i.e. Nephropidae and Palinuridae): 0.5 mg/kg wet weight (except see footnote* 1.0 mg/kg wet weight) [60, 116, 117] *Water and natural mineral water*: maximum 1 μg/L [106, 107]
Intakes	*Contaminated soils*: adults 600–880 and children 351–432 μg/day (Western Bengal, India) [75, 108, 109] *High fish intakes* (μg/day): 297 [110], 224 [111] (Spain), 280 (Japan) [112] *Low fish intakes* (μg/day): 16.7-7- 48.5 (Canada), 7–62 (EU), 89 (UK), 12 (Belgium), 27 μg (Australia), 55 μg (New Zealand) [75]	*MeHg*: 0.14 μg/kg body weight per week (very elderly) to 5.05 μg/kg body weight per week (adolescents) (95th percentile middle bound dietary exposure and based only on the food group fish and other seafood); infants 0.09–0.94 μg/kg body weight per week (depending on human milk consumption) [77] *Inorganic Hg*: 0.25 μg/kg body weight per week (low bound exposure to elderly and very elderly) to 4.06 μg/kg body weight per week (upper bound exposure to toddlers) (95th percentile); infants 0.17–1.94 μg/kg body weight per week (depending on human milk consumption) [77]
Exposure and toxicity	Carcinogenic (group I) [113] TWI inorganic arsenic: 15 μg/kg [114]	Reference dose† MeHg: 0.1 μg/kg [118] TWI MeHg: 1.6 μg/kg body weight or 1.3 μg/kg expressed as mercury [119] and 4 μg/kg inorganic mercury [120]

*Anglerfish (*Lophius* spp.), Atlantic catfish (*Anarhichas lupus*), bonito (*Sarda sarda*), eel (*Anguilla* spp.), emperor, orange roughy, rosy soldierfish (*Hoplostethus* spp.), grenadier (*Coryphaenoides rupestris*), halibut (*Hippoglossus hippoglossus*), marlin (*Makaira* spp.), megrim (*Lepidorhombus* spp.), mullet (*Mullus* spp.), pike (*Esox lucius*), plain bonito (*Orcynopsis unicolor*), poor cod (*Tricopterus minutes*), Portuguese dogfish (*Centroscymnus coelolepis*), rays (*Raja* spp.), redfish (*Sebastes marinus*, *S. mentella*, *S. viviparus*), sailfish (*Istiophorus platypterus*), scabbard fish (*Lepidopus caudatus*, *Aphanopus carbo*), seabream, pandora (*Pagellus* spp.), shark (all species), snake mackerel or butterfish (*Lepidocybium flavobrunneum*, *Ruvettus pretiosus*, *Gempylus serpens*), sturgeon (*Acipenser* spp.), swordfish (*Xiphias gladius*) and tuna (*Thunnus* spp., *Euthynnus* spp., *Katsuwonus pelamis*).

†Reference dose: estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

and bioavailability was high (89%). Conversely, 86% of total As was present as dimethylarsinate in greenhouse-grown rice (using irrigation water contaminated with sodium arsenate), with low bioavailability (only 33%) of the total rice-bound As [68]. Following absorption, the As compounds are methylated to less toxic forms such as MMA and DMA, which are excreted in urine [72].

Table 3.3 The main chemistry forms presents in food, dietetic sources and toxicity of Cd and Pb.

	Cadmium	**Lead**
Inorganic forms	Although Cd may bind to proteins (Cd-MT) and other organic molecules and form salts with organic acids, these compounds are regarded as inorganic with respect to cadmium [88]	Pb(II) (phosphate, carbonate, chloride, hydroxide)
Organic forms		Tetraethyl lead $Pb(C_2H_5)_4$ and tetramethyl lead $Pb(CH_3)_4$ are the most commonly used alkyl-lead compounds [97]. Trialkyl-lead and tetraalkyl-lead compounds are more toxic than inorganic forms of lead but are metabolized to inorganic lead in humans [122]
Food sources	Offal (kidney and liver) and offal products, seafood (bivalve molluscs, i.e. oysters, mussels, crustaceans, cephalopods), fish, seaweed, chocolate, and foods for special diets [88] *Contaminated zones*: cereals and meat contents can be higher than usual content and produce intoxication (itai-itai disease) [87] *MLs in foodstuffs*: recompiled by EFSA [60, 88, 117] *Water and natural mineral water*: maximum 5 μg/L and 3 μg/L, respectively [106, 107]	Cereal products, followed by potatoes, cereal grains (except rice), cereal-based mixed dishes and leafy vegetables and tap water [97] *MLs in foodstuffs*: recompiled by EFSA [60, 97, 117] *Water and natural mineral water*: maximum 10 μg/L [106, 107]
Intakes	*Exposure* Mean: 1.9–3.0 μg/kg body weight per week High: 2.5–3.9 μg/kg body weight per week Vegetarians: 5.4 μg/kg body weight per week Regular consumers of bivalve molluscs and wild mushrooms: 4.6 and 4.3 μg/kg body weight per week, respectively [88]	*Exposure* Mean: 0.36–1.24 μg/kg body weight daily High: 0.73–2.43 μg/kg body weight daily Infants: 0.21–0.94 μg/kg body weight daily Children: 0.80–3.10 (average consumers), up to 5.51 (high consumers) μg/kg body weight daily [97]
Exposure and toxicity	Carcinogenic (group I) [121] PTWI: 7 μg/kg body weight [88]	Probably carcinogenic (group 2A) [122] PTWI: 25 μg/kg body weight [123]

MLs, maximum levels; PTWI, provisional tolerable weekly intake.

Significant interspecies differences have been reported for organic As bioavailability. In rodents, methylarsonate and dimethylarsinate (pentavalent form) are absorbed from the gastrointestinal tract in a proportion of over 40% of the ingested dose, while the trivalent organoarsenicals are generally poorly absorbed [68]. Recently, Juhasz *et al*. [71] found the gastrointestinal absorption of methylarsonate and dimethylarsinate in swine to be 17% and 33%, respectively. Few data are available on the absorption of organic As in humans. In a study involving volunteers who ingested a single oral dose of As (500 μg) as either methylarsonate or dimethylarsinate, the amount of As excreted in urine after 4 days represented 78% and 75% of the ingested dose, respectively, suggesting gastrointestinal absorption of over 75% for pentavalent organoarsenicals. In one male volunteer, 80% of arsenosugar was excreted in urine 4 days after ingestion, affording evidence of almost complete absorption in humans. However, more recent data based on urinary excretion suggest considerable individual variability in the absorption of arsenosugars [68].

The very early studies of As in fish and crustaceans (i.e. mostly arsenobetaine) showed arsenobetaine to be efficiently absorbed, appearing in urine within 2 days (>70% of seafood As). However, there appear to be no precise quantitative data on arsenobetaine absorption in humans. A study with ^{74}As-labelled arsenobetaine administered to six volunteers measured the whole-body content of the labelled As. Unfortunately, the first measurement point was after 1 day, by which time much of the absorbed arsenobetaine would have already been excreted in urine. However, the reported whole-body content after 1 day was about 50% of the ingested dose, and this value dropped steadily over the following

days. These results are consistent with essentially complete absorption of arsenobetaine [68].

The key step in the control of oral As absorption would be its dissolution at the acid pH of the stomach [73]. *In vitro* studies have demonstrated the influence of gastrointestinal pH on As bioaccessibility, the element being 10–30% more soluble at gastric pH than at intestinal pH. The soluble inorganic species of As are quickly absorbed in the gastrointestinal tract of humans and most laboratory animals, and since most of the ingested As is absorbed, the urine As contents would be a good indicator of its oral absorption [74].

The presence of other elements affects the bioavailability of As. Thus, Zn ingestion increases the concentration of metalloproteins that favour As detoxification. A high As intake in relation to Se favours competition between both elements, As replacing Se in Se-dependent enzymes such as GPX, thereby inactivating the latter. As a result, Se deficiency increases As toxicity [75].

Nutritional state also influences the risk of As exposure. Different studies indicate that the provision of vitamin C and methionine reduces the toxicity of As, while vitamin A deficiency increases such toxicity. Diets rich in protein or carbohydrates, or even containing abundant fats, increase the toxic effect [75].

3.4.2 Mercury

The toxicity and absorption of Hg depends on the chemical species involved. Elemental Hg is volatile, and its vapours are easily absorbed via the respiratory route (50–100%), while very little is absorbed through the skin or gastrointestinal tract. Inorganic Hg salts (mercurous I and mercuric II) differ in their properties and absorptions. Most organic Hg compounds are easily absorbed via the respiratory and gastrointestinal routes. After oral intake, methylmercury (MeHg) is much more extensively and rapidly absorbed (practically 100%) than mercuric and mercurous Hg. MeHg and dimethylmercury are the most toxic chemical species, with high bioavailability, and easily concentrate within the food chain. Absorption is also influenced by age, the frequency of meals and the presence of other components in the diet [76].

Exposure to MeHg mostly occurs via the diet, with collection and concentration especially in the aquatic food chain, making populations with a high intake of fish and seafood particularly vulnerable. The 95th percentile dietary exposure is close to or above the tolerable weekly intake (TWI) for all age groups. The dietary exposure estimations in high and frequent consumers of fish (95th percentile, consumers only) was in general approximately twofold higher in comparison with the total population (see Table 3.2) (middle bound exposure 0.54 μg/kg body weight per week in elderly to 7.48 μg/kg body weight per week in other children). Exposure may exceed the TWI by up to approximately sixfold. Unborn children constitute the most vulnerable group. Inorganic Hg is prevalent in most other foodstuffs such as non-alcoholic beverages and composite foods (mainly cereal-based dishes such as cooked pasta and pizza, and prepared salads). Dietary inorganic Hg or MeHg exposure in Europe does not exceed the TWI [77].

A Joint FAO/WHO Expert Consultation on the risks and benefits of fish consumption considered nutrients (*n*-3 long-chain polyunsaturated fatty acids) and specific chemical contaminants (MeHg and dioxin-like compounds) and MeHg in fish species. It was concluded that among women of childbearing age, pregnant women and nursing mothers, considering the benefits of docosahexaenoic acid versus the risks of MeHg, fish consumption lowers the risk of suboptimal neurological development in their offspring compared with not eating fish in most circumstances evaluated. Among infants, young children and adolescents, the evidence was insufficient to derive a quantitative framework of health risks and benefits [78].

In human blood, mercuric Hg is divided between plasma and erythrocytes, with more being present in plasma, whereas MeHg is accumulated to a large extent (>90%) in erythrocytes. In contrast to mercuric Hg, MeHg is able to enter the hair follicle, and to cross the placenta as well as the blood–brain and blood–cerebrospinal fluid barriers, allowing accumulation in hair, the fetus and the brain. Total Hg in hair and blood are routinely used as biomarkers to assess long-term MeHg exposure. A frequently cited total Hg blood-to-hair ratio is 1 : 250. However, important variations exist, especially in people with infrequent fish consumption [77]. The biological half-life of MeHg is 1.5–2 months [76]. Mercuric Hg in the brain is generally the result of either *in situ* demethylation of organic Hg species or oxidation of elemental Hg.

Excretion of absorbed mercuric Hg occurs mainly in urine, whereas the main pathway of excretion of

absorbed MeHg is in stools in the form of mercuric Hg [77]. The results of different toxokinetic studies of MeHg [79–81] suggest that high inorganic Hg levels in urine and their close correlation to the MeHg contents in blood indicate partial demethylation of the methylated Hg compounds, increasing the concentration of inorganic Hg in the kidneys and therefore also in urine. Urinary total Hg might be a suitable biomarker of inorganic (and elemental) Hg, but not of MeHg exposure [77].

Some studies relate slight changes in adult coordination and motor function to relatively low levels of inorganic Hg [82] and organic Hg [83, 84]. In a study on the possible neurotoxic effects of relatively low levels of Hg absorption from fish involving two groups of adults – one with regular tuna fish consumption (Hg level >1 μg/g) and a control group – higher urine Hg levels were recorded in the first group (mean 6.5 μg/g creatinine; range 1.8–21.5) than among the controls (mean 1.5 μg/g creatinine; range 0.5–5.3). In addition, a significant correlation was observed between weekly fish consumption and the blood and urine Hg contents [85]. Most of the *in vitro* and *in vivo* studies have used methylmercuric chloride, which differs in bioavailability, tissue distribution and toxicity from MeHg species present in fish.

The *in vitro* studies shown in Table 3.4 and the studies on the influence of cooking and processing indicate that there is little impact of cooking or processing on the Hg content in foods, and so data for Hg in raw foods are suitable for use in dietary exposure estimates [77]. Torres-Escribano *et al*. [11] evaluated the influence of cooking on Hg bioaccessibility, and found a decrease of around one-half of the original concentration. That might be attributable to alterations in the structural conformation of the fish muscle proteins produced by temperature, which could cause loss of the native protein structure. These changes might impede access of the enzymes used in *in vitro* gastrointestinal digestion to the structures to which Hg is bound in muscle low-molecular-weight thiols, i.e. sulfhydryl group-containing molecules such as cysteine. Maulvault *et al*. [10] also found reductions of up to 40% in the bioaccessible fraction of Hg in fish after it was cooked. Ouédraogo and Amyot [86] in turn found Hg concentrations (dry weight) to be slightly higher in boiled fish, but boiling or frying reduced bioaccessibility by 40–50%, and this reduction was moreover greater (50–60%) in the presence of tea or coffee.

3.4.3 Cadmium

Foodstuffs are the main source of Cd exposure for the non-smoking and non-professional exposure general population, and can contribute 70–90% of the Cd reaching the body. The Cd contents in foods are very low, so the contribution of the diet to daily intake is therefore small [87, 88]. The contents in the main dietetic sources (see Table 3.3) are in the milligram per kilogram range. Although plant foods contain little Cd, their high consumption levels define them as one of the main dietetic sources of Cd [87, 89].

In animal tissues, most of the Cd is bound to Zn-thionein or metallothionein (MT). Little is known of the form in which Cd is found in plants. In part, the element forms complexes with organic acids, metallothioneins and so-called phytochelatins, which are peptides composed of γ-glutamylcysteine units with a terminal carboxyglycine group (poly-(γ-EC) Gs). Phytochelatins and metallothioneins have different structures but similar functions, since they form very stable Cd–cysteine complexes that can be induced by metals and by the synthesis of glutathione or its γ-glutamylcysteine precursors. In foods of plant origin it is not known to what extent the metabolism of Cd-phytochelatins is similar to that of Cd-metallothionein (Cd-MT) [87, 90]. In oysters, Cd is bound to high molecular weight proteins other than metallothionein, and its bioavailability is low, considering that the documented kidney Cd concentrations are low in deceased oyster consumers [90].

The food groups that contribute most to dietary Cd exposure, primarily because of high consumption, are cereals and cereal products, vegetables, nuts and pulses, starchy roots or potatoes, and meat and meat products. The mean dietary exposure across European countries can be seen in Table 3.3. Vegetarians, due to their high consumption of cereals, nuts, oilseeds and pulses, and also regular consumption of bivalve molluscs and wild mushrooms, have higher dietary exposures. The mean exposure for adults across Europe is close to or slightly above the TWI of 2.5 μg/kg body weight. Subgroups such as vegetarians, children, smokers, and people living in highly contaminated areas may exceed the TWI by about twofold. Although the risk of adverse effects on kidney function at an individual level resulting from

Table 3.4 Examples of recent studies of *in vitro* bioavailability of As, Hg, Cd and Pb.

Analyte	Sample matrix	*In vitro* assays/digestion			Analytical techniques	Bioaccessibility (%)	Reference
As: total, inorganic (arsenite + arsenate), arsenite and arsenate	*Edible seaweed* Brown seaweed *Hijikia fusiforme* (*hijiki*); red seaweed *Porphyra* sp. (*nori*); green seaweed *Enteromorpha* sp. (green *nori* flakes)	*Gastric digestion*: pepsin pH 2 (HCl), 120 strokes/min, 37 °C, 2 h *Intestinal digestion*: pancreatin–bile extract pH 7.2 (NaOH). Centrifugation: 15,000 rpm for 30 min at 4 °C			Total and inorganic As: FI-HG-AAS Arsenite and arsenate: HPLC-HG-AFS	Total As (32.0–67.2) and inorganic As (48.6–77.2) and increase after cooking Arsenite and arsenate (36–94 and 5–51, respectively and decrease after cooking	Laparra *et al*. [124, 125]
Arsenite, arsenate, MMA, DMA, AB, AC, TMAO	Seafood reference materials: TORT-2 (lobster hepatopancreas), DOLT-3 (dogfish liver), CRM 6327 (tuna fish), DORM-3 (fish muscle)	*Salivary digestion*: KH_2PO_4 + NaOH pH 6.5, 37 °C, 5 min *Gastric digestion*: pepsin pH 1.2, 37 °C, 1 h *Intestinal digestion*: pancreatin 37 °C, 1 h Centrifugation Continuous online leaching procedure (juices were successively pumped through the sample contained in a mini-column at 37 °C (200 mg solid sample wrapped in a membrane filter placed in a PTFE tubing) directly connected to ICP-MS or batch method (200-mg aliquots of solid sample were placed into test tubes with a certain volume of juices at 37 °C/centrifugation)			ICP-MS	Continous leaching method 42–59 vs. batch method 52–69 Potential contamination was minimized with online leaching procedure	Dufailly *et al*. [126]
MeHg	Swordfish, sardine and tuna	*Gastric digestion*: pepsin pH 1.8, 37 °C, 4 h, shaking periodically *Intestinal digestion*: pancreatin + amylase + bile salts pH 6.8, 37 °C, 4 h, shaking periodically Centrifugation: 1575 *g* for 60 min			LC-ICP-MS	Low bioaccessibility (17, 13 and 9 for swordfish, sardine and tuna, respectively) and no modification during digestion was found for all samples	Cabañero *et al*. [59, 127]
MeHg	Caribou, bearded seal, ringed seal, walrus, narwhal, beluga, Arctic char	Stomach	*Dynamic SHIME* Carbohydrate nutrition solution ($KHCO_3$, NaCl, arabinogalactan, pectin, xylan, potato starch, glucose, yeast extract, peptone, mucin, *L*-cysteine) pH 2, 37 °C, 8 h	*Static SHIME* Pepsin pH 1.5, 37 °C, 2 h, 70 rpm	Hg–thiourea complex (HgTu/LC-CVAFS)	Between 1 (caribou and ringed seal) and 93 (Arctic char) and was independent of food concentration Gastrointestinal microorganisms may influence the speciation of dietary Hg in the intestinal lumen via the conversion of MeHg to Hg(II), although this effect appears to depend on type of food consumed	Laird *et al*. [128]

		Small intestine	Pancreatin 2 h, 37 °C	Pancreatin pH 6.5, 37 °C, 2 h, 70 rpm			
		Colon	Total aerobes 10^8 CFU/mL and total anaerobes 10^8 CFU/mL and with faecal coliforms, clostridia, enterococci, staphylococci, bifidobacteria and fungi at 37 °C	Total aerobes 10^8 CFU/mL and total anaerobes 10^8 CFU/mL and with faecal coliforms, clostridia, enterococci, staphylococci, bifidobacteria and fungi, pH 6.3, N_2, 18 h, 37 °C, filtration 0.45 μm and ultrafiltration 10 kDa, frozen 5% HCl			
Cd	Infant food: cereal- and milk-based diets and two ready-to-use baby dishes	*Gastric digestion*: pepsin pH 5.5 (NaOH) (infant) or 1.8 (HCl) (adults), 37 °C, 4 h, gentle shaking *Intestinal digestion*: pancreatin + bile salts pH 7.4 (HCO_3), 37 °C, 4 h, shaking periodically Centrifugation: 2000 *g* for 60 min at 5 °C *Caco-2 cells*: supernatant of the infant food digests containing ^{109}Cd on the apical side + DMEM on the basolateral side. Radioactive $^{109}CdCl_2$, 180 min, 37 °C. Basolateral solution collected, and radioactivity in the cells and in the basolateral solution measured			Gamma-counter	Infant digestion generally resulted in lower Cd solubility (52%) compared with adult digestion (63%) of the diets. This means less dissociation of Cd from food components and less proteolytic degradation with changes in binding sites for Cd Uptake or transport in Caco-2 cells was generally much lower, varying from 1 to 6 There is no any correlation between the solubility and the BD estimated by Caco-2 cells	Eklund *et al.* [91]

(Continued)

Table 3.4 (Continued)

Analyte	Sample matrix	*In vitro* assays/digestion			Analytical techniques	Bioaccessibility (%)	Reference
Pb	White and red wine	*Gastric digestion*: pepsin pH <3.5, 37 °C, 3 h *Intestinal digestion*: pancreatin + amylase + bile salts pH 7.4, 37 °C, 3 h. For dialysability, dialyser (with a solution of NaCl, equipped with 15,000 nominal MWCO membrane) was immersed in the vessel of digestion			HPLC-AAS	Solubility: white wine 93–94%, white Verde 74–77%; red wine 36–39% Dialysability was lower than the total soluble fraction by about 10 ± 20% of total Pb	Azenha & Vasconcelos [129]
As, Cd, Hg and Pb	SRM: *Fucus* sp. (IAEA-140/TM) and TORT-2		<u>Static</u>	<u>Dynamic TIM</u>	FI-HG-AAS (As) GF-AAS (Cd, Pb) CV-AFS (Hg)	Significant differences between the bioaccessible concentrations obtained by each *in vitro* gastrointestinal digestion method for both samples As: static (71, 72), TIM (47,67) Cd: static (12, 21), TIM (35, 53) Hg: static (3, 4), TIM (8, 21) Pb: static (9, 19), TIM (7, 7)	Torres-Escribano *et al*. [130]
		Gastric	Pepsin pH 2, 37 °C, 120 strokes/min	Lipase/pepsin 37 °C, 120 strokes/min, chyme mixing (reducing the pH from 6.4 to 2 in 2 h)			
		Intestinal	Pancreatin + porcine bile extract , pH 5, 2 h. Centrifuge 15,344 *g* for 30 min at 4 °C	Pancreatin + porcine bile extract: duodenum (pH 6.4), jejunum (pH 6.9) and ileum (pH 7.2), 6 h Centrifuge 15,344 g for 30 min at 4 °C			

AB, arsenobetaine; AC, arsenocholine; DMA, dimethylarsenic; ICP-MS, inductively coupled plasma mass spectrometry; MMA, monomethylarsenic; SRM, standard reference material; TMAO, trimethylarsine oxide; DMEM, Dulbecco's Modified Eagle medium; FI-HG-AAS, flow injection hydride generation atomic absorption spectrometry; GF-AAS, graphite furnace atomic absorption spectrometry; HgTu/LC-CVAFS, liquid chromatography–cold vapour atomic fluorescence spectrometry; HPLC-HG-AFS, high performance liquid chromatography hydride generation atomic fluorescence spectrometry; HPLC-AAS, high performance liquid chromatography atomic absorption spectrometry; LC-ICP-MS, liquid chromatography inductively coupled plasma mass spectrometry; SHIME, simulated human intestinal microbial ecosystem.

dietary exposures across Europe is very low, the current exposure to Cd at the population level should be lowered [88].

Cd absorption after dietary exposure in humans is relatively lower (4–8%) than pulmonary absorption (15–40%) (except in children, where it rises to 37%), but not many details are known about the intestinal absorption mechanisms of Cd [87, 91]. The available information has recently been reviewed by Asagba [92]. Most absorption and toxokinetic studies have been made with inorganic Cd salts, recording Cd retentions in humans of between 3 and 7% [87]. Absorbed Cd is excreted very slowly, with excretion in urine and stools being approximately equal [88].

In the blood, Cd is mainly found in the erythrocytes, where it is predominantly bound to MT, though it may also bind to other thiol-rich low molecular weight peptides or amino acids such as glutathione and cysteine, respectively. Cd bound to albumin is to a large extent taken up by the liver, where the complex is split and only minute amounts reach the proximal renal tubuli. In liver cells, Cd induces the synthesis of MT. Cd is then excreted in bile mainly bound to glutathione (GSH). Redistribution occurs from the liver. Cd is either excreted in bile mainly bound to GSH or released into the plasma as Cd-MT. The Cd-MT complex is filtered through the renal glomeruli and then reabsorbed by the proximal tubular cells until the critical concentration is reached. The critical concentration is represented by the Cd burden at which tubular cells are no longer able to synthesize enough MT to neutralize free Cd^{2+} produced by lysosomal degradation of Cd-MT. When the critical concentration is exceeded, tubular damage results in increased enzymuria, low molecular weight proteinuria, and increased excretion of Cd either as Cd-MT or, probably, also as Cd^{2+} [88].

Over the short term, Cd is taken up and distributed to the liver and kidney (organs which contain about 40–80% of total body Cd), with a very long biological half-life ranging from 10 to 30 years. In humans, the maximum renal Cd content is reached after 40–50 years, with renal cortical levels 10–30 times higher than in the liver, where the Cd content decreases after 30 years. The kidney is the organ most affected by prolonged dietetic Cd exposure, and more Cd accumulates when it is present as Cd-MT than when it is present as inorganic salts [87, 92].

Cd bioavailability, retention and consequently toxicity are affected by several factors such as food chemistry forms, diet composition and nutritional status (low body iron stores) and multiple pregnancies, pre-existing health conditions, or diseases [88, 92, 93].

There is conflicting information on the availability of Cd in Cd-MT for intestinal uptake in relation to that of ionic Cd added to diet or drinking water. When Cd exposure is high (>10 mg/kg), differences in metabolism are detected between organic and inorganic Cd. These differences are dose-dependent, but when intake is low, Cd is released from Cd-MT, followed by absorption in the intestinal mucosa and continuation along the same pathway as inorganic Cd. After prolonged oral exposure, no differences are seen between the renal toxicity produced by inorganic Cd and that caused by Cd-MT. This, and the existence of practically similar metabolism, indicates that dietetic Cd-MT and $CdCl_2$, administered via the oral or intravenous route, have similar toxicity; consequently, the values obtained in rodents exposed to low levels of $CdCl_2$ would be applicable to the estimation of risk associated with the ingestion of Cd-MT [87].

Diets low in proteins, minerals and certain vitamins (A, C, B_6 and B_1) have been reported to increase Cd absorption and toxicity. In contrast, diets rich in fibres have the reverse effect [92]. In recent decades, a number of elements (Ca, P, Mn, Mg, Fe, Zn, Cu, Se) have been reported to interfere with the metabolism of Cd. This element would compete with Ca, Fe and Zn for specific transporters. Thus, diets deficient in calcium increase Cd retention, this being particularly important due to the possible role of Cd in the development of osteomalacia, characteristically seen in Cd intoxication (itai-itai disease). Cd and Zn are mutual antagonists. The absorption of Cd increases when diet Zn content is low, and the content of the latter element in the renal cortex increases with the concentration of Cd. This can be explained by an increase in the synthesis of MT, which binds both metals. The absorption of Cd is greater (24%) and faster in humans with iron deficiency than in those with a satisfactory iron status (1%). Dietetic Fe supplements protect against Cd accumulation and intoxication by reducing the absorption of inorganic Cd released from Cd-MT, without affecting that of intact Cd-MT [87, 89, 94]. A review has recently been published on how these findings

might influence risk assessment and the management of food Cd [92, 95].

Adequate nutritional status protects against the adverse effects of Cd. In this sense, it has been seen that Japanese families that consume rice rich in Cd and poor in Zn developed Fanconi's syndrome (proximal renal tubular proteinuria caused by Cd), while consumers of contaminated oysters (Cd level 5 mg/kg) in New Zealand showed no such syndrome. The main difference between the two populations was the greater dietetic provision of Ca, Fe and Zn in New Zealand [90].

3.4.4 Lead

Lead is commonly present in food and is regulated as a contaminant. Over the past decades, Pb concentrations have decreased significantly due to the phase-out of leaded petrol, other actions, and the concomitant significant decrease in Pb air pollution. As an example, the content of Cd, Pb, Ni, Hg and Se in 83 foods was monitored from 1993 to 1997 in Denmark and compared with similar testing in 1988–1992. A general decrease in Pb concentrations had occurred, whereas the contents of Ca, Ni, Hg and Se were stable or declined only slightly [96]. Food such as cereals, vegetables and tap water were the major sources of exposure to Pb in the general European population. More specifically, the food groups identified as the major contributors to Pb exposure are shown in Table 3.3. Non-dietary exposure to Pb is likely to be of minor importance for the general population in the European Union. House dust and soil can be important sources of exposure to Pb for children [97]. The available evidence for women of childbearing age and vegetarians does not indicate dietary exposure different from that of the general adult population [97].

The absorbed fraction in subjects ranges between 37–70% (radiotracer experiments) and 15–20% (stable isotope experiments). Details of the mechanism of absorption remain to be determined. Absorbed Pb is transported in the blood primarily within erythrocytes, and is then transferred to soft tissues, including the liver and kidneys. Pb can accumulate with age in the bone tissue. From the skeleton, it is released gradually back into the bloodstream, particularly during physiological or pathological periods of bone demineralization such as pregnancy, lactation and osteoporosis, even when Pb exposure has already ceased. Pb can be transferred from the mother to the fetus/infant *in utero* and through breast milk. Half-lives for inorganic Pb in blood and bone are approximately 30 days and 10–30 years, respectively, and excretion is primarily in urine and stools. Pb in blood is considered the best indicator of the concentration of Pb in soft tissues, reflecting recent and, to some extent, past exposure, whereas bone Pb *in vivo* reflects long-term uptake and body burden [97].

Absorption of Pb from the gastrointestinal tract depends on host characteristics (e.g. age, fasting, nutritional Ca and Fe status, pregnancy) and on characteristics of the particles (size, solubility and Pb species). Studies in animals provide additional evidence for age dependency of the gastrointestinal absorption of Pb. Absorption of ingested soluble Pb compounds appears to be higher in children than in adults. In this sense, adults absorb 7–15% of the Pb in the diet, compared with 35–40% in the case of nursing infants and children [74, 97].

Absorption is lower in the presence of food. In the same way as for Cd, Pb absorption is influenced by interactions with minerals and vitamins. An inverse relationship has been observed between low Ca or Fe intake and deficient Fe status and blood Pb content. In rats, Fe deficiency increases Pb absorption, possibly by enhancing its binding to Fe binding carriers, and Pb absorption is enhanced by dietary Ca depletion or the administration of vitamin D. Lactose has a limited effect, whereas lactoferrin may cause an increase in absorption. Similar mechanisms may contribute to Pb–Fe and Pb–Ca absorption interactions and to interactions between Pb and other divalent cations such as Cd, Cu, Mg and Zn [97].

The processes that control the dissolution rate are important in relation to the bioavailability of Pb via the oral route, since bioavailability is dependent on solubility during the limited period of transit through the gastrointestinal tract. The area–volume ratio is greater for small particles than larger particles, and this favours their solubility, bioaccessibility and bioavailability. At the acid pH of the stomach, Pb compounds that are formed in an acid medium (e.g. lead sulfate) tend to be more stable and less bioaccessible than those formed in alkaline media (e.g. lead carbonate or oxide) [74].

Some examples of studies used in *in vitro* gastrointestinal digestion techniques to establish preliminary assessments regarding As, Hg, Cd and Pb bioavailability are shown in Table 3.4.

Abbreviations

AAS: Atomic absorption spectrometry
AB: Arsenobetaine
AC: Arsenocholine
Cd-MT: Cd-metallothionein
DMA: Dimethylarsenic
DMeHg: Dimethylmercury
DHA: Docosahexaenoic acid
DMEM: Dulbecco's modified Eagle medium
EFSA: European Food Safety Authority
ESI Q-TOF MS: Electrospray tandem mass spectrometry
FI-HG-AAS: Flow injection hydride generation atomic absorption spectrometry
GF-AAS: Graphite furnace atomic absorption spectrometry
GPX: Glutathione peroxidase
HgTu/LC-CVAFS: Liquid chromatography cold vapour atomic fluorescence spectrometry
HPLC-AAS: High performance liquid chromatography atomic absorption spectrometry
HPLC-HG-AFS: High performance liquid chromatography hydride generation atomic fluorescence spectrometry
HPLC-ICP-MS: High performance liquid chromatography inductively coupled plasma mass spectrometry
HPP: High pressure processing
IARC: International Agency for Cancer Research
ICP-MS: Inductively coupled plasma mass spectrometry
LC-ICP-MS: Liquid chromatography inductively coupled plasma mass spectrometry
MALDI TOF MS: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MeHg: Methylmercury
MLs: Maximum levels
MMA: Monomethylarsenic
MT: Metallothionein
NRC: National Research Council
SeCys: Selenocysteine
SeMCS: Se-methylselenocysteine
SeMet: Selenomethionine
SeOMet: Selenoximethionine
SHIME: Simulated human intestinal microbial ecosystem
SRM: Standard reference material
TMAO: Trimethylarsine oxide
TWI: Tolerable weekly intake

References

1 Guerra A, Etienne-Mesmin L, Livrelli V, Denis S, Blanquet-Diot S, Alric M. Relevance and challenges in modeling human gastric and small intestinal digestion. *Trends Biotechnol* 2012;**30**:591–600.
2 Van Campen DR, Glahn RP. Micronutrient bioavailability techniques: accuracy, problems and limitations. *Field Crops Res* 1999;**60**:93–113.
3 Fernández-García E, Carvajal-Lérida I, Pérez-Gálvez A. *In vitro* bioaccessibility assessment as a prediction tool of nutrient efficiency. *Nutr Res* 2009;**29**:751–760.
4 Wienk KJH, Marx JJM, Beynen AC. The concept of iron bioavailability and its assessment. *Eur J Nutr* 1999;**38**: 51–75.
5 Watzke HJ. Impact of processing on bioavailability: examples of minerals in foods. *Trends Food Sci Technol* 1998;**9**: 320–327.
6 Viadel B, Barberá R, Farré R. Estimación *in vitro* de la biodisponibilidad mineral de alimentos. *Nutr Clín Diet Hosp* 2004;**24**:9–16.
7 Frontela C, Scarino ML, Ferruzza S, Ros G, Martínez C. Effect of dephytinization on bioavailability of iron, calcium and zinc from infant cereals assessed in the Caco-2 cell model. *World J Gastroenterol* 2009;**15**:1977–1984.
8 Viadel B, Barberá R, Farré R. Effect of cooking and legume species upon calcium, iron and zinc uptake by Caco-2 cells. *J Trace Elem Med Biol* 2006;**20**:115–120.
9 Hemalatha S, Platel K, Srinivasan K. Influence of heat processing on the bioaccessibility of zinc and iron from cereals and pulses consumed in India. *J Trace Elem Med Biol* 2007;**21**:1–7.
10 Maulvault AL, Machado R, Afonso C *et al*. Bioaccessibility of Hg, Cd and As in cooked black seabbard fish and edible crab. *Food Chem Toxicol* 2011;**49**:2808–2815.
11 Torres-Escribano S, Ruiz A, Barrios L, Vélez D, Montoro R. Influence of mercury bioaccessibility on exposure assessment associated with consumption of cooked predatory fish in Spain. *J Sci Food Agric* 2011;**91**:981–986.
12 Cilla A, Lagarda MJ, Alegría A *et al*. Effect of processing and food matrix on calcium and phosphorus bioavailability from milk-based fruit beverages in Caco-2 cells. *Food Res Int* 2011;**44**:3030–3038.
13 Briones-Labarca V, Muñoz C, Maureira H. Effect of high pressure on antioxidant capacity, mineral and starch bioaccessibility of a non conventional food: Prosopis chilensis seed. *Food Res Int* 2011;**44**:875–883.
14 Briones-Labarca V, Venegas-Cubillos G, Ortiz-Portilla S, Chacana-Ojeda M, Maureira H. Effects of high hydrostatic pressure (HPP) on bioaccessibility, as well as antioxidant activity, mineral and starch contents in Granny Smith apple. *Food Chem* 2011;**128**:520–529.
15 Fairweather-Tait FJ, Dainty J. Use of stable isotopes to assess the bioavailability of trace element: a review. *Food Addit Contam* 2002;**19**:939–947.

16 Sandström B, Fairweather-Tait S, Hurrell R, Van Dokkum W. Methods for studying mineral and trace element absorption in humans using stable isotopes. *Nutr Res Rev* 1993;**6**: 71–95.
17 Mellon GA, Fairweather-Tait SJ. Stable isotope methods for studying nutrient mineral metabolism in humans. *Endeavour* 1997;**21**:12–18.
18 Dainty JR. Use of stable isotopes and mathematical modeling to investigate human mineral metabolism. *Nutr Res Rev* 2001;**14**:295–315.
19 Griffin IJ. Using stable isotope and isotope ratio mass spectrometry to study mineral metabolism in humans. *J Anal Atom Spectrom* 2002;**17**:1186–1193.
20 Zheng JJ, Mason JB, Rosenberg IH, Wood RJ. Measurement of zinc bioavailability from beef and a ready-to eat high fiber breakfast cereals in humans: application of a whole-gut lavage technique. *Am J Clin Nutr* 1993;**58**:902–907.
21 Moreda-Piñeiro J, Moreda-Piñeiro A, Romarís-Hortas V *et al*. *In-vivo* and *in-vitro* testing to assess the bioaccessibility and the bioavailability of selenium and mercury species in food samples. *Trends Anal Chem* 2011;**30**:324–345.
22 Narasinga Rao BS, Prabhavathi T. An *in vitro* method for predicting the bioavailability of iron foods. *Am J Clin Nutr* 1978;**31**:169–175.
23 Miller DD, Schricker BR, Van Campen DR. An in vitro method for estimation of iron availability from meals. *Am J Clin Nutr* 1981;**34**:2248–2256.
24 Minekus M, Marteau P, Havenaar R, Huis in't Veld JHJ. A multicompartmental dynamic computer-controlled model simulating the stomach and small intestine. *ATLA* 1995;**23**:19–209.
25 Ekmekcioglu C. A physiological approach for preparing and conducting intestinal bioavailbility studies using experimental systems. *Food Chem* 2002;**76**:225–230.
26 Intawongse M, Dean JR. *In-vitro* testing for assessing oral bioaccesibility of trace metals in soil and food samples. *Trends Anal Chem* 2006;**25**:876–886.
27 Miller DD, Berner LA. Is solubility in vitro a reliable predictor of iron bioavilability? *Biol Trace Elem Res* 1989;**19**: 11–24.
28 Glahn RP, Wien EM, Van Campen DR, Miller DD. Caco-2 cell iron uptake from meat and casein digests parallels *in vivo* studies: use of a novel *in vitro* method for rapid estimation of iron bioavailability. *J Nutr* 1996;**126**:332–339.
29 Glahn RP, Lee OA, Yeung A, Goldman MI, Miller DD. Caco-2 cell ferritin formation predicts nonradiolabeled food iron availability in an *in vitro* digestion/Caco-2 cell culture model. *J Nutr* 1998;**128**:1555–1561.
30 Hur SJ, Lim BO, Decker EA, McClements DJ. *In vitro* human digestion models for food applications. *Food Chem* 2011;**125**:1–12.
31 Viadel B, Barberá R, Farré R. Uptake and retention of calcium, iron, and zinc from raw legumes and the effect of cooking on lentils in Caco-2 cells. *Nutr Res* 2006;**26**: 591–596.
32 Fairweather-Tait S, Lynch S, Hotz C *et al*. The usefulness of *in vitro* models to predict the bioavailability of iron and zinc: a consensus statement from the harvestplus expert consultation. *Int J Vitam Nutr Res* 2005;**75**:371–374.
33 Langerholc T, Maragkoudakis PA, Wollgast J, Gradisnik L, Cencic A. Novel and established intestinal cell line models: an indispensable tool in food science and nutrition. *Trends Food Sci Technol* 2011;**22**:S11–S20.
34 Miller GD, Jarvis JK, McBean LD. Review: The importance of meeting calcium needs with foods. *J Am Coll Nutr* 2001;**20**:168–185.
35 Nicklas TA. Review: Calcium intake trends and health consequences from childhood through adulthood. *J Am Coll Nutr* 2003;**22**:340–356.
36 Guéguen L, Pointillart A. The bioavailability of dietary calcium. *J Am Coll Nutr* 2000;**19**:119S–136S.
37 Allen LH. Calcium bioavailability and absorption: a review. *Am J Clin Nutr* 1982;**35**:783–808.
38 Fairweather-Tait SJ, Teucher B. Iron and calcium bioavailability of fortified foods and dietary supplements. *Nutr Rev* 2002;**60**:360–367.
39 Rafferty K, Walters G, Heaney RP. Calcium fortificants: overview and strategies for improving calcium nutriture of the US population. *J Food Sci* 2007;**72**:152–158.
40 Amaro MA, Cámara F. Iron availability: an updated review. *Int J Food Sci Nutr* 2004;**55**:597–606.
41 de Benoist B, McLean E, Egli I, Cogswell M. *Worldwide prevalence of anaemia* 1993–2005. World Health Organization, Geneva, 2008. Available at http://whqlibdoc.who.int/publications/2008/9789241596657_eng.pdf.
42 Hurrell R, Egli I. Iron bioavailability and dietary reference values. *Am J Clin Nutr* 2010;**91**:1461S–1467S.
43 Heath ALM, Fairweather-Tait SJ. Clinical implications of changes in the modern diet: iron intake, absorption and status. *Best Pract Res Clin Haematol* 2002;**15**:225–241.
44 Rehman SU, Huma N, Tarara OM, Shah WH. Efficacy of non-heme iron fortified diets: a review. *Crit Rev Food Sci Nutr* 2010;**50**:403–413.
45 Martínez-Navarrete N, Camacho MM, Martínez-Lahuerta J, Martínez-Monzó J, Fito P. Iron deficiency and iron fortified foods: a review. *Food Res Int* 2002;**15**:225–231.
46 Salgueiro MJ, Zubillaga M, Lysionek A, Caro R, Weill R, Boccio J. Fortification strategies to combat zinc and iron deficiency. *Nutr Rev* 2002;**60**:52–58.
47 Hilty FM, Teleki A, Krumeich F *et al*. Development and optimization of iron- and zinc-containing nanostructured powders for nutritional applications. *Nanotechnology* 2009;**20**:1–11.
48 Fidler MC, Walczyk T, Davidsson L *et al*. A micronised, dispersible ferric pyrophosphate with high relative bioavailability in man. *Br J Nutr* 2004;**91**:107–112.
49 Salgueiro MJ, Zubillaga M, Lysionek A *et al*. Zinc as an essential micronutrient: a review. *Nutr Res* 2000;**20**:737–755.
50 Akhta S, Anjum FM, Anjum MA. Micronutrient fortification of wheat flour: recent development and strategies. *Food Res Int* 2011;**44**:652–659.

51 Cámara F, Amaro MA. Nutritional aspect of zinc availability. *Int J Food Sci Nutr* 2003;**54**:143–151.

52 Thiry C, Ruttens A, De Temmerman L, Schneider YJ, Pussemier L. Current knowledge in species-related bioavailability of selenium in food. *Food Chem* 2012;**130**:767–784.

53 Allen L, de Benoist B, Dary O, Hurrell R. *Guidelines on food fortification with micronutrients*. World Health Organization. Geneva, 2006. Available at http://whqlibdoc.who.int/publications/2006/9241594012_eng.pdf?ua=1.

54 Vale G, Rodrigues A, Rocha A *et al*. Ultrasonic assisted enzymatic digestion (USAED) coupled with high performance liquid chromatography and electrothermal atomic absorption spectrometry as a powerful tool for total selenium and selenium species control in Se-enriched food supplements. *Food Chem* 2010;**121**:268–274.

55 Finley JW. Review: Increased intakes of selenium-enriched foods may benefit human health. *J Sci Food Agric* 2007;**87**:1620–1629.

56 Rayman MP, Goenaga Infante H, Sargent M. Food-chain selenium and human health: spotlight on speciation. *Br J Nutr* 2008;**100**:238–253.

57 Fairweather-Tait SJ, Collings R, Hurst R. Selenium bioavailability. Current knowledge and future research requirements. *Am J Clin Nutr* 2010;**91**:1484S–1491S.

58 Kápolna E, Fodor P. Bioavailability of selenium from selenium-enriched green onions (Allium fistulosum) and chives (Allium schoenoprasum) after '*in vitro*' gastrointestinal digestion. *Int J Food Sci Nutr* 2007;**58**:282–296.

59 Cabañero AI, Madrid Y, Cámara C. Selenium and mercury bioaccessibility in fish samples: an *in vitro* digestion method. *Anal Chim Acta* 2004;**526**:51–61.

60 Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF.

61 International Agency for Cancer Research. *Some Drinking-water Disinfectants and Contaminants, Including Arsenic. Summary of Data Reported and Evaluation*. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. **84**. IARC, Lyon, 2004. Available at http://monographs.iarc.fr/ENG/Monographs/vol84/.

62 International Agency for Cancer Research. Arsenic and arsenic compounds. In: *A Review of Human Carcinogens: Arsenic, Metals, Fibres, and Dusts*. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. **100**C. IARC, Lyon, 2012. Available at http://monographs.iarc.fr/ENG/Monographs/vol100C/mono100C-6.pdf (accesed July 2013).

63 Suñer MA, Devesa V, Clemente MJ *et al*. Organoarsenical species contents in fresh and processed seafood products. *J Agric Food Chem* 2002;**50**:924–932.

64 Muñoz O, Devesa V, Suñer MA *et al*. Total and inorganic arsenic in fresh and processed fish products. *J Agric Food Chem* 2000;**48**:4369–4376.

65 Bae M, Watanabe C, Inaoka T *et al*. Arsenic in cooked rice in Bangladesh. *Lancet* 2002;**360**:1839–1840.

66 Muñoz O, Diaz OP, Leyton I *et al*. Vegetables collected in the cultivated Andean area of northern Chile: total and inorganic arsenic contents in raw vegetables. *J Agric Food Chem* 2002;**50**:642–647.

67 Das HK, Mitra AK, Sengupta PK, Hossain A, Islam F, Rabbani GH. Arsenic concentrations in rice, vegetables, and fish in Bangladesh: a preliminary study. *Environ Int* 2004;**30**:383–387.

68 European Food Safety Authority. Scientific opinion on arsenic in food. EFSA Panel on Contaminants in the Food Chain (CONTAM). *EFSA J* 2009;**7**:1351–1448.

69 Agency for Toxic Substances and Disease Registry. *Toxicological Profile for Arsenic*. US Department of Health and Human Services, Public Health Service, Atlanta, GA, 2007. Available at http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=22&tid=3.

70 Juhasz AL, Smith E, Weber J *et al*. Application of an *in vivo* swine model for the determination of arsenic bioavailability in contaminated vegetables. *Chemosphere* 2008;**71**:1963–1969.

71 Juhasz AL, Smith E, Weber J *et al*. *In vivo* assessment of arsenic bioavailability in rice and its significance for human health risk assessment. *Environ Health Perspect* 2006;**114**:1826–1831.

72 Tice RR, Yager IW, Andrews P, Crecelius E. A chemical hypothesis for arsenic methylation in mammals. *Chem Biol Interact* 1997;**88**:89–114.

73 Oomen AG, Hack A, Minekus M *et al*. Comparison of five *in vitro* digestion models to study the bioaccessibility of soils contaminants. *Environ Sci Technol* 2002;**36**:3326–3334.

74 Ruby MV, Schoof R, Brattin W *et al*. Advances in evaluating the oral bioavailability of inorganics in soil for use in human health risk assessment. *Environ Sci Technol* 1999;**33**:3697–3705.

75 Roychowdhury T, Tokunaga H, Ando M. Survey of arsenic and other heavy metals in food composites and drinking water and estimation of dietary intake by the villagers from an arsenic-affected area of West Bengal, India. *Sci Total Environ* 2003;**308**:15–35.

76 Gochfeld M. Cases of mercury exposure, bioavailability, and absorption. *Ecotoxicol Environ Saf* 2003;**56**:174–179.

77 European Food Safety Authority. Scientific opinion on the risk for public health related to the presence of mercury and methylmercury in food, EFSA Panel on Contaminants in the Food Chain (CONTAM). *EFSA J* 2012;**10**:2985.

78 FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization). *Report of the Joint FAO/WHO Expert Consultation on the Risks and Benefits of Fish Consumption*. FAO Fisheries and Aquaculture Report No. 978. FAO, Rome, 2011. Available at http://www.fao.org/docrep/015/ba0136e/ba0136e00.htm.

79 Carrier G, Bouchard M, Brunet RC, Caza M. A toxicokinetic model for predicting the tissue distribution and elimination

of organic and inorganic mercury following exposure to methyl mercury in animals and humans. II. Application and validation of the model in humans. *Toxicol Appl Pharmacol* 2001;**171**:50–60.

80 Smith JC, Allen PV, Turner MD, Most B, Fisher FL, Hall LL. The kinetics of intravenously administered methylmercury in man. *Toxicol Appl Pharmacol* 1994;**128**:251–256.

81 Young JF, Wosilait WD, Luecke RH. Analysis of methylmercury disposition in humans utilizing a PBPK model and animal pharmacokinetic data. *J Toxicol Environ Health* 2001;**63**:19–52.

82 Echeverria D, Aposhian HV, Woods JS, Heyer NJ, Aposhian MM, Bittner ACJR. Neurobehavioral effects from exposure to dental amalgam Hg: new distinctions between recent exposure and Hg body burden. *FASEB J* 1998;**12**: 9711–9980.

83 Dolbec J, Mergler D, Sousa Passos CJ, Sousa de Morass S, Lebel J. Methylmercury exposure affects motor performance of a riverine population of the Tapajòs river, Brazilian Amazon. *Int Arch Occup Environ Health* 2000;**73**:95–203.

84 Lebel J, Mergler D, Branches F, Lucotte M, Amorim M, Larribe F. Neurotoxic effects of low-level methylmercury contamination in the Amazonian basin. *Environ Res* 1998; **79**:20–32.

85 Carta P, Flore C, Alivoni R *et al*. Sub-clinical neurobehavioral abnormalities associated with low level of mercury exposure through fish consumption. *Neurotoxicology* 2003;**24**:617–623.

86 Ouédraogo O, Amyot M. Effects of various cooking methods and food components on bioaccessibility of mercury from fish. *Environ Res* 2011;**111**:1064–1069.

87 Groten JP, van Bladeren PJ. Cadmium bioavailability and health risk in food. *Trends Food Sci Technol* 1994;**5**:50–55.

88 European Food Safety Authority. Cadmium in food. Scientific opinion of the Panel on Contaminants in the Food Chain. *EFSA J* 2009;**980**:1–139.

89 McLaughlin MJ, Parker DR, Clarke JM. Metals and micronutrients: food safety issues. *Field Crops Res* 1999;**60**: 143–163.

90 Jonnalagadda SB, Prasada Rao PVV. Toxicity, bioavailability and metal speciation. *Comp Biochem Physiol* 1993;**3**: 585–595.

91 Eklund G, Linden A, Tallkvist J, Oskarsson A. Bioavailability of cadmium from *in vitro* digested infant food studied in Caco-2 cells. *J Agric Food Chem* 2003;**51**:4168–4174.

92 Asagba SO. Role of diet in absorption and toxicity of oral cadmium. A review of literature. *African J Biotechnol* 2009; **8**:7428–7436.

93 Satarug S, Garrett S, Sens MA, Sens DA. Cadmium, environmental exposure and health outcomes. *Environ Health Perspect* 2010;**118**:182–190.

94 Reeves PG, Chaney RL. Marginal nutritional status of zinc, iron and calcium increases cadmium retention in the duodenum and other organs of rats fed rice-based diets. *Environ Sci Technol* 2004;**96**:311–322.

95 Reeves PG, Chaney RL. Bioavailability as an issue in risk assessment and management of food cadmium: a review. *Sci Total Environ* 2008;**398**:13–19.

96 Larsen EH, Andersen NL, Moller A, Petersen A, Mortensen GK, Petersen J. Monitoring the content and intake of trace elements from food in Denmark. *Food Addit Contam* 2002;**19**:33–46.

97 European Food Safety Authority. Scientific opinion on lead in food. EFSA Panel on Contaminants in the Food Chain (CONTAM). *EFSA J* 2010;**8**:1570–1621.

98 Cámara F, Amaro MA, Barberá R, Lagarda MJ. Speciation of bioaccessible (heme, ferrous and ferric) iron from school menus. *Eur Food Res Technol* 2005;**221**:768–773.

99 Wortley G, Leusner S, Good C, Gugger E, Glahn R. Iron availability of a fortified processed wheat cereal. A comparison of fourteen iron forms using an *in vitro* digestion/human colonic adenocarcinoma (CaCo-2) cell model. *Br J Nutr* 2005;**93**:65–71.

100 Sorensen AD, Sorensen H, Sondergaard I, Bukhave K. Non-haem iron availability from pork meat: impact of heat treatments and meat protein dose. *Meat Sci* 2007;**76**: 29–37.

101 Sebastiá V, Barberá R, Farré R, Lagarda MJ. Effects of legume processing on calcium, iron and zinc contentsand dialysabilities. *J Sci Food Agric* 2001;**81**:1180–1185.

102 Perales S, Barberá R, Lagarda MJ, Farré R. Fortification of milk with calcium: effect on calcium bioavailability and interactions with iron and zinc. *J Agric Food Chem* 2006; **54**:4901–4906.

103 Drago SR, Binaghi MJ, Valencia ME. Effect of gastric digestion pH on iron, zinc, and calcium dialyzability from preterm and term starting infant formulas. *J Food Sci* 2005; **70**:107–112.

104 Pedrero Z, Madrid Y, Cámara C. Selenium species bioaccessibility in enriched radish (Raphanus sativus). A potential dietary souce of selenium. *J Agric Food Chem* 2006;**54**: 2412–2417.

105 Hinojosa Reyes L, Ruiz Encinar J, Marchante-Gay JM, García Alonso JE, Sanz-Medel A. Selenium bioaccessibility assessment in selenized yeast after '*in vitro*' gastrointestinal digestion using two-dimensional chromatography and mass spectrometry. *J Chromatogr A* 2006;**1110**: 108–116.

106 Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. http://europa.eu/legislation_summaries/environment/water_protection_management/l28079_en.htm.

107 Directive 2003/40/EC of 16 May 2003 establishing the list, concentration limits and labelling requirements for the constituents of natural mineral waters and the conditions for using ozone-enriched air for the treatment of natural mineral waters and spring waters.http://ec.europa.eu/food/food/labellingnutrition/water/index_en.htm.

108 Chowdhury UK, Rahman MM, Mandal BK *et al*. Groundwater arsenic-contamination and human sufferings

in West Bengal, India and Bangladesh. *Environ Sci* 2001;**8**: 393–415.

109 Roychowdhury T, Uchino T, Tokunaga H, Ando M. Survey of arsenic in food composites from an arsenic-affected area of West Bengal, India. *Food Chem Toxicol* 2002;**40**:1611–1621.

110 Jalon M, Urieta I, Macho ML, Azpiri M. *Metales Pesados y Arsénico*. En: Vigilancia de la Contaminación Química de los Alimentos en la Comunidad Autónoma del País Vasco 1990–1995. Vitoria-Gazteiz, Servicio Central de Publicaciones del Gobierno Vasco, 1997, pp. 29–43.

111 Llobet JM, Falcó G, Casas C, Teixidó A, Domingo JL. Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain. *J Agric Food Chem* 2003;**51**:838–842.

112 Tsuda T, Inoue T, Kojima A, Aoki S. Market basket and duplicate portion estimation of dietary intakes of cadmium, mercury, arsenic, copper, manganese and zinc by Japanese adults. *J AOAC Int* 1995;**78**:1363–1368.

113 International Agency for Cancer Research. *Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Vols 1–42*. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Suppl. 7. IARC, Lyon, 1987.

114 World Health Organization. *Evaluation of certain food additives and contaminants. 33rd report of the Joint FAO/WHO Expert Committee on Food Additives*. WHO Technical Report Series 776. WHO, Geneva, 1989.

115 Caussy D, Gochfeld M, Gurzau E, Neagu C, Ruedel H. Lessons from case studies of metals: investigating exposure, bioavailability, and risk. *Ecotoxicol Environ Saf* 2003;**56**: 45–51.

116 Council Regulation (EEC) No. 315/93 of 8 February 1993 laying down Community procedures for contaminants in food. Available at http://europa.eu/legislation_summaries/food_safety/contamination_environmental_factors/l21113_en.htm

117 Commission Regulation (EC) No. 629/2008 of 2 July 2008 amending Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs. Available at http://www.iss.it/binary/lcdr2/cont/Regulation%20629_2008.1215519858.pdf

118 National Research Council. *Toxicological Effects of Methylmercury*. Committee on the Toxicological Effects of Methylmercury, Board on Environmental Studies and Toxicology, Commission on Life Sciences, National Research Council, National Academy Press, Washington, DC, 2000.

119 Joint FAO/WHO Expert Committee on Food Additives. *Sixty-first Scientific Meeting*, Rome, 10–19 June 2003. Available at ftp://ftp.fao.org/es/esn/jecfa/jecfa61sc.pdf.

120 Joint FAO/WHO Expert Committee on Food Additives. Methylmercury. In: *Safety Evaluation of Certain Contaminants in Food*. WHO Food Additives Series, 63. FAO, Rome/WHO, Geneva, 2011, pp. 605–684. Available at http://whqlibdoc.who.int/publications/2011/9789241660631_eng.pdf.

121 International Agency for Cancer Research. *Beryllium, Cadmium, Mercury, and Exposures in the Glass Manufacturing Industry*. Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. **58**. IARC, Lyon, 1993.

122 International Agency for Cancer Research. *Inorganic and Organic Lead Compounds*. Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. **87**. IARC, Lyon, 2006.

123 World Health Organization. *Safety evaluation of certain food additives and contaminants: lead*. WHO Food Additives Series, 44. WHO, Geneva, 2000. Available at http://www.inchem.org/documents/jecfa/jecmono/v44jec12.htm (accesed July 2013).

124 Laparra JM, Velez D, Montoro R, Barberá R, Farré R. Estimation of arsenic bioaccessibility in edible seaweed by an *in vitro* digestion method. *J Agric Food Chem* 2003;**51**: 6080–6085.

125 Laparra JM, Vélez D, Barberá R, Farré R, Montoro R. Bioaccessibility of inorganic As species in raw and cooked Hizikia fusiforme seaweed. *Appl Organomet Chem* 2004;**18**: 662–669.

126 Dufailly V, Guérin T, Noël L, Frémy JM, Beauchemin D. A simple method for the speciation analysis of bio-accessible arsenic in seafood using on-line continuous leaching and ion exchange chromatography coupled to inductively coupled plasma mass spectrometry. *J Anal Atom Spectrom* 2008; **23**:1263–1268.

127 Cabañero A, Madrid Y, Cámara C. Mercury–selenium species ratio in representative fish samples and their bioaccessibility by an *in vitro* digestion method. *Biol Trace Elem Res* 2007;**119**:195–211.

128 Laird BD, Shade C, Gantner N, Chan HM, Siciliano SD. Bioaccessibility of mercury from traditional northern country foods measured using an *in vitro* gastrointestinal model is independent of mercury concentration. *Sci Total Environ* 2009;**407**:6003–6008.

129 Azenha MAGO, Vasconcelos MTSD. Assessment of the Pb and Cu *in vitro* availability in wines by means of speciation procedures. *Food Chem Toxicol* 2000;**38**:899–912.

130 Torres-Escribano S, Blanquet-Diot S, Denis S *et al*. Comparison of a static and a dynamic *in vitro* model to estimate the bioaccessibility of As, Cd, Pb and Hg from food reference materials Fucus sp. (IAEA-140/TM) and Lobster hepatopancreas (TORT-2). *Sci Total Environ* 2011;**409**: 604–611.

CHAPTER 4

Human risk assessment and regulatory framework for minerals in food

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Abstract: There is a huge body of evidence showing that heavy metals are important environmental pollutants that are related to diverse health outcomes. Although there are various sources and pathways for human exposure, the diet is the main route for the more relevant toxic metals. For elements such as Cd, Pb, As, Hg, Ni, Sn, V, Se and Mo, some international agencies have established health-based guidance values, including tolerable daily intake or reference dose, that are used for risk characterization. One of the more relevant methodologies for exposure assessment to metals are total diet studies, which can be performed at regional or national level and reveal exposures to heavy metals from the main food groups through the combination of concentration and consumption data. Apart from this external exposure methodology, human biomonitoring is an emergent approach that delivers information about the internal dose and is increasingly used for risk characterization. Assessment of the risk from heavy metals provides scientific support for many risk management activities including legislation, monitoring and warning systems.

Keywords: metals, dietary exposure, total diet studies, risk assessment, human biomonitoring, legislation

4.1 Introduction

The general population is exposed to a range of metals through food, the water supply and other environmental matrices. The human risk assessment of exposure to metals usually follows the classical approach of estimating the external exposure and comparing it with health-based guidance values (HBGVs) [1]. Apart from this conventional approach to assessing exposure, there is a growing interest in evaluating exposures to environmental chemicals using biomonitoring data (internal exposure approach), so current relevant efforts are being conducted to evaluate the presence of these compounds in biological specimens (urine, blood, breast milk) [2]. Figure 4.1 shows this dual approach to exposure and risk assessment of chemicals.

The diet is the main route of exposure to the more relevant toxic metals and metalloids such as As, Be, Cd, Cr(VI), Pb and Hg. Similarly, food is a major route of exposure to minor toxic metals, namely Sb, Ba, Tl, Sn or U, and to essential or probable essential trace elements with potential toxicity at excess intakes such as Co, Cu, Fe, Mn, Mo, Ni, Se, Cr(III), Zn and V. Over the last decades the scientific community and different international and national agencies have developed methodologies [3, 4] and have been setting HBGVs [5] to protect the general population against potential risks associated with exposure to toxic metals and other relevant toxic chemicals.

The aim of this chapter is to provide an overview of the risk assessment of toxic elements for which the diet is a predominant or major route of exposure. This includes an outline of the different steps of the risk assessment for metals, including hazard identification and characterization, exposure through foods, and risk characterization. Likewise, the use of biomonitoring in a risk-assessment context is introduced, with a discussion on biomarker characterization and a summary of the

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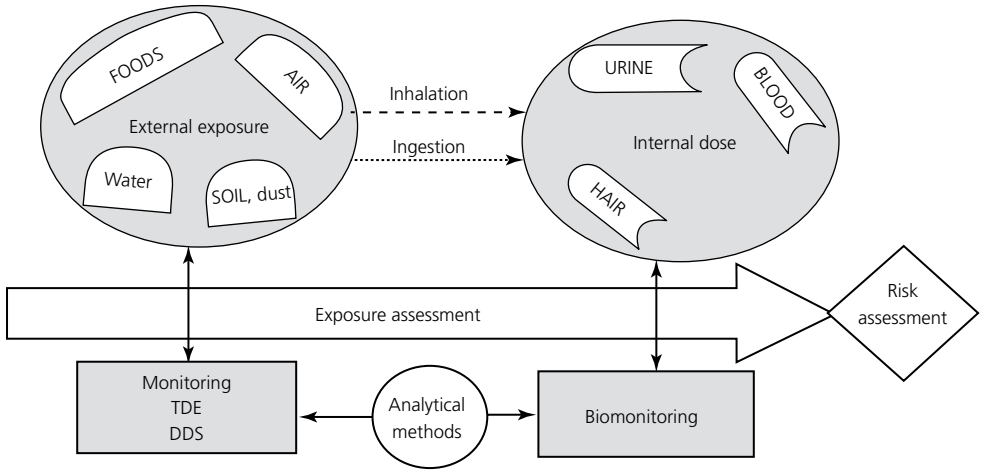


Figure 4.1 Internal and external approach for human risk assessment to chemicals. TDS, total diet studies; DDS, duplicate diet studies.

major biomonitoring programmes. Finally, a summary on risk management, including up-to-date information on the legal regulation of trace elements in food, is provided.

4.2 Dietary exposure and risk assessment of trace elements

There is currently an international acceptance to consider risk analysis as the most effective tool for dealing with food safety issues and for improving food control systems [3]. Risk analysis has been defined by the Codex Alimentarius Commission (CAC) as 'a process consisting of three components: risk assessment, risk management and risk communication' [4]. Risk assessment of food chemicals aims to characterize the potential hazards and the associated risk to life and health resulting from the exposure of humans to chemicals present in food. Risk assessment is a central scientific component of the risk analysis paradigm, and has been divided into the following steps: hazard identification, hazard characterization, exposure assessment and risk characterization [6].

4.2.1 Hazard identification and characterization

Various international agencies such as the World Health Organization (WHO) [7], the United States Environmental Protection Agency (USEPA) [1], the European Food Safety Authority (EFSA) [8] and the Agency for Toxic Substances and Disease Registry (ATSDR) [9] are evaluating the weight of evidence for the adverse health effects of metals (hazard identification). Hazard characterization (dose–response assessment) aims to identify the reference point or point of departure (NOAEL, LOAEL or BMDL) and to establish HBGVs [10]. Both toxicological studies in animals [11] and epidemiological studies (more frequently available for the most toxic heavy metals such as Cd, Hg and Pb and metalloids such as As) [12] are used for setting up HBGVs for the most sensitive end points (e.g. reproductive toxicity, neurotoxicity, lung cancer). Yet a threshold approach has usually been applied for assessment of non-cancer end points. For example, for metals such as Cd, Be, Mn, Se or Mo, it is assumed that a threshold level of exposure exists below which no significant health effects are observed.

Many terms and definitions have been proposed for the HBGV developed from the dose–response relationship in toxicological studies. The most frequent threshold values used in Europe and by the WHO include acceptable daily intake (ADI), tolerable intake (TI), which may in turn be daily (TDI) or weekly (TWI), and provisional tolerable weekly intake (PTWI) [13]. However, the USEPA has replaced ADI and TDI with the term 'reference dose' (RfD) [14]. Other terms used include minimal risk level (MRL) used by the ATSDR [15], and tolerable upper level intake (UL) [16], the latter used for nutrients. These HBGVs are derived by applying uncertainty factors (UFs) to the point of departure [17]. Table 4.1

Table 4.1 Health-based guidance values for elements.

Element	Agency	POD/UF	HBGV	Reference
As-i	FAO/WHO	$BMDL_{05}$: 0.3 μg/kg b.w. daily	PTWI (withdrawn)	[25]
As-i	ATSDR/USEPA		MRL/RfD: 0.0003 mg/kg daily	[26]
As-i	EFSA	$BMDL_{01}$: 0.3–8 μg/kg b.w. daily	MOE	[24]
Ba	USEPA	$BMDL_{05}$/300	RfD: 0.2 mg/kg daily	[27]
Ba	ATSDR		MRL: 0.2 mg/kg daily	[28]
Be	ATSDR		MRL: 0.002 mg/kg daily	[29]
Be	WHO	$BMDL_{10}$ = NOAEL/300	TDI: 0.002 mg/kg b.w. daily	[30]
Cd	ATSDR	NOAEL/10	MRL:1×10^{-4} mg/kg daily	[31]
Cd	USEPA	NOAEL/10	RfD: 1×10^{-3} mg/kg daily	[32]
Cd	EFSA	$BMDL_{5}$	TWI:2.5 μg/kg b.w.	[5]
Cd	FAO/WHO	Reference Point	PTWI: 7 μg/kg b.w.	[7]
Co		POD/30	RfD: 0.03 mg/kg daily	[33]
Cr(VI)	ATSDR		MRL: 9×10^{-4} mg/kg daily	[34]
Cu	USEPA	NOAEL/2	UL: 5 mg/day	[35]
Cu	FAO/WHO		PMTDI: 0.5 mg/kg b.w.	[36]
Fe	FAO/WHO		PMTDI: 0.8 mg/kg b.w.	[37]
MeHg	EFSA	NOEL (46 μg Hg/L blood)/6.4	PTWI :1.3 μg/kg b.w., as Hg	[38]
i-Hg	EFSA	$BMDL_{10}$/100	PTWI : 4 μg/kg b.w., as Hg	[38]
Mn	USEPA	NOAEL (food)/1	RfD: 0.14 mg/kg daily	[39]
Mo	USEPA	LOAEL/30	RfD: 0.005 mg/kg daily	[40]
Mo	EFSA	NOAEL/100	UL: 0.01 mg/kg daily	[41]
Mg	EFSA	NOAEL/1	UL: 250 mg/day	[35]
Ni	WHO	LOAEL	TDI: 12 μg/kg b.w. daily	[42]
Ni	IOM	NOAEL/300	UL: 17 μg/kg b.w. daily	[16]
Pb	EFSA	$BMDL_{01}$	MOEs	[43]
Pb	WHO	Reference Point	PTWI (withdrawn)	[7]
Sb	USEPA-IRIS	LOAEL/1000	RfD: 2×10^{-4} mg/kg daily	[44]
Se	USEPA	NOAEL/3	RfD: 0.005 mg/kg daily	[45]
Se	EFSA	NOAEL/3	UL: 0.03 mg/day	[35]
Sn	ATSDR	NOAEL/100	MRL: 0.3 mg Sn/kg daily (as stannous chloride	[46]
Sn	FAO/WHO		PTWI: 14 mg/kg b.w.	[47]
Tl	USEPA	LOAEL/3000	RfD: 3×10^{-6} mg/kg daily	[48]
U	WHO	LOAEL (0.06 mg/kg b.w)/100	TDI: 0.6 μg/kg daily	[49]
V	USFNB		TUIL: 1.8 mg/day (adult)	[16, 50]
Zn	USEPA	NOAEL/3	RfD: 0.3 mg/kg daily	[51]

FAO, Food and Agricultural Organization; As-i, inorganic arsenic; b.w., body weight; POD, point of departure; UF, uncertainty factor; HBGV, human-based guidance value; TUIL, tolerable upper intake level; IOM, Food and Nutrition Board of the Institute of Medicine, USA; USFNB, United States Food Nutrition Board; MeHg, Methylmercury.

shows the main HBGVs established by different international food and environmental agencies for the most relevant food-related toxic elements.

Conversely, for cancer end points and for substances that are both genotoxic and carcinogenic, a non-threshold approach is being currently applied [18, 19]. There are several approaches for risk assessment of substances that are both genotoxic and carcinogenic [20–22]. EFSA recommends use of the margin of exposure (MOE) approach [23], which employs a reference point on a dose–reference curve taken from animal toxicological or human epidemiological studies. MOE is defined as the ratio of the reference point and human intake data.

The MOE approach has been used by EFSA in a recent risk assessment of inorganic As in the EU population. The PTWI established by the Joint WHO/FAO Expert Committee on Food Additives (JEFCA) was considered no longer appropriate because inorganic As causes

cancer of the lung, urinary bladder and skin. Using key epidemiological studies, EFSA has established a range of benchmark dose lower level confidence limit ($BMDL_{01}$) values between 0.3 and 8 μg/kg body weight per day for cancers of the lung, skin and bladder [24]. Consequently to estimate the dietary risk an assessment of MOE between the identified reference point from the human data and the estimated dietary exposure was proposed. Meanwhile JEFCA calculated a $BMDL_{05}$ of 3 μg/kg body weight per day based on data from an epidemiology study taking the increased incidence of lung cancer as a health end point [25]. On the other hand, the ATSDR has established an MRL for As of 0.0003 mg/kg body weight per day for chronic duration exposure (≥1 year) to inorganic arsenic [26].

The same MOE approach was applied by EFSA to the risk assessment of dietary Pb. The PTWI for Pb of 25 μg/kg body weight established by JEFCA, and endorsed by EFSA and other international agencies, was withdrawn because it could no longer be considered health protective [7]. Although it has not been possible to establish a new PTWI because there was no evidence of a threshold for a number of critical end points (cardiovascular effects and nephrotoxicity in adults, neurodevelopment effects in children), some reference points or BMDs have been estimated. In children a dietary exposure corresponding to a decrease of 1 point of the Intelligence Quotient (IQ) was estimated to be 0.6 μg/kg body weight per day. In adults a dietary exposure of 1.3 μg/kg body weight per day was estimated to be associated with an increase in systolic blood pressure (SBP) of 1 mmHg (critical end point) [7]. Likewise, EFSA also used epidemiological data to establish the 95th BMD of 1% extra risk ($BMDL_{01}$) as a reference point or point of departure for risk characterization of Pb. For adults, a $BMDL_{01}$ of 1.50 μg/kg body weight per day (SBP end point) and 0.63 μg/kg body weight per day for chronic kidney disease were estimated. In turn, a $BMDL_{01}$ of 0.50 μg/kg body weight per day has been established for children (reduction 1 point IQ) [8].

For Cd, a TWI of 2.5 μg/kg body weight was established by the EFSA's Panel on Contaminants in the Food Chain [52]. In 2010 the JECFA established a provisional tolerable monthly intake (PTMI) of 25 μg/kg body weight, which corresponds to a weekly intake of 5.8 μg/kg body weight [53]. Both assessments are based on the same human epidemiological data, but present some methodological differences [5]. The ATSDR has adopted an MRL of 1×10^{-4} mg/kg per day for chronic-duration oral exposure to Cd (≥1 year) that is similar to USEPA's RfD. The ATSDR uses the no-observed-adverse-effect level (NOAEL) to derive MRLs for Cd and other hazardous substances.

An oral TDI for Be of 0.002 mg/kg body weight per day has been reported by WHO derived from a toxicology study in dogs, applying a UF of 300. Although the International Agency for Research on Cancer (IARC) assigned Be to Group I (carcinogenic to humans), this relates to its carcinogenicity via inhalation, not via the oral route, so derivation of a quantitative cancer risk estimate for oral exposure has been precluded [30]. The ATSDR has also derived an MRL of 0.002 mg/kg body weight per day for chronic-duration oral exposures (≥1 year).

Methylmercury (MeHg) is the most toxic form of Hg present in foods, with significant presence mainly in fish and seafood. In a recent revision on the appropriateness of the JEFCA's PTWI of 1.6 μg/kg body weight [54], EFSA established a PTWI of 1.3 μg/kg body weight, expressed as Hg [38]. This HBGV was grounded on some epidemiological studies that considered the association between methylmercury exposure and the neurodevelopmental outcomes after prenatal exposure (end point) that provided a no-observed-effect level (NOEL) of about 11 mg/kg maternal hair. This value was converted into a maternal blood Hg concentration of 46 μg/L, and in the PTWI using a one-compartment toxicokinetic model and a total uncertainty value of 6.4. For inorganic Hg, JEFCA established a PTWI of 4 μg/kg body weight [25] that was considered appropriate by EFSA [38].

WHO has proposed a TDI for Ni of 12 μg/kg body weight, derived from a LOAEL established after oral provocation of fasted patients on an empty stomach [55].

The main forms of chromium are Cr(0), Cr(III), and Cr(VI). In epidemiological studies, an association has been found between exposure to Cr(VI) by the inhalation route and lung cancer. IARC has classified Cr(VI) in Group 1 (human carcinogen). The ATSDR have derived an MRL of 0.0009 μg/g body weight per day for chronic-duration (≥1 year) exposure to Cr(VI) [34].

The general population is exposed to Ba through consumption of drinking water and food. Following long-term exposure to Ba, nephrotoxicity was observed in rats and mice. Using benchmark dose modelling, a $BMDL_{05}$ of 63 mg/kg per day was selected as the point of departure by the US EPA [27]. To calculate the RfD, a UF of 300 was applied, obtaining an RfD of 0.2 mg/kg

per day. The same value has been derived by ATSDR for its MRL for chronic-duration oral exposure [56].

Tin has not been shown to be nutritionally essential for humans. Inorganic Sn is considered to be of low toxicological concern, due to very low absorption. It mainly results in local irritation. Data related to long-term toxicity are limited. In 2005, the ATSDR set an intermediary MRL (<1 year of exposure) of 0.3 mg/kg body weight per day on the basis of haematological effects in rats after administration of the $SnCl_2$ form in feed for 13 weeks [46]. Organic forms (organotins) are more toxic than inorganic forms. The critical toxicological end point for risk assessment was considered to be immunotoxicity. By applying a safety factor of 100, a group TDI of 0.25 μg/kg body weight for tributyltin (TBT), dimethyltin, triphenyltin and di-*n*-octyltin compounds was established by EFSA (based on TBT oxide molecular mass). The group TDI is 0.1 μg/kg body weight when expressed as Sn content or 0.27 μg/kg body weight when expressed as TBT chloride [57].

Absorption in humans has been estimated to be less than 5% vanadium ingested from the diet based on the low concentration of V normally present in urine compared with the daily intake and faecal levels [58]. V has a very low oral absorption (<1%). Oral exposure resulted in increased red blood cells, increased blood pressure, and mild neurological effects in animals. The ATSDR has proposed a MRL of 0.01 mg/kg body weight per day[59].

Some trace elements such as Se, Mo, Mn, Fe, Co, Cu, Mg and Zn are essential micronutrients. The risk assessment of these elements has to take into account the two ends of the dose–response relationship, the risk of deficiency and the risk of toxicity [60, 61]. Dietary reference intakes (DRIs) have been established by the Food and Nutrition Board of the Institute of Medicine (IOM) in the USA. These are reference values that are quantitative estimates of nutrient intakes to be used for planning and assessing diets for apparently healthy people. They include recommended dietary allowances (RDAs), upper intake level (UL) as well as three other types of reference values [16]. On the other hand, WHO, USEPA and other international agencies have carried out risk assessments on the toxicity end of the spectrum, establishing different HBGVs. Se is an essential element in human nutrition [62]. An RDA of 55 μg/day and a UL of 400 μg/day have been established. Its deficiency and toxicity concentrations are very close, so taking into account the main toxic symptoms associated with Se (selenosis), USEPA set a RfD for Se of 5 μg/kg body weight per day based on an epidemiological study [45].

Molybdenum is an essential element that acts as a cofactor for certain enzymes. An adequate intake of 65 μg/day has been proposed for adult men and women by EFSA [41]. Mo is considered to be of low toxicity. Chronic exposure to excess of Mo is characterized by high uric acid levels in serum and urine. USEPA has established an RfD for chronic oral exposure of 5 μg/kg body weight per day [40]. Manganese is an essential nutrient required as a cofactor for a variety of enzymes. Mn occurs naturally in foods and is authorized for addition to food [63]. Oral exposure to Mn can induce neurotoxicity [64]. An RfD for chronic oral exposure of 0.14 mg/kg body weight per day has been derived for total Mn by the USEPA [65].

4.2.2 Exposure assessment and risk characterization

The assessment of dietary exposure to trace elements, as for other harmful chemicals in the food chain, includes determining the concentration of specific forms of the elements in the diet, and their combination with the data from consumption of the different categories of foods that make up the staple diet of the general population or of specific subgroups [66]. Figure 4.2 shows this exposure and risk assessment process.

A complete review of the assessment of dietary exposure to chemicals has been published by Kroes *et al.* [67]. Some guidance documents have been produced in recent years at international level on how exposure assessment should be conducted [19, 68]. A comprehensive review of the various methods used for exposure assessment has been published by the International Programme on Chemical Safety (IPCS) of the WHO [69]. Likewise, Dorne *et al.* [70] have recently published an in-depth review of risk assessment of heavy metals.

The accuracy of any exposure assessment will depend on the precision of the two calculation inputs: chemical concentration and food consumption. To assess the concentration of metals in foods the most widely used approaches include (i) total diet study (TDS), (ii) duplicate diet study (DDS), and (iii) monitoring and surveillance programmes.

TDS has become increasingly relevant, and consists of collecting and analysing a representative 'market basket' of foods commonly prepared for normal consumption,

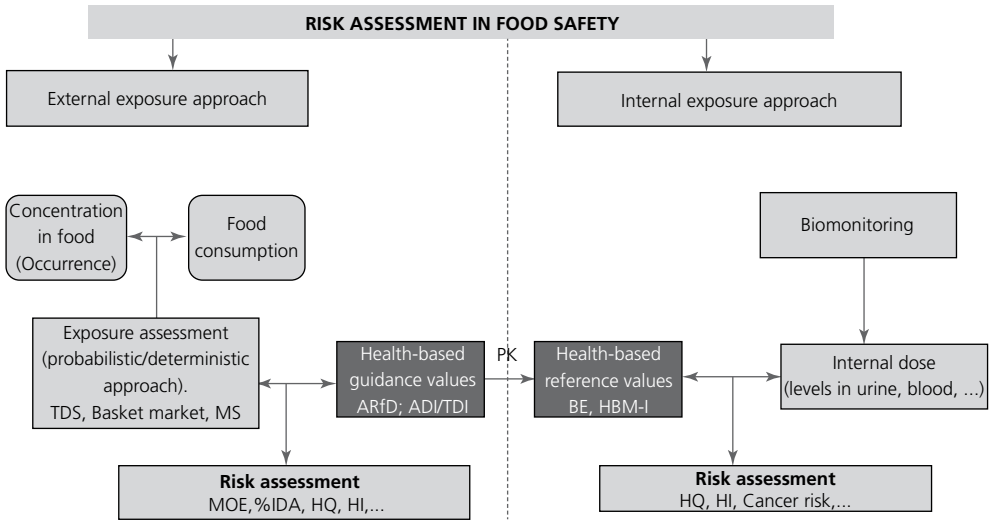


Figure 4.2 Framework for risk assessment of metals in the human population combining exposure assessment (external and internal) with toxicological effects. PK, toxicokinetics; MS, monitoring programmes; HQ, hazard quotient; HI, hazard index; BE, biomonitoring equivalents; MOE, margin of exposure; HBM-I, Health related human biomonitoring values.

pooling the food items into representative food groups and analysing them for a chemical substance. These studies can usually be performed at a regional or national level, and follow standardized methods recommended by the WHO and the EFSA [86, 87]. TDS differs from other chemical surveillance programmes in that it addresses contaminants in diet as a whole rather than in individual foods, and the exposure assessment focuses on the long-term average intake by the population. EFSA, FAO and WHO have recently harmonized the methodology of the TDS in order to improve the comparability at international level [87].

In some studies, the occurrence data available for risk assessment is not specifically generated for this purpose, but derived from the monitoring and surveillance programmes implemented to check for regulatory compliance to specific legislated maximum levels [43, 88]. Likewise, sometimes the foods have not been processed as for consumption before analysis, or the total diet of the population has not been investigated. These studies that differ somewhat from the more standardized TDS have been named TDS-like studies [86].

The duplicate portion or DDS is a technique in which test-persons consume their ordinary diet, but for subsequent analysis they also prepare a duplicate portion of all food products as consumed. Collected foods items are weighed, non-edible parts removed and samples homogenized, resulting in several pooled food samples per day for analysis [68, 72].

Different exposure scenarios can be defined in risk assessment studies. Some of them are related to the handling of non-detected or non-quantified values. For example, in the 'lower-bound' scenario, the foods that do not contain detectable metals are assumed to contain zero concentrations, whereas in the 'upper bound' scenario these foods are assumed to contain levels at limit of detection (LOD) or at half this limit [69].

Diverse approaches are used to combine consumption data, estimated through consumption surveys at an individual or household level, with chemical concentration data. The most frequently applied assessment for metal exposure is the deterministic approach [6] that combines estimated data points (mean, median) for both consumption and metal concentration. A more refined method aimed at assessing the actual exposure characterizes the full range of food consumption as well as the full range of chemical concentration in the foods, and then appropriate statistical models are used to evaluate the probabilities of consumers showing each level of exposure (probabilistic approach) [89–91].

Risk characterization is the final step in the risk assessment process. It is defined by the WHO [92] as the qualitative or quantitative estimation, including attendant uncertainties, of the probability of occurrence of known

and potential adverse effects in a given population. Risk characterization provides estimates of the potential human risk under different exposure scenarios. The risk characterization compares dietary exposures with the HBGV established in the hazard characterization, and different metrics such as the percentage of the ADI or RfD, the hazard quotient (HQ) or the hazard index (HI) currently used for threshold metals such as Hg or Cd. For chemicals that are genotoxic carcinogens or metals such as As or Pb, the MOE is calculated by dividing the point of departure (BMDL, etc.) with the human exposure [24].

Tables 4.2 and 4.3 show estimated dietary exposures to relevant and essential metals from significant and recent studies published in several countries. A brief discussion on the human exposure and risk characterization of Cd, Pb, As, Hg, Ni, Sn, V, Se and Mo is presented in the following sections.

Cadmium

In a recent study, JEFCA [7] concluded that the mean Cd exposure for adults ranged from 2.2 to 12 μg/kg body weight per month. This JEFCA report includes information at a national and regional level submitted by various countries such as Australia, China, Japan, the USA, Chile, Lebanon and Republic of Korea, and data provided by EFSA for Europe. For the European region the mean exposure was 9.1 μg/kg body weight per month. The exposure was estimated using national food consumption data but not the GEMS/Food consumption cluster diets [93], because the national data provide more refined estimates.

The median of the means of exposure of individual European countries (16 countries) estimated by EFSA [52] was 2.27 μg/kg body weight per week (range 1.89–2.96 μg/kg body weight per week). The mean high exposure was 3.02 μg/kg body weight per week. This exposure for adults across Europe is close to, or for some population groups slightly exceeds, the TWI of 2.5 μg/kg body weight established by EFSA. For some subgroups such as vegetarians, children and smokers and people living in highly contaminated areas, the exposure may exceed the TWI [94] by about twofold. To assess Cd exposure, the occurrence data were provided by the EU member states from monitoring results from 2003 to 2007. The consumption data were used as reported in EFSA's Concise European Food Consumption Database [95]. The food groups that contributed the most to Cd exposure were cereals and cereal products, vegetables, nuts and pulses.

Table 4.2 shows estimates of Cd dietary exposure from studies that used a more refined approach, because they utilized more representative contamination values of the food groups and more accurate data for consumption (use of national or regional surveys). These are mainly TDS and, with the exception of the study carried out in Catalonia (Spain) by Domingo *et al*. [72], the estimated exposures are well below the PTWI. In the study of Domingo *et al*. [72] a DDS approach was performed.

Lead

In 2011, JEFSA obtained estimates of exposures to Pb based on TDS for nine countries (including China, USA and Australia) or on other studies that had assessed levels in food, including the study of exposure in Europe (19 countries) conducted by EFSA [7]. The data from all these 28 countries were mostly based on food as consumed. For the adult population, mean exposures ranged from 0.02 to 3 μg/kg body weight per day. Mean exposures for children ranged from 0.03 to 9 μg/kg body weight per day. Chile was the country that presented the highest exposures, with milk and milk products, fruit, bread and sugar as the main food categories that contributed the most to dietary exposure of Pb.

Pb levels in food have declined over time in many developed countries. The JEFSA report summarizes the temporal trends in Pb exposure in five countries, showing declines in exposures since 1980 of 75% in New Zealand, 95% in the UK and 50% in France and Canada. The USA reported declines in teenage males from 70 μg/day in 1976 to 3.45 μg/day in 2000. In addition to the decreased levels in food owing to lower Pb levels in the environment after the phase-out of leaded petrol and other legislative control measures, some of the decline in exposure may actually be due to the improved sensitivity of the analytical methods and the treatment of left-censored values (<LOD).

EFSA has estimated a mean lifetime dietary exposure to Pb of 0.68 μg/kg body weight per day in the overall European population. Exposure was highest in toddlers and older children, being 1.32 and 1.03 μg/kg body weight per day, respectively [43]. The major contributing food categories, following the FoodEx classification [95], were grains and grain-based products (16.1%), milk and dairy products (10.4%), non-alcoholic beverages (10.2%), and vegetables and vegetable products

Table 4.2 Exposure to the main heavy metals and metalloids in some recent relevant studies (levels in μg/kg body weight per day).

Trace element	Country, year/type of study	Mean/P95, adult	Mean/P95, children	Main contributors (adults)	Reference
Ag	France, 2006/TDS	2.65/4.78	3.47/6.59	Molluscs and crustaceans (24%) Children: milk (9%), water (8%)	[71]
As-T	France, 2006/TDS	0.78/1.79	1.21/2.91	Fish (30–42%), molluscs (7–17%), water (6–8%)	[71]
As-i	France, 2006/TDS	0.28/0.5	0.39/0.76	Water (≥19%), non-alcoholic beverages (15%) Children: milk (15%)	[71]
As-i	Catalonia (Spain)/DDS	0.37 (assuming 11% of As-T)			[72]
As-i	Catalonia (Spain), 2008/TDS (MBS)	0.23 (estimated from As-T, assuming 2% in fish, 3.5% in shelfish and 70% in others)		Fish and seafood (44%), cereals (41%)	[73]
As-T	Catalonia (Spain), 2008/TDS (MBS)	4.6		Fish and seafood (98%), cereals (2.9%)	[73]
As	Vancouver (Canada), 2007/TDS	0.97			[74]
As-T	UK, 2006/TDS	1.65/6.85	1.95/8.24	Fish (88%)	[75]
As-i	UK, 2006/TDS	0.028–0.093/0.071–0.165	0.055–0.158/0.128–0.291	Miscellaneous cereals (23%), beverages (19%)	[75]
As-T	New Zealand, 2009/TDS	1.61–1.67	1.52–1.62	Seafood (92%)	[76]
As-i	New Zealand, 2009/TDS	0.27–0.3	0.34–0.37		[76]
As-i	Hong Kong, 2010/TDS	0.22/0.38		Cereals and their products (53.5%), non-alcoholic beverages (13%)	[77]
As-T	Australia, 2008/TDS	0.55/1.13 (P90th)	0.88/2.02 (P90th)	Seafood (32–41%), rice and rice products (11–13%)	[78]
Be	Vancouver (Canada), 2007/TDS	0.002			[74]
Ba	Vancouver (Canada), 2007	9.3			[74]
Ba	Pavia (Italy), 2004/TDS-DDS	5.23 (70 kg)		Milk and dairy products (47%), fruit and vegetables (18%)	[79]
Ba	UK, 2006/TDS	9.40/45.29	14.36/64.75	Nuts (46%) no other >10%	[75]
Cd	France, 2006/TDS	0.16/0.27	0.24/0.45	Bread and dried bread products (13–22%), potatoes and potato products (12–13%), vegetables (8–10%)	[71]
Cd	Catalonia (Spain), 2010/DDS	0.83			[72]
Cd	Catalonia (Spain), 2008/TDS (MBS)	0.27		Fish and seafood (21%), tubers (19%)	[73]
Cd	Vancouver (Canada), 2007/TDS	0.22			[74]
Cd	Pavia (Italy), 2004/TDS-DDS	0.19		Cereals and tubers (55%), fruits and vegetables (30%)	[79]
Cd	UK, 2006/TDS	0.15/0.27	0.29/0.53	Potatoes (24%), miscellaneous cereals (21%), bread (19%)	[75]

Cd	Sweden, 1999/TDS	0.142		Cereals products (48%), potatoes (19%)	[80]
Cd	Lebanon, 2008/TDS	0.226/0.336 (P97.5th)		Vegetables (46.8%), bread and cereals (30.9%)	[81]
Cd	New Zealand, 2009/TDS	0.18–0.186	0.37–0.38	Potatoes and related products (24%), breads (10%)	[76]
Cd	Australia, 2008/TDS	0.106/0.189 (P90th)	0.211/0.33 (P90th)	Root vegetables (21–27%), savoury snacks (6–17%)	[78]
Cr	France/TDS	3.95/5.9	11.15/16.65	Bread (8%), dried bread products(5%), alcoholic beverages (5%) Children: milk (9%), pasta (6%)	[71]
Cr	UK, 2006/TDS	0.28–0.37/0.50–0.62	0.51–0.65/1.03–1.22	Sugars and preserves (16%), beverages (13%), miscellaneous cereals (13%)	[75]
Cr	Tarragona County (Spain)	1.26		Fish and seafood (74%), meat (26%)	[82]
Cs	Vancouver (Canada), 2007/TDS	0.20			[74]
MeHg	France, 2006/TDS	0.017/0.061	0.022/0.097	Fish and seafood products	[71]
Hg-i	France, 2006/TDS	0.18/0.29	0.26/0.47	Foods other than seafood products	[71]
Hg-T	Catalonia (Spain)/DDS	0.67			[72]
MeHg	Catalonia (Spain), 2008/TDS (MBS)	0.14 (estimated from 90% Hg total)		Fish and seafood (53%), meat and meat products (15%)	[73]
Hg-T	Catalonia (Spain), 2009/TDS (MBS)	0.16		Fish and seafood (53%), meat and meat products (9.9%)	[73]
Hg-T	UK, 2006/TDS	0.05/0.13	0.08/0.18	Fish (25%), beverages (25%)	[75]
Hg-T	New Zealand, 2009/TDS	0.07–0.125	0.09–0.185	Fish and shellfish (73%)	[76]
MeHg	New Zealand, 2009/TDS (fish and shellfish only)	0.047–0.048	0.065		[76]
MeHg	EFSA (European countries)	0.01–0.16/0.07–0.44	0.012–0.24/0.094–0.72	Only from fish and seafood	[38]
MeHg	Australia, 2008/TDS	0.060/0.148 (P90th)	0.061/0.141 (P90th)	Seafood (crumbed) (41%), fish (38%)	[78]
Pb	France, 2006/TDS	0.2/0.35	0.27/0.57	Alcoholic beverages (14%), bread and dried bread products (13%), water (11%) Children: milk (11%), water (11%), non-alcoholic beverages (10%)	[71]
Pb	Catalonia (Spain), 2010/DDE	0.33			[72]
Pb	Catalonia (Spain), 2008/TDS (MBS)	1.45			[73]
Pb	Vancouver (Canada), 2007/TDS	0.12			[74]
Pb	Pavia (Italy), 2004/TDS	0.78		Cereals and tubers (51%), fruits and vegetables (17%)	[79]
Pb	UK, 2006/TDS	0.10/0.18	0.15/0.30	Beverages (17%), bread (16%), other vegetables (16%)	[75]

(Continued)

Table 4.2 (Continued)

Trace element	Country, year/type of study	Mean/P95, adult	Mean/P95, children	Main contributors (adults)	Reference
Pb	Sweden, 1999/TDS (MBS)	0.1		Cereal products (24%), fruit (18%), strong beer, wine, spirits (15%)	[80]
Pb	Lebanon, 2008/TDS	0.118/0.178 (P97.5th)		Vegetables (47%), bread and cereals (31%)	[81]
Pb	New Zealand, 2009/TDS	0.114–0.157	0.2–0.28	Grains (26–30%)	[76]
Pb	Australia, 2008/TDS	0.132/0.47 (P90th)	0.172/0.25 (P90th)	Water (non-bottled) (10%), coffee (15%), savoury pastries (7%)	[78]
Sb	France, 2006/TDS	0.004(UB)/0.07	0.006(UB)/0.10	Beverage other than coffee (13%), sugars and sugar derivatives (11%) Children: pastries and cakes (11%)	[71]
Sb	UK, 2006/TDS	0.03/0.06(P97.5th)	0.049/0.096	Total population: meat products (24%), beverages (20%)	[75]
Pd	UK, 2006/TDS	0.009/0.016	0.016/0.032	Miscellaneous (13%), beverages (20%)	[75]
Tl	Vancouver (Canada), 2007/TDS	0.014			[74]
Tl	UK, 2006/TDS	0.012/0.021	0.016/0.035	Beverages (23% no detected), potatoes (19%)	[75]
Sn-T	France, 2006/TDS	3.9/17	7.3/31.9	Cooked fruits (adults 33%, children 54%), fruit (adults 20%, children 17%)	[71]
Sn-T	Pavia (Italy), 2004/TDS-DDS	22.4		Cereals and tubers (97%)	[79]
Sn-T	Tarragona County (Spain)	0.49		Fish and seafood (24%), pulses (24%)	[82]
U	Vancouver (Canada), 2007/TDS	0.07			[74]
U	Catalonia (Spain)/MBS	0.221	0.29	Meat and meat products (27%), cereals (19.8%), dairy products (15.7%)	[83]

TDS, total diet study; DDS, duplicate diet study; MBS, market basket study; T, total; i, inorganic; P, percentile; P95, 95th percentile; MeHg, methylmercury.

(8.4%). Likewise, EFSA has evaluated the trends in Pb occurrence in food over time, estimating a reduction of about 23% between 2003 and 2010.

There is no threshold for the potential critical health effects of Pb, so the PTWI of 25 μg/kg body weight per day was withdrawn [8]. However, EFSA identified a 95th percentile lower confidence limit of the benchmark dose of 1% extra risk ($BMDL_{01}$) of 0.50, 1.50 and 0.63 μg/kg body weight per day for development of neurotoxicity in young children, cardiovascular effects in adults and nephrotoxicity in adults, respectively. Taking into account these $BMDL_{01}$, and the exposures for the different population groups and scenarios, MOEs range from 0.51 to 4.2, and consequently the possibility of effects in some European consumers cannot be excluded.

Arsenic

Estimation of the dietary exposure to As in various studies focuses on both total and inorganic As. The latter is the more toxic form in which As can appear in foods. The 72nd meeting of JEFCA [25] aimed to estimate the dietary exposure to inorganic As, and states that the main factors influencing dietary exposure to inorganic As are water supply, type of food consumed and food-preparation methods. In general, the ranges of dietary exposure for North America and Europe were similar but lower than those reported for Asian and some South American countries. In a small community in Chile the exposure to dietary inorganic As (drinking water included) ranged from 2.08 to 21.48 μg/kg body weight per day. For China, the reported mean derived from a TDS (including drinking water) exposure ranged from 0.24 to 0.76 μg/kg body weight per day for the adult population. Similar ranges were reported for Japan.

Using data reported from 15 European countries, EFSA estimates that the exposure to inorganic As from food and water ranged from 0.13 to 0.56 μg/kg body weight per day for average consumers, and from 0.37 to 1.22 μg/kg body weight per day for 95th percentile consumers. Dietary exposure in children under 3 years of age is estimated to be twofold to threefold that of adults [25]. In this report, EFSA has made a number of assumptions for the contribution of inorganic As to total As because 89% of the results were reported as total As. For food commodities other than fish and seafood the proportion of inorganic As was assumed to be 70% of total As. In fish and seafood the relative proportion of inorganic As is small, so a fixed value for As of 0.03 mg/kg in fish and 0.1 mg/kg in seafood were used.

The estimated dietary exposures to inorganic As for average and high-level consumers in Europe are within the range of $BMDL_{01}$ values (0.3–8 μg/kg body weight per day) and therefore there is little or no MOE and the possibility of risk to some consumers cannot be excluded. The main sources of inorganic As intake are cereal grains and cereal-based products, food for special dietary uses (e.g. algae), bottled water, coffee and beer, rice and rice-based products, fish and vegetables. Table 4.2 shows the exposures to total and inorganic As in studies carried out in different countries and the main food categories that contribute to dietary exposure. Fish and seafood are the main contributor to total As, but only a small fraction (0.36–3%) [73, 75] is organic As (mostly arsenobetaine), which is assumed to be of no toxicological concern [96].

Mercury

Using national TDSs, JEFCA has summarized the exposure assessments to total Hg in different countries, including Australia, Canada, China, France, the USA, the UK or Spain [25]. Estimated mean dietary exposure to total Hg ranged from 0.07 to 5.81 μg/kg body weight per week. The contribution of fish and shellfish to the total dietary exposure ranged from 40 to 100% when samples with non-detectable concentrations were assigned to zero concentration. Almost all dietary exposure to methylmercury (the most toxic form) is from fish and seafood that contain this organic Hg species in a very high proportion (90%) [97]. In a previous report [98], the JEFCA assessed the dietary exposure to methylmercury using a deterministic approach and this resulted in values ranging from 0.3 to 1.0 μg/kg body weight per week.

EFSA has reported the dietary exposure to methylmercury based only on the consumption of fish and other seafood [38]. The study was based on the occurrence data provided by 20 European countries covering the period 2004–2011 and applying conversing factors to calculate methylmercury and inorganic Hg from the analysed total Hg. The mean methylmercury dietary exposure varied from 0.06 μg/kg body weight per week in the elderly to 1.57 μg/kg body weight per week in toddlers. The 95th percentiles ranged from 0.14 in the very elderly to the highest maximum of 5.05 μg/kg body weight per week in adolescents. The mean dietary exposure does not exceed the TWI for methylmercury (1.3 μg/kg body weight per week), with the exception of toddlers and other children in some surveys. The medians of 95th percentile dietary exposure across surveys are close to or above the TWI for all age groups.

The report also stated that high consumers of fish meat may exceed the TWI by up to approximately sixfold.

Likewise, the EFSA also reported the mean estimated exposure to inorganic Hg in Europe, which ranged from 0.13 to 2.16 μg/kg body weight per week. These values do not exceed the TWI for inorganic Hg (4 μg/kg body weight per week).

Nickel

Nickel is an essential micronutrient for plant growth and several species of vertebrates, and is therefore present in a wide range of primary crops, animals and foodstuffs [99]. People are exposed to Ni mainly via food and drinking water, although soil and dust ingestion and air inhalation are other pathways that can contribute to the total exposure. Ni is usually measured in food as total Ni, so there is uncertainty as to the chemical form, although it is normally considered to be in the form of complex bound organic Ni, which may be less bioavailable than other forms.

Average dietary exposure to Ni across Europe is in the range of 70–250 μg/day [99]. However, in a TDS study carried out in Pavia (Italy), the mean exposure in adults (70 kg) was estimated at 360 μg/day [79]. Whereas the mean daily intake of Ni was 4.3 μg/kg body weight in Canada [74], Nasreddine *et al*. [81] have reported a mean and 97.5th percentile of 1.80 μg/kg body weight per day and 2.91 μg/kg body weight per day in Lebanon. In the UK, the Ni dietary exposure of the population was estimated to be 130 μg/day [75], and has decreased since 1976 (330 mg/day), although it has remained relatively stable since 1982. In recent studies (see Table 4.2), the estimates of dietary exposure were below the WHO TDI (12 μg/kg body weight per day), so it is possible to conclude that these dietary exposures to Ni were unlikely to be of toxicological concern. Cereals, beverages and vegetables are the main groups contributing to to the dietary intake of Ni, although the percentage of each group varies in different countries (Table 4.2).

Tin

Tin occurs naturally in foods as stannous and stannic salts, and has not been shown to be nutritionally essential for humans. The absorption of inorganic compounds of Sn from the gastrointestinal tract in humans is very low (2%), and consequently inorganic Sn compounds have low systemic toxicity in humans [100].

Data on Sn intake are limited. The French population's mean exposure to total Sn is estimated at 3.9 μg/kg body weight per day in adults and 7.3 μg/kg body weight per day in children. At the 95th percentile, exposure is 17.0 μg/kg body weight per day [71]. In the UK, the mean intake in adults from food is estimated at 1.8 mg/day, ranging up to about 6 mg/day, and appears to be decreasing [100]. In a northern Italian area, the average daily exposure in an adult population was estimated to be 1.57 mg. The estimated dietary intake of Sn in 25 pairs of Japanese children and their parents, using 7-day duplicate diet composites, were 162 and 152 μg/day, respectively [101]. Turconi *et al*. [79] have reviewed different studies on data exposure to Sn, concluding that the daily exposure ranged from 0.03 to 3.2 mg/day. In all the studies mentioned above, the average daily estimates are less than 3% of the PTDI established by WHO (140 mg/day).

The main dietary sources of Sn for adults and children are compotes and cooked foods (33% and 54%, respectively) in the French TDS and cereals and tubers (97%) in the Italian area. Pulses (24%) and seafood (24%) were the food groups that contributed the most to diet exposure to Sn in Catalonia (Spain) [82].

Vanadium

About 80% of exposure to V comes from the diet. There are limited data on V concentration in foods and on the daily dietary intake of V. In the second French TDS, 48% of the samples presented levels of V lower than the LOD or the limit of quantification (LOQ). The mean exposure of the French population was estimated at 0.86 μg/kg body weight per day in adults, and 1.06 μg/kg body weight per day in children. V could only be detected in fish and seafood in a study performed in Tarragona (Spain), where the intake was estimated at 0.41 μg/kg body weight per day. Likewise, the average daily exposure in adults estimated in an Italian TDS ranged from 12.4 to 17.9 μg, which is less than 0.1% of the health-based guidance value of 1.8 mg/day established by the IOM [16].

Water and alcoholic beverages have been reported as the main contributors to exposure to V in France, while in Italy cereals and fruit and vegetables appear to be the main sources of dietary exposure (see Table 4.2).

Selenium

Diet is the major source of this essential element. The Se content of plants is directly related to its content in soil, whereas the levels in animal products are linked to the content of the animal's diet [62]. In the TDS performed in the UK [75], the miscellaneous cereal group (16%)

and meat-product group (15%) made the greatest contribution to the population exposure, which was estimated to range between 0.048 and 0.058 mg/day. These were well below the UL of 0.3 mg/day set by EFSA [35] or the 0.4 mg/day established by the IOM. In studies carried out in Canada, Sweden and New Zealand (Table 4.3), the mean intake for adults ranged from 0.74 to 3.2 μg/kg body weight per day, which means that exposures to Se are not of toxicological concern.

Molybdenum

Estimates of daily intake vary widely regionally depending on the soil type. Results from TDSs in different countries including Italy [79], France and the UK [75] have shown that cereals and cereal-based products including bread are the major food contributors to dietary Mo intake in adults. In the UK the group of miscellaneous cereals contribute about one-third to the total Mo intake, and bread 19%. Further contributors to Mo intake are fruit and vegetables (~15%). EFSA has compiled data from studies carried out in several European countries that have used different approaches to estimate Mo intakes [41]. Results show that the mean Mo intake for adults ranges from 58 to 157 μg/day, with an average above 100 μg/day. In children, mean intakes were reported to be 74.9 μg/day in France (3–17 years) and 4.8 μg/kg body weight per day in the UK. Mo intakes in the USA range from 240 μg/day for adult men to 100 μg/day for women [102]. All these values are clearly below the RfD (0.35 mg/day) or the UL (0.7 mg/day) for adults of 70 kg.

Figure 4.3 depicts the risk characterization for the relevant metals derived from the average of the mean values for the adult population in the studies described in Tables 4.2 and 4.3. For Pb and As-i, the $BMDL_{01}$ and $BMDL_{05}$ have been used, respectively, as a surrogate of the HBGV. Cd, As-I and Pb present the most high risk, with exposures very close to the health reference value, and consequently some percentage of the population will have intakes above the threshold values.

4.3 Human biomonitoring for risk assessment of metals

Human biomonitoring (HBM), defined as the measurement of chemicals or their metabolites in human tissues or specimens, is an excellent tool for assessing and controlling human exposure to environmental pollutants [2, 103]. Data from HBM studies are at present widely available and are increasingly used for assessing environmental exposures and their effects on human health. HBM delivers information about the dose really taken up from the environment (internal dose), and provides an integrated measure of exposure from every route and pathway of exposure (Figure 4.4).

The main goals of national biomonitoring programmes and biomonitoring studies include establishing reference ranges for concentrations of chemicals in the general population, detecting trends in population exposure levels, identifying populations with exposures above background, and assessing the effectiveness of regulatory and environmental risk management [104, 105]. But one of the most valuable potential applications of HBM is interpreting HBM data in a public health risk context, helping in the assessment of which chemicals may be of concern for public health [106].

For the proper development of HBM studies, a number of requirements should be fulfilled, such as the choice of an appropriate biomarker able to reflect exposure to the parent compound [107], and the selection of a suitable biological specimen (blood, urine, etc.). Likewise, some information on the advantages and disadvantages of available biomarkers, information about the main routes and sources of exposure and some pharmacokinetic information could be helpful for interpreting the results.

According to the National Research Council [2], biomarkers are divided into three categories: biomarkers of exposure, effect, and susceptibility. A biomarker of exposure is the chemical itself or a substance-specific metabolite, or the product of an interaction between a chemical and some target molecule or cell that is measured in a biological material. In studies of community exposure to environmental contaminants, biomarkers of exposure are largely used. Ideally a valid biomarker of exposure should be sensitive (capable of measuring the contaminant after exposure), specific for the chemical of interest, of biological relevance, practical (the biological sample obtainable), inexpensive (low cost of analyses) and available [108]. Selecting an appropriate biomarker of exposure requires a wide knowledge of the toxicokinetics of the substance (absorption, distribution, metabolism and excretion) in order to choose the appropriate metabolite and the biological matrix, and to consider the time from exposure to measurement. In addition, several factors can confuse the use

Table 4.3 Exposure to essential and probably essential metals in some recent relevant studies (levels in μg/kg body weight per day).

Element	Country, year/type of study	Mean/P95, adult	Mean/P95, children	Main contributors, adults	Reference
Co	France, 2006/TDS	0.18/0.31	0.31/0.62	Coffee (11%) Children: chocolate (12%)	[71]
Co	Vancouver (Canada), 2007	0.21			[74]
Co	Pavia (Italy), 2004/TDS-DDS	0.41		Cereals and tubers (41%), fruit and vegetables (27%); meat, fish, seafood, eggs	[79]
Co	Sweden, 1999/ TDS (MBS)	0.157		Cereal products (26%); sugar, sweets, ice cream (25%)	[80]
Co	Lebanon, 2008/TDS	0.162/0.248 (P97.5th)		Vegetables (31.3%), potatoes (20.9%)	[81]
Cu	Vancouver (Canada), 2007/TDS	4.3			[74]
Cu	Pavia (Italy), 2004/TDS-DDS	16.2		Fruit and vegetables (39%); meat, fish, seafood and eggs (22%); cereals and tubers (25%)	[79]
Cu	UK, 2006/TDS	17.23/34.37	29.41/54.92	Miscellaneous cereals (21%), bread (14%), potatoes (10%)	[75]
Cu	Sweden, 1999/TDS (MBS)	16.42		Cereal products (31%), fruit (14%)	[80]
Cu	Lebanon, 2008/TDS	15.77/25.88 (P97.5th)		Bread and cereals (44.1%), meat and poultry (13.3%)	[81]
Cr	Sweden, 1999/TDS (MBS)	0.357		Sugar, sweets, ice cream (34%); cereals products (16%)	[80]
Cr	Australia, 2008/TDS	1.82/2.54	2.69/3.69	White breads (11%), milks and cream (7%)	[78]
Fe	Pavia (Italy), 2004/TDS-DDS	157		Cereals and tubers (42%); meat, fish, and seafood, eggs (28%)	[79]
Fe	Sweden, 1999/TDS (MBS)	131		Cereal products (37%), meat (22%)	[80]
Fe	New Zealand, 2003–2004/TDS	182.8		Red meat (24%), breads (16%)	[84]
Fe	Lebanon, 2008/TDS	185.7 (70 kg)/309.3 (P97.5th)		Bread and cereals (37.5%), meat and poultry (26.6%)	[81]
Mn	UK, 2006/TDS	67/124	106/201	Beverages (41%), miscellaneous cereals (20%)	[75]
Mn	Vancouver (Canada), 2007	54			[74]
Mn	Pavia (Italy), 2004/TDS	23		Cereals and tubers (62%), fruit and vegetables (20%)	[79]
Mn	Sweden, 1999/TDS (MBS)	50		Cereal products (53%), fruits (15%)	[80]
Mn	Lebanon, 2008/TDS	29.14/46.28 (P97.5th)		Bread and cereals (47.6%), vegetables (17.1%)	[81]
Mo	Pavia (Italy), 2004/TDS	1.14		Cereals and tubers (45%), fruit and vegetables (25%)	[79]
Mo	UK, 2006/TDS	1.64/3.08	3.05/5.82	Miscellaneous cerals (33%), bread (19%)	[75]

Ni	France, 2006/TDS	2.33/3.76	3.83/7.44	Alcoholic beverages (9%), water (8%), vegetables (7%) Children: chocolate (10%), water (7%), dairy-based deserts (7%)	[71]
Ni	Vancouver (Canada), 2007/TDS	4.3			[74]
Ni	Pavia (Italy), 2004/TDS-DDS	5.15		Cereals and tubers (34%), milk and dairy products (21%)	[79]
Ni	UK, 2006/TDS	1.49–1.63/3.01–3.08	2.62–3.05/5.27–5.82	Beverages (21%), miscellaneous cereals (16%), sugars and preserves (14%)	[75]
Ni	Sweden, 1999/TDS (MBS)	1.42		Sugar, sweets, ice cream (30%); cereal products (29%)	[80]
Ni	Lebanon, 2008/TDS	1.80 (70 kg)/2.91 (P97.5th)		Bread and cereals (43.3%), vegetables (20%)	[81]
Se	Vancouver (Canada), 2007	3.2			[74]
Se	UK, 2006/TDS	0.83–0.95/1.65–1.79	1.27–1.44/2.60–2.84	Miscellaneous cereals (16%), meat products (15%), bread (11%)	[75]
Se	Sweden, 1999/TDS (MBS)	0.74		Fish (21%), meat (20%)	[80]
Se	New Zealand, 2003–2004/TDS	0.95		Seafood (15%), chicken (13%)	[84]
Se	New Zealand, 2008/TDS	1.07–1.17	1.95–2.1	Grains (21%), chicken (19%), seafood (12%)	[76]
Se	Australia, 2008/TDS	2.3/3.13	3.13/4.9	Milk and cram (12%); beef, veal and venison (10%)	[78]
V	France, 2006/TDS	0.86/1.47	1.06/2.10	Water (20%), alcoholic beverages (15%)	[71]
V	Pavia (Italy), 2004/TDS-DDS	0.174		Cereals and tubers (37%), fruits and vegetables (23%)	[79]
V	Tarragona County (Spain), 2003/MBS	0.41		Fish and seafood (100%)	[82]
Zn	Vancouver (Canada), 2007	214			[74]
Zn	Pavia (Italy), 2004/TDS-DDS	171		Meat, fish, seafood, eggs (28%); cereals and tubers (26%)	[79]
Zn	Sweden, 1999/TDS (MBS)	161		Meat products (31%), dairy products (29%)	[80]
Zn	Lebanon, 2008/TDS	156 (70 kg)/265 (P97.5th)		Meat and poultry (36.5%), bread and cereals (28%)	[85]

TDS, total diet study; DDS, duplicate diet study; MBS, market basket study; P, percentile; P95, 95th percentile.

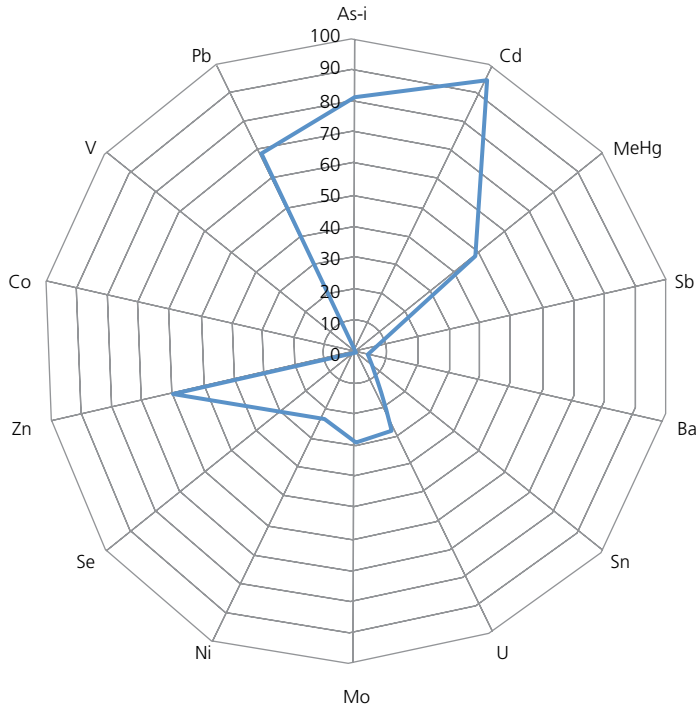


Figure 4.3 Risk characterization (% HBGV) for relevant metals derived from the studies included in Tables 4.2 and 4.3. The exposure values have been calculated as the average of the means for adults, and the more conservative HBGV has been selected.

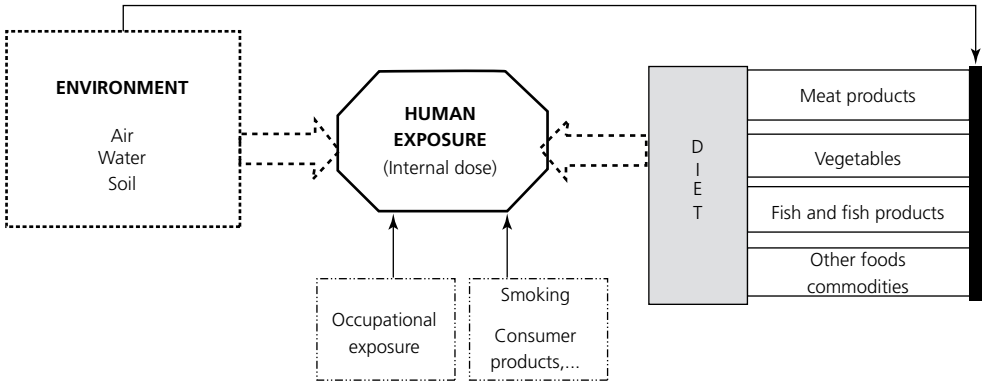


Figure 4.4 Sources of human exposures to metals.

and interpretation of biomarkers of exposure. For instance, there may be more than one source of exposure to the substance; the biomarker may be a metabolite of another xenobiotic substance, or it may be difficult to identify individuals exposed to substances that are commonly found in body fluids (e.g. essential mineral nutrients such as Cu, Zn, and Se).

The biological half-life of a substance and the duration and route of exposure strongly influence biomarker levels by the time samples can be taken [109]. Some metals such as Hg, Pb and Cd are bioaccumulative in humans [110]. Methylmercury has a half-life in blood of roughly 50 days, and it is incorporated into growing hair [111]; consequently the measure of total Hg in both matrices

provides information on accumulated dose of organic Hg. The skeleton acts as a storage compartment of Pb, supplying 40–70% of the Pb in blood. The half-life of Pb in blood is 1–2 months, whereas it is from years to decades in the skeleton [112]. The other mineral elements have been classified as non-bioaccumulative chemicals [110].

Urine and blood (or its components) are the most commonly used matrices in HBM programmes and studies for assessing exposure to metals. For example, the national biomonitoring programmes developed in the USA [113] and Canada [104] measure metals and trace elements in both matrices. Urine is accessible in large volumes, allowing the determination of very low concentrations of chemicals caused by environmental exposure. Owing to the difficulties of 24-hour urine voids, first morning void or spot urine samples are most generally used [109]. However, the variability of both the volume of urine and the concentration of chemicals from void to void need to be taken into account. Rivera-Nuñez *et al*. [114] have evaluated six As species (arsenobetaine, arsenite, arsenate, methylarsenious acid, methylarsenic acid, and dimethylarsenic) in first morning void and in spot urine samples, concluding that both sample types can be used without preference when evaluating As exposure in epidemiological studies. In addition to the magnitude of exposure, several factors influence the interindividual variability of biomarkers in spot samples [115]. One of the main factors is the elimination half-life relative to the exposure interval, and this has to be considered when designing biomonitoring strategies and evaluating the results [106].

Blood-based matrices (whole blood, plasma, serum) are the most usual samples for the biomonitoring of human exposure to more persistent and lipophilic compounds such as organochlorine pesticides, polybrominated diphenyl ethers (PBDEs) or dioxins [116, 117]. For bioaccumulative metals such as Pb, blood is considered to be the most suitable biomarker of exposure reflecting a combination of recent and several years before exposures [8]. Blood is an ideal matrix because it is in contact with every tissue and is in equilibrium with tissues where chemicals are deposited. However, blood collection is invasive, which may make its collection difficult, mainly from infants and small children.

Non-invasive matrices [118] such as scalp hair [119, 120], axillary hair [121], nails [122] or saliva have been proposed [123]. Apart from these matrices, breast milk is used in studies that aim to monitor neonatal and maternal exposures to different metals including Pb, Cd or Hg [124–128]. Some organizations and authors have developed a framework based on weight of evidence for selecting appropriate exposure biomarkers for biomonitoring studies [129].

4.3.1 Biomarker characterization

Table 4.4 summarizes the biomarkers currently used by HBM programmes as well as the studies on exposure assessment of the main metals related to the diet. Those that present a higher toxicological relevance and constitute a significant potential threat to human health are As, Pb, Hg and Cd [55].

Humans are exposed to various forms of As, including inorganic (arsenite, arsenate) and organic forms. Inorganic As is an established human carcinogen [146] and the main sources of exposure are drinking water and food. Seafood is the main source of non-toxic organic As (arsenobetaine, arsenocholine, arsenosugars). The most widely used As biomarker is urinary total As (As-U). Urinary As levels reflect recent exposures and are correlated with As intake from drinking water and dietary sources. However, As biomarkers also include analysis in blood, hair or nails [147]. Absorbed As is primarily excreted in urine, with a half-life of around 4 days. As is determined in urine as total or speciated [132]. Measuring total As in human urine assesses the combined exposure to every route and every species of As. Measurable species resulting from inorganic As are arsenate, arsenite, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). The main organic species are arsenobetaine, arsenocholine and trimethylarsine oxide (TMAO) [148].

Non-smokers are primarily exposed to Cd through food. In the European population the two highest contributors to dietary exposure are cereals and cereal products, and vegetables, nuts and pulses [94]. Cd absorption after dietary exposure in humans is relatively low (3–5 %) but it is efficiently retained in the kidney (half-life 10–30 years) and the concentration is proportional to that in urine. Both total Cd in urine (Cd-U) and in blood (Cd-B) have generally been the selected biomarkers for assessing Cd exposure [52, 149]. Usually, Cd-U concentration is the biomarker of chronic exposure and it is directly correlated with renal cortex Cd concentrations (the critical dose metric associated with Cd-induced proteinuria) [133]. Cd-B is considered

Table 4.4 Biomarkers of exposure to metals.

Metal	Matrix*	Source of exposure/biomonitoring information	Reference
Antimony (Sb)	U, B	The general population is exposed to Sb mainly through ingestion of food, but also from water, air or dust. Sb is most commonly measured in blood and urine. Levels of urinary Sb reflects recent exposure	[130, 131]
Arsenic (As)	U	The general population's exposure to inorganic As results from drinking water. The organic forms (much less toxic) are found in seafood, and are represented by arsenobetaine, arsenocholine and arsenosugars. Measurement of urinary As levels is generally accepted as the most reliable indicator of recent As exposure. Urinary As levels are correlated with As intakes from drinking water and dietary sources. Arsenobetaine and dimethylarsinic acid (DMA) are the main contributors to the total urinary As. Recent and frequent fish consumption led to higher As values in urine, mainly owing to increased levels of arsenobetaine and arsenocholine	[26, 96, 132]
Barium (Ba)	U	The general population can be exposed to low amounts of Ba in food and drinking water. Ba can be measured in bone, blood, urine, and faeces. However, there are no data correlating Ba levels in these tissues with specific exposure levels. Urinary Ba reflects recent exposure	[27, 28]
Beryllium (Be)	U	Be in blood, urine and biological tissues can be used as biomarkers of exposure. Urinary Be levels represent recent and accumulated exposure	[29]
Cadmium (Cd)	U, B	Cd exposure reflecting the internal dose is monitored in either blood or urine. Cd concentrations in urine reflect both cumulative exposure and the concentration of Cd in the kidney. Cd levels in blood are believed to be an indicator of recent exposure and Cd accumulated over time. Blood Cd may not be as directly correlated with the critical dose metric (renal cortex concentration) associated with critical toxic response as urinary Cd	[52, 133]
Caesium (Cs)		Food and drinking water are the largest sources of exposure to Cs in the general population. Urinary Cs levels reflect recent exposure	[134]
Cobalt (Co)	U	Cobalt is an essential human element. Urinary levels of Co decline rapidly 24 hours after exposure ceases. Urinary levels mainly reflect recent exposure	[135]
Cupper (Cu)	B, U, H/N	Dietary intake is the main source of exposure to this essential trace element. Serum Cu levels may only be reflective of recent exposure. Cu levels in hair and nails can also be used to assess exposure to this metal, and may reflect exposure over longer periods of time	[136]
Chromium (Cr)		The general population may be exposed to Cr daily through food, drinking water, and air. Cr(III) is an essential element and is normally present in blood and urine. Elevations in Cr in blood and urine are considered the most reliable biomarkers of exposure	[34]
Lead (Pb)	B	Blood Pb concentration is the most widely used and considered the most reliable biomarker of Pb exposure. Blood Pb levels reflect both recent intake and equilibration with stored Pb in other tissues, particularly in the skeleton. Elevated blood Pb concentration (e.g. >10 µg/dL) is an indication of excessive exposure in infants and children and is considered to be excessive for women of childbearing age	[8, 112, 137]
Manganese (Mn)	B, U	For the general population, exposure to this essential element is mainly through diet. At low levels, blood Mn concentrations would be related to intake from food, water, and air; large differences in individual blood Mn levels would be observed. Concentrations in blood and urine can be used to evaluate exposure to Mn	[16, 138]

Mercury (Hg)	U, B, hair	Exposure of the general population to methylmercury is primarily through consumption of contaminated fish and seafood. To a much lesser extent, the general population is exposed to inorganic Hg from sources such as dental amalgams. The total blood Hg concentration is due mostly to the dietary intake of methylmercury, while Hg in urine reflects mainly exposure to inorganic Hg. Total Hg in scalp hair has been widely used as a biomarker to assess long-term methylmercury exposure. A frequently cited total Hg blood to hair ratio is 1 : 250, although large variations exist, especially in people with infrequent fish consumption	[38, 119, 139]
Molybdenum (Mo)	U, B	Mo is an essential trace element in humans. Ingestion of food is a route of exposure for the general population. Plasma Mo concentrations reflect longer-term Mo intake, but urinary excretion is related to recent exposure	[41]
Nickel (Ni)	U, B	The general population may be exposed to Ni in ambient air, food and water. Serum and urine Ni levels are the most useful biomarkers of Ni exposure	[140]
Selenium (Se)		Se in an essential nutrient. More than 98% of the total daily intake of Se occurs through food consumption. Levels in urine and blood reflect both short- and long-term exposure	[141]
Tin (Sn)	U	The general population can be exposed to Sn compounds (inorganic or organic) through the diet or by contact with contaminated soil. Absorbed inorganic Sn distributes to soft tissues and bones. Elimination of inorganic Sn from blood is relatively rapid (days), and is excreted primarily in urine. Measurement of urinary Sn may allow detection of long-term exposures to Sn	[46]
Tl (Thallium)	U	For the general population exposure to Tl occurs mainly from eating food. Tl is a highly toxic compound. High Tl concentrations have recently been detected in individual samples of mineral water collected for official food surveillance	[142, 143]
U (Uranium)	U	The main sources of exposure to U for the general population are dietary intake and drinking water. The primary biomarker of U is the detection of total U or individual U isotopes in the urine as it is excreted in urine mostly as uranyl ions. Twenty-four hour urine samples are considered to be the best biomarker for assessing U body burden. However, spot samples normalized to creatinine concentration could be representative of 24-hour U levels. Levels of urinary U reflect recent and accumulated exposure	[144]
Vanadium (V)	U, B	The general population is most likely to be exposed to V through the ingestion of contaminated food. V in urine and blood can be used as biomarkers of exposure	[59]
Zinc (Zn)	U,B	Zn is an essential element. Non-occupational exposure to Zn is mainly through food. Both serum and urine of humans could be used as biomarker of exposure to Zn	[145]

*B, blood; U, urine; H, hair; N, nails.

to represent both recent exposure and Cd accumulation over time.

Pb exposure has decreased substantially since the early 1970s, mainly because leaded gasoline and lead-based paints were phased out and the use of lead solder in food cans was virtually eliminated. Current potential sources of Pb exposure include drinking water from distribution systems containing lead pipes, and ingestion of food grown in areas with high levels of Pb in air, water or soil. Pb in blood (Pb-B) is the preferred indicator for evaluating human exposure to Pb, although other matrices such as urine, bone, hair and teeth have also been used [8]. The Pb-B level reflects a combination of recent exposure (short half-life pool in blood and soft tissues is about 30 days) and that which occurred several years earlier (long half-life pool in the skeleton in the range of 10–30 years) [112]. Urinary Pb levels may reflect recently absorbed Pb, though there is greater individual Pb variation in urine than in blood and greater potential for contamination [113]. Pb in hair has sometimes been used as a biomarker of exposure in some studies. However, as it is susceptible to external contamination, it is not a useful index of internal dose [150].

The ingestion of methylmercury, predominantly from fish and other seafood, constitutes the main source of dietary Hg exposure in the general population. Less than 15% of inorganic Hg (mainly from dental amalgams and demethylation of methylmercury) is absorbed by the human gastrointestinal tract, whereas the fraction of methylmercury absorbed is about 95%. The total blood Hg concentration (Hg-B) is due mostly to the dietary intake of organic forms, particularly methylmercury, and generally reflects short-term exposure (2–5 months). However, in populations with frequent fish consumption, Hg-B could be an indicator of average intake over time [151]. Urinary Hg consists mainly of inorganic Hg, and might be a suitable biomarker of inorganic Hg exposure [113, 152]. However, urinary total Hg (Hg-U) has also been related to fish consumption and may result from demethylation of methylmercury [153]. Hg concentration in hair has also been widely used as a biomarker of exposure for evaluating Hg accumulation in the body [154]. Total Hg in hair (Hg-H) reflects long-term average methylmercury exposure [155]. Likewise, total toenail and fingernail Hg are used as biomarkers for long-term methylmercury exposure [156, 157].

4.3.2 Biomonitoring programmes and studies

Nowadays many countries such as the USA [113], Canada [105], Germany [158], France [159], Slovenia [160], Czech Republic [161], Israel [162] or Flanders [163] have established HBM programmes that report representative levels of different biomarkers in non-occupationally exposed populations. These complement a range of several monitoring activities that study the levels of chemicals in water, food and air, and estimate the external exposure and risk assessment. HBM programmes are mainly used to detect time and spatial trends and differences between subpopulations, and aim to evaluate and support interventions, and in some cases like Germany to establish some statistical reference values. However, the different approaches taken by countries have meant the results are not always comparable. To overcome this situation, the European Comission is committed to developing, along with member states, a coherent approach (standardization in methodology, validated biomarkers, data integration and interpretation) to HBM in Europe [164, 165]. Thus, European scientists, authority experts and other stakeholders have created the COPHES consortium to develop a harmonized approach for HBM in Europe [166].

Likewise, a substantial number of HBM studies are currently running with significant resources assigned [119, 167–169]. However, these research projects pursue different scientific goals and use diverse methodologies, and it is therefore hard to compare the generated data. Moreover, in some cases data collected under research projects have a limited objective and their results are not available for more general HBM activities. Table 4.5 summarizes the metal levels in different biological matrices from relevant HBM programmes and studies.

The interpretation of biomarker levels from metal exposure is hampered by the general lack of screening criteria for evaluating such data in a health-risk context. Biomonitoring data are mainly interpreted in terms of exposure trends and identification of population groups with an unusually high exposure. Among the concepts that have been established for the interpretation of HBM data, the reference values (RV_{95}) proposed by the German Human Biomonitoring Commission are specially relevant [179]. This is a statistical value based on the 95th population percentile of the concentration level of the corresponding biomarker obtained from the reference population [180]. Table 4.6 shows a summary of

Table 4.5 Human biomonitoring of metals in relevant national programmes and studies.

Biomarker*	*n* (age, years)	Blood average (μg/L)/% < LOQ	Urine average (μg/L)/% < LOQ	Urine average(μg/g creatinine)/% < LOQ	Country/year	Reference
Sb-U	5492 (6–79)	—	0.08/22.40	0.08/22.45	Canada/2007–2009	[104]
Sb-U	2558 (6–79)	—	0.080	0.080	USA/2003–2004	[113]
Sb-U	2528 (13–16)	0.33/6.3	—	—	Latium Region (Italy)/2009	[170]
As-U	—	1.41/7.24	23.07/0.18	25.82/0.18	Canada/2007–2009	[104]
As-U	2557 (6–79)	—	8.30	8.24	USA/2003–2004	[132]
As-U	4052 (25–69)	—	3.87/5	—	Germany/1998	[158]
As-U	1354 (6–14)	—	4.29/2	—	Germany/2003–2006	[158]
As-B	252 (13–15)	0.82/9	—	—	Latium Region (Italy)/2009	[170]
As-U	960 (12–75)	—	—	1.29	Andalucía (Spain)	[171]
Ba-U, recent exposure	—	—	1.50 (GM)	1.48	USA/2003–2004	[113]
Be-U	2465	—	<LOD, 100%	<LOD/100	USA/2003–2004	[113]
Be-B	252 (13–15)	0.053/14	—	—	Latium Region (Italy)/2009	[170]
Cd-U/Cd-B	—	0.77/2.91	0.55/9.71	0.57/9.73	Canada/2007–2008	[104]
Cd-B/Cd-U	8372/2543	0.304	0.211	0.21	USA/2003–2004	[113]
Cd-U	1118 (18–58)	0.6 (smokers 1.3; non-smokers 0.4)	—	0.29 (*n* = 657)	Czech Republic/2001–2003	[172]
Cd-U	165 (23–66)	—	—	0.25	Madrid (Spain)/2009–2010	[173]
Cd-B	1679 (14–15)	0.39	—	—	Flanders (Belgium)/2003	[174]
Cd-U	1535 (50–65)	—	—	0.62	Flanders (Belgium)/2003–2006	[163]
Cd-B	1535 (50–65)	0.42	—	—	Flanders (Belgium)/2003–2006	[163]
Cd-U	400 (18–80)	—	—	0.46	UK/2005	[175]
Cd-B/Cd-U	3973/4052 (25–69)	0.43/5	0.243/3	—	Germany/1998	[158]
Cd-B/Cd-U	1245/1354 (6–14)	<0.12/53	0.071/27	—	Germany/2003–2006	[158]
Cd-B	252 (13–15)	0.26/3.5	—	—	Latium Region (Italy)/2009	[170]
Cd-B	960 (12–75)	0.14	—	—	Andalucía (Spain)	[171]
Cd-B	539 (18–65) (non-smokers)	0.08/65	—	—	Sao Paulo (Brazil)/2006	[176]
Cs-U	2558	—	4.67	4.64	USA/2003–2004	[113]
Co-U	2558	—	0.316	0.314	USA/2003–2004	[113]
Co-B	252 (13–15)	0.09/0	—	—	Latium Region (Italy)/2009	[170]

(Continued)

Table 4.5 (Continued)

Biomarker*	*n* (age, years)	Blood average (μg/L)/% < LOQ	Urine average (μg/L)/% < LOQ	Urine average (μg/g creatinine)/% < LOQ	Country/year	Reference
Cu-B/Cu-U	5318/5429 (6–79)	927/0	11.89/0.29	12.44/0.29	Canada/2003–2004	[104]
Cu-B	215 (18–89)	1036	—	—	Sardinia (Italy)/2009	[177]
Pb-B	8373 (6–79)	1.43	0.636	0.632	USA/2003–2004	[113]
Pb-B	—	1.66/0.02	0.76/7.54	0.81/7.56	Canada/2007–2008	[104]
Pb-B	1118 (18–58)	33	—	—	Czech Republic/2001–2003	[172]
Pb-B	333 (8–10)	31	—	—	Czech Republic/2001–2003	[172]
Pb-U	165 (23–66)	—	—	1.11	Madrid (Spain)/2009–2010	[173]
Pb-B	1679 (14–15)	22	—	—	Flanders (Belgium)/2003	[174]
Pb-B	1534 (50–65)	39.6	—	—	Flanders (Belgium)/2003–2006	[163]
Pb-B	3974 (25–69)	31.6 (GM)/6	—	—	Germany/1998	[158]
Pb-B	1245 (6–14)	15.7(GM)/0	—	—	Germany/2003–2006	[158]
Pb-B	252 (13–15)	9.50/0	—	—	Latium Region (Italy)/2009	[170]
Pb-B	539 (18–65)	23.65	—	—	Sao Paulo (Brazil)/2006	[176]
Hg-B/i-Hg-U	5319/5444 (6–79)	1.42/11.64	—/88.16	—/49.63	Canada/2007–2009	[104]
Hg-B/Hg-U	8373/2538	0.797	0.447	0.443	USA/2003–2004	[113]
i-Hg-B	8147	0.7 (95th)	—	—	USA/2003–2004	[113]
Hg-B	1118 (18–58)	0.82	—	0.61 ($n = 657$)	Czech Republic/2001–2003	[172]
Hg-B	333 (8–10)	0.43	—	0.45 ($n = 619$)	Czech Republic/2001–2003	[172]
Hg-U	170 (23–66)	—	—	1.23	Madrid (Spain)/2009–2010	[173]
Hg-U	400 (18–80)	—	—	1.12	UK/2005	[175]
Hg-B/Hg-U	3973/4052	0.61/31	0.40/12	—	Germany/1998	[158]
Hg-B; Hg-U	1240 (6–14)	0.24/40	0.10/54	—	Germany/2003–2006	[158]
Hg-B	252 (13–15)	0.84/5.5	—	—	Latium Region (Italy)/2009	[170]
Hg-U	960 (12–75)	—	—	1.16 (GM)	Andalucía (Spain)	[171]
Hg-H	316 (8–10)	—	—	—	Czech Republic/2008	[178]
Hg-B	593 (18–65)	0.993	—	—	Sao Paulo (Brazil)/2006	[176]
Mo-B	5319 (6–79)	0.76/0.09	53.08/0	53.64/0	Canada/2007–2009	[104]
Mo-U	2558 (6–79)	—	39.7	39.4	USA/2003–2004	[113]

Mo-B	252 (13–15)	1.12/15.8	—	—	Latium Region (Italy)/2009	[170]
Ni-B/Ni-U	5319/5491 (6–79)	0.75/6.69	1.62/3.15	1.77/3.16	Canada/2007–2009	[104]
Ni-B	252 (13–15)	1/21	—	—	Latium Region (Italy)/2009	[170]
Pt-U	2558 (6–79)	—	<LOD	<LOD	USA/2003–2004	[113]
Pt-B	252 (13–15)	9.87/3.1	—	—	Latium Region (Italy)/2009	[170]
Se-B	5319 (6–79)	204.1/0	63.05/0.46	65.22/0.46	Canada/2007–2009	[104]
Tl-U	215 (18–89)	0.04/0	—	—	Latium Region (Italy)/2009	[170]
Tl-U	2558 (6–79)	—	0.155	0.154	USA/2003–2004	[113]
Tl-U	252 (13–15)	0.041 (GM)	—	—	Latium Region (Italy)/2009	[170]
W-U	2558	—	0.071	0.07	USA/2003–2004	[113]
W-U	252 (13–15)	0.033 (GM)/0	—	—	Latium Region (Italy)/2009	[170]
U-U	2557	—	0.008	0.007	USA/2003–2004	[113]
U-U	5491	0/93.02	0/87.05	0/87.6	Canada/2007–2009	[104]
U-B	252 (13–15)	5.21/0	—	—	Latium Region (Italy)/2009	[170]
V-U	—	—	0/90.37	0/90.58	Canada/2007–2009	[104]
V-B	252 (13–15)	0.080 (GM)/2.7	—	—	Latium Region (Italy)/2009	[170]
Zn-B/Zn-U	5319/5492 (6–79)	6.44/0	389.50/0.78	376.88/0.78	Canada/2007–2009	[104]
Zn-B	960 (12–75)	979.6	—	—	Andalucía (Spain)	[171]

*B, blood; U, urine; LOQ, limit of quantification.

Table 4.6 Human biomonitoring assessment values.

Biomarker	Population group	RV_{95}* (µg/L)	BE (µg/L)	HBM-I* (µg/L)	HBM-II* (µg/L)
Sb-U	Children	0.3			
As-U	Children and adults who did not each fish 48 h prior to sample collection	15			
iAs-U			6.4		
Cd-U	Non smoking children	0.2	1.2–1.5†	0.5	2
	Non-smoking adults	0.8	(1.7–2 µg/g creatinine)†	1	4
Cd-B	Non smoking children	<0.3			
	Non-smoking adults	1.0			
Pb-B (blood level intervention: 10 µg/dL)‡	Children	35		S	S
	Women	70			
	Men	90			
Hg-U	Children without dental amalgam filling	0.4		7§	25§
	Adult without dental amalgam fillings	1.0			
Hg-B	Children who ate fish ≤3 times per month	0.8	5.8¶	5§	15§
	Adults who ate fish ≤3 times per month	2.0			
Hg-H				1 µg/g‖	
Ni-U	Children	4.5			
	Adults	3			
Pt-U	Adults without dental inlays, crowns or bridges elements made of precious metals	0.01			
Tl-U	Children	0.6		5	
	Adults	0.5			
	General population				
U-U	Children	0.04			
	Adults	0.03–0.06			

*From Schulz *et al*. [180].
†From Hays *et al*. [133].
‡Canada.
§Children and adults.
¶From Aylward *et al*. [184].
‖Hg hair safe limit (US EPA), 1997.
Age range of children, 3–14 years; age range of adults, 18–69 years, except for Th (20–29 years).
RV, reference values; BE, biomonitoring equivalents; HBM-I, Health related human biomonitoring values proposed by the HMB Commission of Germany B, blood; U, urine; S, suspended; i-As, inorganic arsenic; Hg-H, total mercury in hair.

the RV_{95} derived by the HBM Commission for metal biomarkers. Although reference values are not based on health-exposure guidelines and consequently are not used for risk characterization, if some individuals or groups presented levels higher than the RV_{95}, this would indicate that there is a higher-than-usual internal exposure and a number of actions would have to be promoted to explain these high values (e.g. specific sources of exposure, specific point in time or geographical area).

However, this RV_{95} is linked to the reference (German) population and may vary across countries and regions, and consequently should not be used for risk assessment

of other national programmes or studies developed in other countries. Apart from the biomonitoring programmes of Canada and the USA, which provide extensive information on the 95th percentiles of urine and blood concentrations of different metals, other authors have proposed reference ranges for biomarkers of metals such as Cd-U and Hg-U within UK populations [181], Cd-B and Pb-B for the general population of Sardinia (Italy) [182] or 26 trace elements in the urine of the adult population living in Belgium [183].

4.3.3 Risk characterization using biomonitoring

The application of HBM data to exposure assessment and risk characterization has attracted major interest in recent years. The International Life Science Institute organized in 2004 an International Biomonitoring Workshop focused on the use of biomonitoring data in the context of risk assessment [185]. Some case studies were discussed including organic chemicals that are short-lived in the environment [186, 187] as well as stable and bioaccumulative chemicals like inorganic As [147]. Scientists concluded that from the standpoint of exposure assessment purposes, biomonitoring should guarantee the validity of the biomarker of exposure selected, use an analytical method with sufficient specificity and sensitivity, implement a sampling strategy that includes consideration of toxicokinetics, have sufficient information about sources, pathways/routes of exposure, and have available data on chemical toxicokinetics in humans. Likewise, to use biomonitoring with a view to risk assessment, it is necessary that some epidemiological information supporting the link between human exposures and human health outcomes be available, and/or sufficient toxicology data on the relationship between the biomarker of exposure and a known health effect.

The biomonitoring data can be used for risk characterization if biomonitoring risk assessment values are available for the different chemicals. These values could be epidemiologically based (existing for only a few chemicals) or derived from existing HBGVs, such as the biomonitoring equivalents (BE) proposed by Hays and Aylward [188] or the health-related human biomonitoring values (HBM-I, HBM-II) proposed by the HBM Commission of Germany [180] (see Figure 4.1). BE is defined as the concentration or range of concentrations of a chemical or its metabolites in a biological matrix that is consistent with existing health-exposure guidance values such as those mentioned above. BE values have now been derived for about 90 substances [180].

The definition of HBM-I is very similar to that of BE, and describes the concentration in the body matrix of a substance below which no adverse health effect should be expected (no action would be needed from a toxicological perspective if concentrations are below the HBM-I value). HBM-I values are derived from ADI or TDI after toxicokinetic extrapolation. HBM-II is tied to biomarker concentrations which may be accompanied by adverse health effects [189]. Table 4.6 summarizes the BE and the HBM-I and HBM-II developed by Hays *et al*. [190] and the HBM Commission [180], respectively.

Taking into account these BE and HBM-I values, Aylward *et al*. [184] have performed an across-chemical risk assessment using biomonitoring data reported in the National Report on Human Exposure to Environmental Chemicals (NER) [191]. The authors compare levels of approximately 130 analytes (including Cd, inorganic As, Hg and Th) in the NER with BE and similar risk assessment values (HBM-I). The risk assessment was expressed in terms of hazard quotient (HQ = (biomarker)/BE). For metals, HQ values were close to or above 1 for Cd (in smokers) and inorganic As. However, the authors noted that inorganic As has a short half-life in the body and consequently its concentration distribution, which is expected to show high intraindividual variability, may not be informative of long-term average biomarker concentration in individuals.

In all the studies summarized in Table 4.5, the average levels of urinary Cd are below the BE of 1.7 µg/g creatinine established by Hays *et al*. [133]. Likewise, the levels of total Hg in blood in the studies performed in the USA, Czech Republic, Spain, Germany, Italy, Brazil or Canada are much lower than the BE of 5.8 µg/L proposed by Aylward *et al*. [184] or the 15 µg/L proposed by the German Human Biomonitoring Commission [180] (see Table 4.5). A similar conclusion for the concentrations of Hg in urine could be deduced.

4.4 Risk management and regulatory framework

Risk management is defined by the Food and Agriculture Organization (FAO) and the WHO [4] as the process of weighing policy alternatives in the light of the results of risk assessment and, if required, selecting and

implementing appropriate control options, including regulatory measures. In this context, risk management includes activities related to monitoring and control plans and regulations taking into account the precautionary principle.

Concern with the presence of contaminants in food has increased over the years, especially in the case of metals. However, legislation is usually introduced when a serious and potentially widespread hazard is identified. Consequently, regulations around the world referring specifically to metals in food include only a few metals such as As, Cd, Pb, Hg or Sn.

This section presents an overview of the legislation in force in Europe and the USA, as well as those international food standards for metals developed by the WHO/FAO through the Codex Alimentarius [192]. Furthermore, some specific European risk management tools such as the monitoring programmes and the rapid alert system for food and feed (RASFF) are presented.

4.4.1 Legislative framework and regulated levels in food

European Union (EU)

EU food legislation has two primary types of legislative acts, directives and regulations. Regulations are the most direct form of EU law. They have binding legal force and are directly applicable in all member states. Directives are addressed to national authorities, who must decide the form and methods to make them part of national law.

Regarding contaminants, Commission Regulation No. 1881/2006 set maximum levels (MLs) for certain contaminants in foodstuffs, including Pb, Cd, Hg and Sn [193]. In addition, Directive 1998/83/EC about the quality of water intended for human consumption set the parametric values for Sb, As, B, Cd, Cr, Cu, Pb, Hg, Ni, Se, Al, Fe, Mn and Na in drinking water [194]. Natural mineral waters are not included in this Directive, and maximum limits for 12 metals have been established in Directive 2003/40/EC [195]. Regarding food contact materials, Directive 84/500/EEC sets migration limits for Cd and Pb which might be released from decoration and/or glazing on ceramic objects [196]. A permanent update of this legislation can be found at the website of the EU [197].

Focusing on Commission Regulation (EC) No. 1881/2006, the MLs defined for heavy metals reflect the results of dietary exposure assessments carried out previously [198, 199] and have the main goal of reducing the presence of these metals as much as possible. The corresponding MLs are summarized in Table 4.7.

MLs are generally set for the edible part of the foodstuffs concerned, unless otherwise specified. For dried, diluted, processed and compound foodstuffs, no specific MLs have been established and therefore specific concentration and dilution factors should be taken into account.

Codex Alimentarius

The change from previous centuries, when food was produced and distributed locally, to the present century, where a huge variety and quantity of food is traded around the world, has led to the international harmonization of food standards.

In this context, the Codex Alimentarius Commision, established by the FAO and the WHO in 1963, develops internationally accepted food standards, guidelines, codes of practice and other recommendations. The guiding principle is to protect the health of consumers and ensure fair practices in food trade [200]. Standards may be described as 'vertical' or commodity standards for specific commodities and 'horizontal' or general standards applicable to all foods. The Codex General Standard for Contaminants and Toxins in Food and Feed [201] is an example of a horizontal standard. Specifically, Annex II of this standard contains the ML for some heavy metals such as As, Cd, Pb, Hg and Sn and the guideline levels (GLs) for methylmercury in certain foodstuffs about which there is particular concern. The proposed MLs and GLs are shown in Table 4.8. The Codex ML for a contaminant in a food or feed commodity is the maximum concentration of that substance recommended by the CAC to be legally permitted in that commodity. A Codex GL is the maximum level of a substance in a food or feed commodity which is recommended by the CAC to be acceptable for commodities moving in international trade [200].

Codex Alimentarius has become the global reference point for consumers, food producers and processors, national food control agencies and the international food trade. It promotes the harmonization of food standards and ensures their global implementation. The Codex Alimentarius is recognized by the World Trade Organization as an international reference point for resolving trade disputes in international laws concerning food safety and consumer protection [202].

Table 4.7 Maximum levels (MLs) of metals in foodstuffs (mg/kg wet weight) in the European Union.*

Foodstuffs	ML
Lead	
Raw milk, heat-treated milk and milk for the manufacture of milk-based products	0.020
Infant formulae and follow-on formulae	0.020
Meat (excluding offal) of bovine animals, sheep, pig and poultry	0.10
Offal of bovine animals, sheep, pig and poultry	0.50
Muscle meat of fish	0.30
Crustaceans: muscle meat from appendages and abdomen. In case of crabs and crab-like crustaceans (Brachyura and Anomura), muscle meat from appendages	0.50
Bivalve molluscs	1.5
Cephalopods (without viscera)	1.0
Legume vegetables, cereals and pulses	0.20
Vegetables, excluding brassica vegetables, leaf vegetables, fresh herbs, fungi and seaweed. For potatoes the maximum level applies to peeled potatoes	0.10
Brassica vegetables, leaf vegetables and the following fungi: *Agaricus bisporus* (common mushroom), *Pleurotus ostreatus* (oyster mushroom), *Lentinula edodes* (shiitake mushroom)	0.30
Fruit, excluding berries and small fruit	0.10
Berries and small fruit	0.20
Fats and oils, including milk fat	0.10
Fruit juices, concentrated fruit juices as reconstituted and fruit nectars	0.050
Wine (including sparkling wine, excluding liqueur wine), cider, perry and fruit wine	0.20
Aromatized wine, aromatized wine-based drinks and aromatized wine-product cocktails	0.20
Food suplements	3.0
Cadmium	
Meat (excluding offal) of bovine animals, sheep, pig and poultry	0.050
Horsemeat, excluding offal	0.20
Liver of bovine animals, sheep, pig, poultry and horse	0.50
Kidney of bovine animals, sheep, pig, poultry and horse	1.0
Muscle meat of fish, excluding species listed below	0.050
Muscle meat of the following fish: mackerel (*Scomber* spp.), tuna (*Thunnus* spp., *Euthynnus* spp., *Katsuwonus pelamis*), binique (*Sicyopterus lagocephalus*)	0.10
Muscle meat of the following fish: bullet tuna (*Auxis* spp.)	0.15
Muscle meat of the following fish: anchovy (*Engraulis* spp.), swordfish (*Xiphias gladius*), sardine (*Sardina pilchardus*)	0.25
Crustaceans: muscle meat from appendages and abdomen. In case of crabs and crab-like crustaceans (Brachyura and Anomura), muscle meat from appendages	0.50
Bivalve molluscs	1.0
Cephalopods (without viscera)	1.0
Cereal grains excluding wheat and rice	0.10
Wheat grains, rice grains, soy beans, wheat bran and wheat germ for direct consumption	0.20
Vegetables and fruit, excluding leaf vegetables, fresh herbs, leafy brassica, fungi, stem vegetables, root and tuber vegetables and seaweed	0.050
Stem vegetables (excluding celery), root and tuber vegetables (excluding celeriac, parsnips, salsify and horseradish). For potatoes the maximum level applies to peeled potatoes	0.10
Leaf vegetables, fresh herbs, leafy brassica, celery, celeriac, parsnips, salsify, horseradish and the following fungi: *Agaricus bisporus* (common mushroom), *Pleurotus ostreatus* (oyster mushroom), *Lentinula edodes* (shiitake mushroom)	0.20
Fungi, excluding those listed in the previous point	1.0
Food supplements excluding food supplements listed below	1.0
Food supplements consisting exclusively or mainly of dried seaweed, products derived from seaweed, or of dried bivalve molluscs	3.0

(Continued)

Table 4.7 (Continued)

Foodstuffs	ML
Mercury	
Fishery products and muscle meat thereof, excluding species listed in the next point. The maximum level for crustaceans applies to muscle meat from appendages and abdomen. In case of crabs and crab-like crustaceans (Brachyura and Anomura), it applies to muscle meat from appendages	0.50
Muscle meat of the following fish: anglerfish (*Lophius species*), Atlantic catfish (*Anarhichas lupus*), bonito (*Sarda sarda*), eel (*Anguilla species*), emperor, orange roughy, rosy soldierfish (*Hoplostethus species*), grenadier (*Coryphaenoides rupestris*), halibut (*Hippoglossus hippoglossus*), kingklip (*Genypterus capensis*), marlin (*Makaira species*), megrim (*Lepidorhombus species*), mullet (*Mullus species*), pink cusk eel (*Genypterus blacodes*), pike (*Esox lucius*) plain bonito (*Orcynopsis unicolor*), poor cod (*Tricopterus minutes*), Portuguese dogfish (*Centroscymnus coelolepis*), rays (*Raja species*), redfish (*Sebastes marinus, S. mentella, S. viviparus*), sail fish (*Istiophorus platypterus*), scabbard fish (*Lepidopus caudatus, Aphanopus carbo*), seabream, pandora (*Pagellus species*), shark (*all species*), snake mackerel or butterfish (*Lepidocybium flavobrunneum, Ruvettus pretiosus, Gempylus serpens*), sturgeon (*Acipenser species*), swordfish (Xiphias gladius), tuna (*Thunnus species, Euthynnus species, Katsuwonus pelamis*)	1.0
Food supplements	0.10
Tin (inorganic)	
Canned foods other than beverages	200
Canned beverages, including fruit juices and vegetable juices	100
Canned baby foods and processed cereal-based foods for infants and young children, excluding dried and powdered products	50
Canned infant formulae and follow-on formulae (including infant milk and follow-on milk), excluding dried and powdered products	50
Canned dietary foods for special medical purposes intended specifically for infants, excluding dried and powdered products	50

*Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

4.4.2 Monitoring, sampling and methods of analysis

This section is focused on the monitoring and other related activities carried out by the EU. Regarding official controls, Regulation (EC) No. 882/2004 [203] establishes that, in order to have a global and uniform approach, member states should set and implement multi-annual national control plans in accordance with broad guidelines drawn up at Community level. These guidelines should promote coherent national strategies, and identify risk-based priorities and the most effective control procedures. Examples of this include the Multi-Annual National Control Plan for the UK [204], the National Plan of Official Control of the Food Chain in Spain [205] and the Multi annual Control Plan of Belgium [206].

In addition, competent authorities are to ensure that they carry out their activities with a high level of transparency. Furthermore, member states should be required to present an annual report to the Commission with information on the implementation of the multi-annual national control plans. A data-collection programme issues a variety of reports published by EFSA [207].

Sampling and analytical methods play a crucial role in the quality of data, including accuracy and precision, on the investigation of the levels of metals in food commodities. Regarding sampling and analysis, Regulation (EC) No. 882/2004 establishes some general and relevant Community rules. In the course of sampling, precautions shall be taken to avoid any changes which would affect the levels of contaminants, adversely affect the analytical determination or make the aggregate samples unrepresentative. In the EU, methods of sampling for the official control of levels of Pb, Cd, Hg and inorganic Sn in foodstuffs have to fulfill the sampling methods described in Regulation (EC) No. 333/2007 [208].

In terms of analysis, competent authorities are assisted by official control laboratories, national reference laboratories and community reference laboratories. Official laboratories are designated by the competent authorities for the purpose of analysing samples taken during official controls. However, competent authorities may only designate laboratories that operate and are assessed and accredited in accordance with the following European Standards: EN ISO/IEC 17025, EN 45002 and EN 45003.

Table 4.8 Maximum level (ML) (mg/kg) and guideline level (GL, only for methylmercury) (mg/kg) for metals in food.*

Food product	ML
Mercury	
Natural mineral waters	0.001†
Salt, food grade	0.1
Methylmercury	
Fish	0.5‡
Predatory fish	1‡
Arsenic	
Edible fats and oils	0.1
Margarine	0.1
Minarine	0.1
Named animal fats	0.1
Olive oil, refined	0.1
Olive oil, virgin	0.1
Olive, residue oil	0.1
Vegetable oils, crude	0.1
Vegetable oils, edible	0.1
Natural mineral waters	0.01†
Salt, food grade	0.5
Cadmium	
Brassica vegetables	0.05
Bulb vegetables	0.05
Fruiting vegetables, curcubits	0.05
Fruting vegetables, other than curcubits	0.05
Leafy vegetables	0.2
Legume vegetables	0.1
Potato	0.1
Pulses	0.1
Root and tuber vegetables	0.1
Stalk and stem vegetables	0.1
Cereal grains, except buckwheat, cañihua and quinoa	0.1
Rice, polished	0.4
Wheat	0.2
Marine bivalve molluscs	2
Cephalopods	2
Natural mineral waters	0.003
Salt, food grade	0.5
Lead	
Assorted (sub)tropical fruits, edible peel	0.1
Assorted (sub)tropical fruits, inedible peel	0.1
Berries and other small fruits	0.2
Citrus fruits	0.1
Pome fruits	0.1
Stone fruits	0.1
Brassica vegetables	0.3
Bulb vegetables	0.1
Fruiting vegetables, cucurbits	0.1
Fruiting vegetables, other than curcurbits	0.1
Leafy vegetables	0.3
Legume vegetables	0.2
Pulses	0.2
Root and tuber vegetables	0.1
Canned fruit cocktail	1
Canned grapefruit	1
Canned mandarin oranges	1
Canned mangoes	1
Canned pineapple	1
Canned raspberries	1
Canned strawberries	1
Canned tropical fruit salad	1
Jams (fruit preserves) and jellies	1
Mango chutney	1
Table olives	1
Canned asparagus	1
Canned carrots	1
Canned green beans and canned wax beans	1
Canned green peas	1
Canned mature processed peas	1
Canned mushrooms	1
Canned palmito	1
Canned sweetcorn	1
Canned tomatoes	1
Pickled cucumbers	1
Processed tomato concentrates	1.5
Fruit juices	0.05
Cereal grains, except buckwheat, cañihua and quinoa	0.2
Canned chestnuts and canned chestnuts purée	1
Meat of cattle, pigs and sheep	0.1
Poultry meat	0.1
Cattle, edible offal of	0.5
Pig, edible offal of	0.5
Poultry, edible offal of	0.5
Edible fats and oils	0.1
Fish	0.3
Margarine	0.1
Minarine	0.1
Named animal fats	0.1
Olive oil, refined	0.1
Olive oil, virgin	0.1
Olive oil, residue oil	0.1
Poultry fats	0.1
Vegetable oils, crude	0.1
Vegetable oils, edible	0.1
Milks	0.02
Secondary milk products	0.02
Natural mineral waters	0.01
Infant formula	0.02
Salt, food grade	2
Wine	0.2

(Continued)

Table 4.8 (Continued)

Food product	ML
Tin	
Canned foods (other than beverages)	250
Canned beverages	250
Canned citrus fruits	250
Jams, jellies and marmalades	250
Canned stone fruits	250
Canned vegetables	250
Canned fruit cocktail	250
Canned mangoes	250
Canned pineapple	250
Canned raspberries	250
Canned strawberries	250
Canned tropical fruit salad	250
Mango chutney	250
Table olives	250
Canned mushrooms	250
Canned tomatoes	250
Pickled cucumbers	250
Processed tomato concentrates	250
Fruit juices	250
Cereal grains, except buckwheat, cañihua and quinoa	250
Canned chestnuts and canned chestnuts purée	250
Cooked cured chopped meat (tinplate containers)	200
Cooked cured chopped meat (other containers)	50
Cooked cured ham (tinplate containers)	200
Cooked cured ham (other containers)	50
Cooked cured pork shoulder (tinplate containers)	200
Cooked cured pork shoulder (other containers)	50
Corned beef (tinplate containers)	200
Corned beef (other containers)	50
Luncheon meat (tinplate containers)	200
Luncheon meat (other containers)	50

*CODEX (1995) Codex General Standard for Contaminants and Toxins in Food and Feed.
†Expressed in mg/L.
‡GLs are intended for methylmercury in fresh or processed fish and fish products moving in international trade.

All of them should have implemented quality assurance programmes in order to fulfil the requirements established by Regulation (EC) No. 882/2004.

The methods of analysis used for food control purposes shall comply with the general criteria of points 1 and 2 of Annex III to Regulation (EC) No. 882/2004. In addition, as no specific methods for the determination of metals in foodstuffs are prescribed at Community level, laboratories may select any validated method of analysis (where possible, the validation shall include a certified reference material) provided the selected method meets the specific performance criteria set out in Table 4.9.

4.4.3 The European Community's Rapid Alert System for Food and Feed

As part of the risk management activities implemented by the EU, Regulation EC/178/2002 [209], better known as the General Food Law, established the Rapid Alert System for Food and Feed (RASFF). Under the RASFF system, members, such as the national food control authorities of EU member states, are obliged to notify the European Commission of any measure taken regarding food safety should there be any information about a serious health risk deriving from food or feed. In particular, RASFF members are to notify any measures taken such as withdrawing or recalling food or feed products from the market in order to protect consumers' health and whether rapid action is required. They must also notify any specific agreements with the operator responsible and if, as a consequence, food or feed should not be placed on the market. Additionally, they should disclose if the measure is taken on account of a serious risk or if the product in question is placed on the market under specific conditions.

The European Commission publishes weekly overviews and annual reports on the number of notifications and the pertaining categories of food products and hazards. Figure 4.5 depicts both the number of total notifications and notifications referring to metals in food during the period 2008–2013. Metal notifications represent 7–14% of the overall notifications and they include chemical, biological and physical hazards, alongside hygiene hazards and issues concerning quality, fraud, defective packaging and labelling.

Figure 4.6 shows the distribution of metal notifications between the different foodstuffs during the period 2008–2012. It clearly shows that Hg in fish is the most reported hazard in relation to heavy metals, followed by Cd in fishery products (fish, crustaceans and cephalopods).

The distribution of notifications of heavy metals related to migration from food contact materials is shown in Figure 4.7. Cr, followed by Pb and Cd, are the most frequently informed heavy metals in food contact materials. For the migration of Pb and Cd from ceramics, EU legal limits are in force.

Table 4.9 Performance criteria for methods of analysis for lead, cadmium, mercury and inorganic tin in the EU.*

Parameter	Value/comment
Applicability	Foods specified in Regulation (EC) No. 1881/2006
LOD	For inorganic tin less than 5 mg/kg
	For other elements less than one-tenth of the maximum level in Regulation (EC) No. 1881/2006, except if the maximum level for lead is less than 100 µg/kg. For the latter, less than one-fifth of the maximum level
LOQ	For inorganic tin less than 10 mg/kg
	For other elements less than one-fifth of the maximum level in Regulation (EC) No. 1881/2006, except if the maximum level for lead is less than 100 µg/kg. For the latter, less than two-fifth of the maximum level
Precision	HORRATr† or HORRATR‡ values of less than 2
Recovery	The result may be reported uncorrected for recovery if evidence is provided by ideally making use of suitable certified reference material that the certified concentration allowing for the measurement uncertainty is achieved (i.e. high accuracy of measurement). In case the result is reported uncorrected for recovery this shall be mentioned
Specificity	Free from matrix or spectral interferences

*Commission Regulation (EC) No. 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs.
†HORRATr: the observed RSDr divided by the RSDr value estimated from the Horwitz equation using the assumption $r = 0.66R$, where r is repeatability, R is reproducibility and RSDr is relative standard deviation calculated from results generated under repeatability conditions.
‡HORRATR: the observed RSDR value divided by the RSDR value calculated from the Horwitz equation, where RSDR is relative standard deviation calculated from results generated under reproducibility conditions.



Figure 4.5 Number of RASFF notifications (total and metals) during the period 2008–2012.

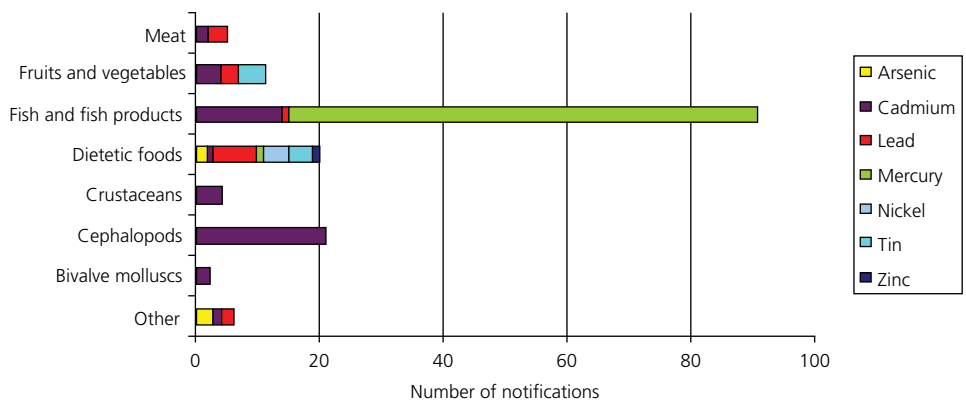


Figure 4.6 RASFF heavy metals in food notifications during the period 2008–2012. (See insert for colour representation of the figure.)

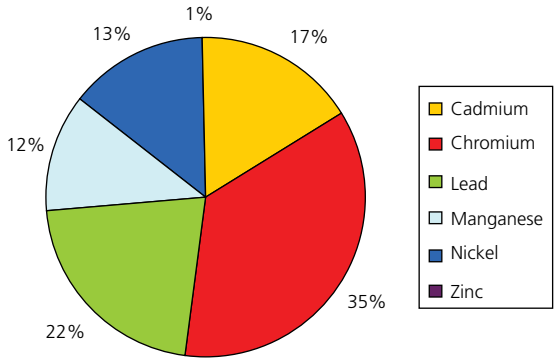


Figure 4.7 RASFF notifications on heavy metals in food contact materials during the period 2008–2012. (See insert for colour representation of the figure.)

4.5 Conclusions and future perspectives

This chapter has highlighted the principles and methods for the assessment of risk by trace metals. This includes the classical external exposure approach and an emergent approach that uses biomonitoring data as a tool for risk assessment. For the purposes of risk assessment, knowing the average intake of a metal is not enough. When the average intake is not far from the HBGV, variability in food consumption and in occurrence should be taken into account in the exposure assessment. Consequently, it seems appropriate to consider probabilistic dietary exposure assessments on a more routine basis [209], mainly when refinements are needed. In this context, a more accurate measure of the uncertainty and variability associated with each risk assessment seems to be necessary in order to be able to provide enough information to risk managers.

Although some international guidelines have been developed for performing harmonized TDS, significant methodological differences between studies still remain. These are mainly related to the type of dietary surveys that provide the consumption data, the statistical methodologies for the estimation of usual intakes from short-term dietary data, the classification of food groups and the use of composites, the pre-analytical treatment of samples, the performance of the analytical methods or the treatment of left-censored data. Further standardization efforts should be performed at an international level to make better comparison between studies feasible.

There is increasing use of biomonitoring as a tool for studying the exposure of the general population to chemicals. However, to interpret HBM data in a risk-assessment context, some metrics are required (health-based reference values). The development of BE, HBM-II or other related health-based reference values for the various metals or other chemicals are critical for more widespread use of biomonitoring in food safety risk assessment. Biomonitoring does not replace the use of the current risk assessment paradigm based on external exposure, (e.g. biomonitoring does not provide information on pathways and routes of exposure), but having metrics such as BE or HBM-II for the different chemicals could help to provide more insight on the biomonitoring data in a public health risk context.

Abbreviations

ADI: Acceptable daily intake: estimate of the amount of a substance in food or drinking water that can be ingested over a lifetime without appreciable risk

ATSDR: Agency for Toxic Substances and Disease Registry

BMD: Benchmark dose

BMDL: Benchmark dose lower confidence limit

EFSA: European Food Safety Authority

HI: Hazard index: sum of more than one hazard quotient for multiple substances and/or multiple exposure pathways. The HI is calculated separately for chronic, sub-chronic, and shorter-duration exposures

HQ: Hazard quotient: ratio of a single substance exposure level over a specified time period (e.g. sub-chronic) to a reference dose for that substance derived from a similar exposure period

JEFCA: Joint WHO/FAO Expert Committee on Food Additives

LOAEL: Lowest-observed-adverse-effect level

LOD: Limit of detection

LOQ: Limit of quantitation

MOE: Margin of exposure: ratio of a specific point on a dose–response curve for adverse effects obtained in animal experiments (in the absence of human epidemiological data) and human intake data. The preferred reference points describing the dose–response relationship are the BMD and BMDL

MRL: Minimal risk level

NOAEL: No-observed-adverse-effect level

PMTDI: Permissible maximum tolerable intake: permissible human exposure as a result of natural occurrence of the substance in food and drinking water. For trace essential elements, a range is expressed; the lower value represents the level of essentiality and the upper value the PMTDI [61]. Calculated for contaminants with no cumulative properties

PTWI: Provisional tolerable weekly intake

RDA: Recommended daily allowance: average daily intake level that is sufficient to meet the nutrient requirements of nearly all healthy individuals in a particular life stage and gender group

RfD: Reference dose: estimate of the daily exposure to which the human population may be continually exposed over a lifetime without appreciable risk of deleterious effect

TWI: Tolerable weekly intake

UL: Tolerable upper intake level: highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population

USEPA: United States Environmental Protection Agency

USFNB: United States Food Nutrition Board

WHO: World Health Organization

References

1 Environmental Protection Agency. *Framework for Metals Risk Assessment*. US EPA, Washington, DC, 2007. Available at http://www.epa.gov/raf/metalsframework/pdfs/metals-risk-assessment-final.pdf.

2 National Research Council. *Human Biomonitoring for Environmental Chemicals*. National Academy of Sciences, Washington, DC, 2006.

3 FAO/WHO. *Food Safety Risk Analysis. A Guide for National Food Safety Authorities*. FAO, Rome, 2006. Available at ftp://ftp.fao.org/docrep/fao/009/a0822e/a0822e00.pdf.

4 FAO/WHO. *Principles and Methods for the Risk Assessment of Chemicals in Food. Chapter 2: Risk assessment and its role in risk analysis*. WHO, Geneva, 2009. Available at http://whqlibdoc.who.int/ehc/WHO_EHC_240_5_eng_Chapter2.pdf.

5 European Food Safety Authority. Statement on tolerable weekly intake for Cd. *EFSA J* 2011;**9**(2):1975.

6 Dorne JL, Fink-Gremmels J. Human and animal health risk assessments of chemicals in the food chain: comparative aspects and future perspectives. *Toxicol Appl Pharmacol* 2013;**270**:187–195.

7 FAO/WHO. *Evaluation of certain food additives and contaminants. WHO Technical Report Series 960*. WHO, Geneva, 2011. Available at http://whqlibdoc.who.int/trs/who_trs_960_eng.pdf.

8 European Food Safety Authority. Scientific opinion on lead in food. *EFSA J* 2010;**8**(4):1570.

9 Agency for Toxic Substances and Disease Registry. Available at http://www.atsdr.cdc.gov/ (accessed October 2013).

10 European Food Safety Authority. Use of the benchmark dose approach in risk assessment. *EFSA J* 2009;**1150**:1–72.

11 Organisation for Economic Cooperation and Development. *OECD Guidelines for the Testing of Chemicals*. 2009. Available at http://www.oecd-ilibrary.org/content/package/chem_guide_pkg-en (accessed October 2013).

12 Kossowska B, Dudka I, Gancarz R, Antonowicz-Juchniewicz J. Application of classic epidemiological studies and proteomics in research of occupational and environmental exposure to lead, cadmium and arsenic. *Int J Hyg Environ Health* 2013;**216**:1–7.

13 FAO/WHO. Principles and Methods for the Risk Assessment of Chemicals in Food. Chapter 5: Dose–response assessment and derivation of health based guidance values. WHO, Geneva, 2009. Available at http://whqlibdoc.who.int/ehc/WHO_EHC_240_8_eng_Chapter5.pdf.

14 Environmental Protection Agency. A Review of the Reference Dose and Reference Concentration Processes. EPA, Washington, DC, 2002.

15 Agency for Toxic Substances and Disease Registry. Toxic Substances Portal. Minimal Risk Levels (MRLs). Available at http://www.atsdr.cdc.gov/mrls/index.asp (accessed September 2013).

16 Institute of Medicine. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. National Academies Press, Washington, DC, 2001.

17 Dorne JLCM, Renwick AG. The refinement of uncertainty/safety factors in risk assessment by the incorporation of data on toxicokinetic variability in humans. *Toxicol Sci* 2005;**86**:20–26.

18 Dorne JL. Metabolism, variability and risk assessment. *Toxicology* 2010;**268**:156–164.

19 European Food Safety Authority. Opinion of the scientific committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. *EFSA J* 2005;**282**:1–31.

20 Barlow S, Renwick AG, Kleiner J *et al*. Risk assessment of substances that are both genotoxic and carcinogenic. Report of an International Conference organized by EFSA and WHO with support of ILSI Europe. *Food Chem Toxicol* 2006;**44**:1636–1650.

21 O'Brien J, Renwick AG, Constable A *et al*. Approaches to the risk assessment of genotoxic carcinogens in food: a critical appraisal. *Food Chem Toxicol* 2006;**44**:1613–1635.

22 Environmental Protection Agency. *Guidelines for Carcinogen Risk Assessment*. EPA, Washington, DC, 2005.

23 European Food Safety Authority. Opinion of the scientific committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. *EFSA J* 2005;**282**:1–31.
24 European Food Safety Authority. Scientific opinion on arsenic in food. *EFSA J* 2009;**7**(10):1351.
25 WHO. *Evaluation of Certain Contaminants in Food*. WHO, Geneva, 2011. Available at http://whqlibdoc.who.int/trs/who_trs_959_eng.pdf.
26 Agency for Toxic Substances and Disease Registry. Toxicological profile for arsenic, August 2007. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=22&tid=3.
27 Environmental Protection Agency. *Toxicological Review of Barium and Compounds*. EPA, Washington, DC, 2005.
28 Agency for Toxic Substances and Disease Registry. *Toxicological Profile for Barium and Barium Compounds*. ATSDR, Atlanta, GA, 2007.
29 Agency for Toxic Substances and Disease Registry. Toxicological profile for beryllium, September 2002. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=185&tid=33.
30 WHO. Beryllium in drinking-water. Available at http://www.who.int/water_sanitation_health/dwq/chemicals/beryllium_in_drinking2.pdf.
31 Agency for Toxic Substances and Disease Registry. Toxicological profile for cadmium, September 2012. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=48&tid=15.
32 Environmental Protection Agency. Integrated Risk Information System. Cadmium. Available at http://www.epa.gov/iris/subst/0141.htm (accessed December 2013).
33 Finley BL, Paustenbach DJ, Gaffney SH. Derivation of a chronic oral reference dose for cobalt. *Regul Toxicol Pharmacol* 2012;**64**:491–503.
34 Agency for Toxic Substances and Disease Registry. Toxicological profile for chromium, September 2012. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=62&tid=17.
35 European Food Safety Authority. *Tolerable Upper Intake Levels for Vitamins and Minerals*. EFSA, Parma, Italy, 2006. Available at http://www.efsa.europa.eu/en/ndatopics/docs/ndatolerableuil.pdf.
36 WHO. Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Copper. Available at http://www.inchem.org/documents/jecfa/jecmono/v17je31.htm (accessed November 2013).
37 WHO. Joint FAO/WHO Expert Committee on Food Additives. Iron. Available at http://www.inchem.org/documents/jecfa/jeceval/jec_1132.htm (accessed November 2013).
38 European Food Safety Authority. Scientific opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA J* 2012;**10**(12):2985.
39 Agency for Toxic Substances and Disease Registry. Toxicological profile for manganese, September 2012. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=102&tid=23.
40 Environmental Protection Agency. Integrated Risk Information System. Molybdenum. Available at http://www.epa.gov/iris/subst/0425.htm (accessed September 2013).
41 European Food Safety Authority. Scientific opinion on dietary reference values for molybdenum. *EFSA J* 2013;**11**(8):3333.
42 WHO. Guidelines for drinking-water quality, 3rd edn: incorporating first and secnd addenda, Vol. **1**, Recommendations. WHO, Geneva, 2008. Available at http://www.who.int/water_sanitation_health/dwq/fulltext.pdf?ua=1.
43 European Food Safety Authority. Lead dietary exposure in the European population. *EFSA J* 2012;**10**(7):2831.
44 Environmental Protection Agency. Integrated Risk Information System. Antimony. Available at http://www.epa.gov/iris/subst/0006.htm (accessed December 2013).
45 Environmental Protection Agency. Integrated Risk Information System. Selenium and Compounds. Available at http://www.epa.gov/iris/subst/0472.htm (accessed September 2013).
46 Agency for Toxic Substances and Disease Registry. Toxicological profile for tin, August 2005. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=543&tid=98.
47 Joint FAO/WHO Expert Committee on Food Additives. Tin (addendum). In: Safety Evaluation of Certain Food Additives and Contaminants. WHO Food Additives Series 46. WHO, Geneva, 2001, pp. 307–360. Available at http://www.inchem.org/documents/jecfa/jecmono/v46je01.htm.
48 Environmental Protection Agency. Integrated Risk Information System. Thallium (I), soluble salts. Available at http://www.epa.gov/iris/subst/1012.htm (accessed November 2013).
49 WHO. Uranium in Drinking-water. WHO, Geneva, 2012. Available at http://www.who.int/water_sanitation_health/publications/2012/background_uranium.pdf.
50 European Food Safety Authority. Vanadium citrate, bismaltolato oxo vanadium and bisglycinato oxo vanadium added for nutritional purposes to foods for particular nutritional uses and foods (including food supplements) intended for the general population and vanadyl sulphate, vanadium pentoxide and ammonium monovanadate added for nutritional purposes to food supplements. *EFSA J* 2008;**634**:1–15.
51 Environmental Protection Agency. *Toxicological Review of Zinc and Compounds*. EPA, Washington, DC, 2005.
52 European Food Safety Authority. Cadmium in food. *EFSA J* 2009;**980**:1–139.
53 FAO/WHO. *Safety Evaluation of Certain Food Additives and Contaminants*. WHO Food Additive Series 64. WHO, Geneva, 2011.
54 WHO. *Evaluation of Certain Food Additives and Contaminants*. WHO Technical Report Series 940. WHO, Geneva, 2007.
55 WHO. Guidelines for drinking-water quality, 3rd edn: incorporating first and secnd addenda, Vol. **1**, Recommendations. WHO, Geneva, 2008. Available at http://www.who.int/water_sanitation_health/dwq/fulltext.pdf?ua=1.
56 Agency for Toxic Substances and Disease Registry. Toxicological profile for barium, August 2007. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=327&tid=57.
57 European Food Safety Authority. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission to assess the health risks to consumers

associated with exposure to organotins in foodstuffs. *EFSA J* 2004;**102**:1–119.
58 European Food Safety Authority. Vanadium citrate, bismaltolato oxo vanadium and bisglycinato oxo vanadium added for nutritional purposes to foods for particular nutritional uses and foods (including food supplements) intended for the general population and vanadyl sulphate, vanadium pentoxide and ammonium monovanadate added for nutritional purposes to food supplements. *EFSA J* 2008;**634**:1–15.
59 Agency for Toxic Substances and Disease Registry. Toxicological profile for vanadium, September 2012. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=276&tid=50.
60 Renwick AG, Walker R. Risk assessment of micronutrients. *Toxicol Lett* 2008;**180**:123–130.
61 Goldhaber SB. Trace element risk assessment: essentiality vs. toxicity. *Regul Toxicol Pharmacol* 2003;**38**:232–242.
62 Navarro-Alarcon M, Cabrera-Vique C. Selenium in food and the human body: a review. *Sci Total Environ* 2008;**400**:115–141.
63 European Food Safety Authority. Manganese ascorbate, manganese aspartate, manganese bisglycinate and manganese pidolate as sources of manganese added for nutritional purposes to food supplements. *EFSA J* 2009;**1114**:1–23.
64 European Food Safety Authority. Scientific opinion on dietary reference values for manganese. *EFSA J* 2013;**11**:3419.
65 Environmental Protection Agency. Integrated Risk Information System. Manganese. Available at http://www.epa.gov/iris/subst/0373.htm.
66 Dorne JLCM, Bordajandi LR, Amzal B, Ferrari P, Verger P. Combining analytical techniques, exposure assessment and biological effects for risk assessment of chemicals in food. *Trends Anal Chem* 2009;**28**:695–707.
67 Kroes R, Müller D, Lambe J *et al*. Assessment of intake from the diet. *Food Chem Toxicol* 2002;**40**:327–385.
68 European Food Safety Authority. Overview of the procedures currently used at EFSA for the assessment of dietary exposure to different chemical substances. *EFSA J* 2011;**9**(12):2490.
69 WHO. Principles and Methods for the Risk Assessment of Chemicals in Food. Chapter 6: Dietary Exposure Assessment of Chemicals in Food. WHO, Geneva, 2009. Available at http://whqlibdoc.who.int/ehc/WHO_EHC_240_9_eng_Chapter6.pdf.
70 Dorne JL, Kass GE, Bordajandi LR *et al*. Human risk assessment of heavy metals: principles and applications. *Met Ions Life Sci* 2011;**8**:27–60.
71 Arnich N, Sirot V, Riviere G *et al*. Dietary exposure to trace elements and health risk assessment in the 2nd French Total Diet Study. *Food Chem Toxicol* 2012;**50**:2432–2449.
72 Domingo JL, Perello G, Giné Bordonaba J. Dietary intake of metals by the population of Tarragona County (Catalonia, Spain): results from a duplicate diet study. *Biol Trace Elem Res* 2012;**146**:420–425.
73 Martorell I, Perello G, Marti-Cid R, Llobet JM, Castell V, Domingo JL. Human exposure to arsenic, cadmium, mercury, and lead from foods in Catalonia, Spain: temporal trend. *Biol Trace Elem Res* 2011;**142**:309–322.
74 Health Canada. Canadian Total Diet Study, October 2013. Available at http://www.hc-sc.gc.ca/fn-an/surveill/total-diet/index-eng.php.
75 Rose M, Baxter M, Brereton N, Baskaran C. Dietary exposure to metals and other elements in the 2006 UK Total Diet Study and some trends over the last 30 years. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 2010;**27**:1380–1404.
76 New Zealand Ministry of Agriculture and Forestry. *2009 New Zealand Total Diet Study. Agricultural compound residues, selected contaminant and nutrient elements*. Ministry of Agriculture and Forestry, Wellington, New Zealand, 2011. Available at http://www.foodsafety.govt.nz/elibrary/industry/total-diet-study.pdf.
77 Wong WWK, Chung SWC, Chan BTP, Ho YY, Xiao Y. Dietary exposure to inorganic arsenic of the Hong Kong population: results of the first Hong Kong total diet study. *Food Chem Toxicol* 2013;**51**:379–385.
78 Food Standards Australia New Zealand. The 23rd Australian Total Diet Study. Available at http://www.foodstandards.gov.au/publications/pages/23rdaustraliantotald5367.aspx.
79 Turconi G, Minoia C, Ronchi A, Carla Roggi C. Dietary exposure estimates of twenty-one trace elements from a total diet study carried out in Pavia, Northern Italy. *Br J Nutr* 2009;**101**:1200–1208.
80 Becker W, Jorhem L, Sundström B, Grawé KP. Contents of mineral elements in Swedish market basket diets. *J Food Comp Anal* 2011;**24**:279–287.
81 Nasreddine L, Nashalian O, Naja F *et al*. Dietary exposure to essential and toxic trace elements from a total diet study in an adult Lebanese urban population. *Food Chem Toxicol* 2010;**48**:1262–1269.
82 Bocio A, Domingo JL. Human exposure to metals through the diet in Tarragona, Spain. *Biol Trace Elem Res* 2005;**104**:193–201.
83 Bellés M, Linares V, Perelló G, Domingo JL. Human dietary exposure to uranium in Catalonia, Spain. *Biol Trace Elem Res* 2013;**152**:1–8.
84 Thomson BM, Vannoort RW, Haslemore RM. Dietary exposure and trends of exposure to nutrient elements iodine, iron, selenium and sodium from the 2003–4 New Zealand Total Diet Survey. *Br J Nutr* 2008;**99**:614–625.
85 Nasreddine L, Hwalla N, El Samad O *et al*. Dietary exposure to lead, cadmium, mercury and radionuclides of an adult urban population in Lebanon: a total diet study approach. *Food Addit Contam* 2006;**23**:579–590.
86 EFSA/FAO/WHO. State of the art on total diet studies based on the replies to the EFSA/FAO/WHO questionnaire on national total diet study approaches. Supporting Publications 2011:206. Available at http://www.efsa.europa.eu/en/supporting/doc/206e.pdf.
87 European Food Safety Authority. Towards a harmonised total diet study approach: a guidance document. *EFSA J* 2011;**9**(11):2450.

88 Yusà V, Suelves T, Ruiz-Atienza L, Cervera ML, Benedito V, Pastor A. Monitoring programme on cadmium, lead and mercury in fish and seafood from Valencia, Spain: levels and estimated weekly intake. *Food Addit Contam Part B* 2008;**1**:22–31.

89 Tressou J, Crépet A, Bertail P, Feinberg MH, Leblanc JC. Probabilistic exposure assessment to food chemicals based on extreme value theory. Application to heavy metals from fish and sea products. *Food Chem Toxicol* 2004;**42**: 1349–1358.

90 Kim M, Wolt JD. Probabilistic risk assessment of dietary cadmium in the South Korean population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 2011;**28**:62–70.

91 Slob W. Probabilistic dietary exposure assessment taking into account variability in both amount and frequency of consumption. *Food Chem Toxicol* 2006;**44**:933–951.

92 WHO. Principles and Methods for the Risk Assessment of Chemicals in Food. Environmental Health Criteria 240. WHO, Geneva, 2009.

93 WHO. Global Environment Monitoring System. Food Contamination Monitoring and Assessment Programme (GEMS/Food). Available at http://www.who.int/foodsafety/areas_work/chemical-risks/gems-food/en/ (accessed November 2013).

94 European Food Safety Authority. Cadmium in food. Scientific Opinion of the Panel on Contaminants in the Food Chain. *EFSA J* 2009;**980**:1–139.

95 European Food Safety Authority. The EFSA Comprehensive European Food Consumption Database. Available at http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm (accessed November 2013).

96 WHO. *Arsenic and Arsenic Compounds*, 2nd edn. Environmental Health Criteria 224. WHO, Geneva, 2001.

97 Marti-Cid R, Llobet JM, Castell V, Domingo JL. Dietary intake of arsenic, cadmium, mercury, and lead by the population of Catalonia, Spain. *Biol Trace Elem Res* 2008;**125**:120–132.

98 FAO/WHO. Evaluation of Certain Food Additives and Contaminants: 61st report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series 922. WHO, Geneva, 2004.

99 De Brouwere K, Buekers J, Cornelis C, Schlekat CE, Oller AR. Assessment of indirect human exposure to environmental sources of nickel: oral exposure and risk characterization for systemic effects. *Sci Total Environ* 2012;**419**:25–36.

100 European Food Safety Authority. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the tolerable upper intake level of tin. *EFSA J* 2005;**254**:1–25.

101 Aung NN, Yoshinaga J, Takahashi JI. Dietary intake of toxic and essential trace elements by the children and parents living in Tokyo Metropolitan Area, Japan. *Food Addit Contam* 2006;**23**:883–894.

102 WHO. *Molybdenum in Drinking-water*. Background document for development of WHO Guidelines for Drinking-water Quality. WHO, Geneva, 2011. Available at http://www.who.int/water_sanitation_health/dwq/chemicals/molybdenum.pdf.

103 Calafat M, Manori XY, Silva J, Kuklenyik Z, Needham LL. Human exposure assessment to environmental chemicals using biomonitoring. *Int J Androl* 2006;**29**:166–171.

104 Health Canada. *Second Report on Human Biomonitoring of Environmental Chemicals in Canada*. Available at http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/chms-ecms-cycle2/index-eng.php.

105 Haines DA, Murray J. Human biomonitoring of environmental chemicals: early results of the 2007–2009 Canadian Health Measures Survey for males and females. *Int J Hyg Environ Health* 2012;**215**:133–137.

106 Hays SM, Aylward LL. Interpreting human biomonitoring data in a public health risk context using Biomonitoring Equivalents. *Int J Hyg Environ Health* 2012;**215**:145–148.

107 Angerer J, Ewers U, Wilhelm M. Human biomonitoring: state of the art. *Int J Hyg Environ Health* 2007;**210**:201–228.

108 Metcalf SW, Orloff KG. Biomarkers of exposure in community settings. *J Toxicol Environ Health A* 2004;**67**:715–726.

109 Barr DB, Wang RY, Needham LL. Biologic monitoring of exposure to environmental chemicals throughout the life stages: requirements and issues for consideration for the National Children's Study. *Environ Health Perspect* 2005;**113**:1083–1091.

110 Needham LL, Ozkaynak H, Whyatt RM *et al*. Exposure assessment in the National Children's Study: introduction. *Environ Health Perspect* 2005;**113**:1076–1082.

111 Li P, Feng X, Qiu G, Wan Q. Hair can be a good biomarker of occupational exposure to mercury vapor: simulated experiments and field data analysis. *Sci Total Environ* 2011;**409**:4484–4488.

112 Agency for Toxic Substances and Disease Registry. Toxicological profile for lead, August 2007. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=96&tid=22.

113 Centers for Disease Control. Fourth National Report on Human Exposure to Environmental Chemicals. CDC, Atlanta, GA, 2009.

114 Rivera-Núñez Z, Meliker JR, Linder AM, Nriagu JO. Reliability of spot urine samples in assessing arsenic exposure. *Int J Hyg Environ Health* 2010;**213**:259–264.

115 Kile ML, Hoffman E, Hsueh Y-M *et al*. Variability in biomarkers of arsenic exposure and metabolism in adults over time. *Environ Health Perspect* 2009;**117**:455–460.

116 Porta M, Puigdomènech E, Ballester F *et al*. Monitoring concentrations of persistent organic pollutants in the general population: the international experience. *Environ Int* 2008;**34**:546–561.

117 Shen H, Ding G, Han G *et al*. Distribution of PCDD/Fs, PCBs, PBDEs and organochlorine residues in children's blood from Zhejiang, China. *Chemosphere* 2010;**80**:170–175.

118 Esteban M, Castano A. Non-invasive matrices in human biomonitoring: a review. *Environ Int* 2009;**35**:438–449.

119 Freire C, Ramos R, Lopez-Espinosa MJ *et al*. Hair mercury levels, fish consumption, and cognitive development in preschool children from Granada, Spain. *Environ Res* 2010;**110**:96–104.

120 Gonzalez-Munoz MJ, Pena A, Meseguer I. Monitoring heavy metal contents in food and hair in a sample of young Spanish subjects. *Food Chem Toxicol* 2008;**46**: 3048–3052.

121 Gil F, Hernández AF, Márquez C *et al*. Biomonitorization of cadmium, chromium, manganese, nickel and lead in whole blood, urine, axillary hair and saliva in an occupationally exposed population. *Sci Total Environ* 2011;**409**: 1172–1180.

122 Hussein Were F, Njue W, Murungi J, Wanjau R. Use of human nails as bio-indicators of heavy metals environmental exposure among school age children in Kenya. *Sci Total Environ* 2008;**393**:376–384.

123 Costa de Almeida GR, Umbelino de Freitas C, Barbosa F Jr, Tanus-Santos JE, Gerlach RF. Lead in saliva from lead-exposed and unexposed children. *Sci Total Environ* 2009;**407**:1547–1550.

124 Garcia-Esquinas E, Pérez-Gómez B, Fernández MA *et al*. Mercury, lead and cadmium in human milk in relation to diet, lifestyle habits and sociodemographic variables in Madrid (Spain). *Chemosphere* 2011;**85**:268–276.

125 Liu K-S, Hao JH, Xu YQ *et al*. Breast milk lead and cadmium levels in suburban areas of Nanjing, China. *Chin Med Sci J* 2013;**28**:7–15.

126 Chien LC, Han BC, Hsu CS *et al*. Analysis of the health risk of exposure to breast milk mercury in infants in Taiwan. *Chemosphere* 2006;**64**:79–85.

127 Vieira SM, de Almeida R, Holanda IB *et al*. Total and methylmercury in hair and milk of mothers living in the city of Porto Velho and in villages along the Rio Madeira, Amazon, Brazil. *Int J Hyg Environ Health* 2013;**216**:682–689.

128 Örün E, Yalçın SS, Aykut O *et al*. Breast milk lead and cadmium levels from suburban areas of Ankara. *Sci Total Environ* 2011;**409**:2467–2472.

129 Zelenka MP, Barr DB, Nicolich MJ *et al*. A weight of evidence approach for selecting exposure biomarkers for biomonitoring. *Biomarkers* 2011;**16**:65–73.

130 Agency for Toxic Substances and Disease Registry. Toxicological profile for antimony, December 1992. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=332&tid=58.

131 European Union Risk Assessment Report. Diantimony trioxide. Risk assessment. Swedish Chemicals Inspectorate, Sundbyberg, Sweden, May 2008. Available at https://echa.europa.eu/documents/10162/13630/trd_rar_sweden_diantimony_trioxide_en.pdf.

132 Caldwell KL, Jones RL, Verdon CP, Jarrett JM, Caudill SP, Osterloh JD. Levels of urinary total and speciated arsenic in the US population: National Health and Nutrition Examination Survey 2003–2004. *J Expo Sci Environ Epidemiol* 2009;**19**:59–68.

133 Hays SM, Nordberg M, Yager JW, Aylward LL. Biomonitoring Equivalents (BE) dossier for cadmium (Cd) (CAS No. 7440-43-9). *Regul Toxicol Pharmacol* 2008;**51**:S49–S56.

134 Agency for Toxic Substances and Disease Registry. Toxicological profile for cesium, April 2004. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=578&tid=107

135 Agency for Toxic Substances and Disease Registry. Toxicological profile for cobalt, April 2004. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=373&tid=64.

136 Agency for Toxic Substances and Disease Registry. Toxicological profile for copper, September 2004. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=206&tid=37.

137 Jones RL, Homa DM, Meyer PA *et al*. Trends in blood lead levels and blood lead testing among US children aged 1 to 5 years, 1988– 2004. *Pediatrics* 2009;**123**:376.

138 Agency for Toxic Substances and Disease Registry. Toxicological profile for manganese, September 2012. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=102&tid=23.

139 Agency for Toxic Substances and Disease Registry. Toxicological profile for mercury, March 1999. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=115&tid=24.

140 Agency for Toxic Substances and Disease Registry. Toxicological profile for nickel, August 2005. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=245&tid=44.

141 Institute of Medicine. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. National Academy Press, Washington, DC, 2000.

142 Agency for Toxic Substances and Disease Registry. ToxFAQs for thallium, July 2013. Available at http://www.atsdr.cdc.gov/toxfaqs/TF.asp?id=308&tid=49.

143 Agency for Toxic Substances and Disease Registry. Toxicological profile for thallium, July 1992. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=309&tid=49.

144 Agency for Toxic Substances and Disease Registry. Toxicological profile for uranium, February 2013. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=440&tid=77.

145 Agency for Toxic Substances and Disease Registry. Toxicological profile for zinc, August 2005. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=302&tid=54.

146 International Agency for Research on Cancer. Arsenic and arsenic compounds. In: *IARC Monographs on the Evaluation of Carcinogenic Risk to Humans, Vol. 100C*. IARC, Lyon, 2012.

147 Hughes MF. Biomarkers of exposure: a case study with inorganic arsenic. *Environ Health Perspect* 2006;**114**:1790–1796.

148 Haysa SM, Aylward LL, Gagné M, Nong A, Krishnan K. Biomonitoring Equivalents for inorganic arsenic. *Regul Toxicol Pharmacol* 2010;**58**:1–9.

149 Jarup L, Akesson A. Current status of cadmium as an environmental health problem. *Toxicol Appl Pharmacol* 2009;**238**: 201–208.

150 WHO. *Health Risks of Heavy Metals from Long-range Transboundary Air Pollution*. WHO, Copenhagen, 2007.

151 Roman HA, Walsh TL, Coull BA *et al*. Evaluation of the cardiovascular effects of methylmercury exposures: current evidence supports development of a dose–response function for regulatory benefits analysis. *Environ Health Perspect* 2011;**119**:607–614.

152 Berglund M, Ling B, Björnberg KA, Palm B, Einarsson O, Vahter M. Inter-individual variations of human mercury exposure biomarkers: a cross-sectional assessment. *Environ Health* 2005;**4**:1–11.

153 Passos CJ, Mergler D, Lemire M, Fillion M, Guimarães JR. Fish consumption and bioindicators of inorganic mercury exposure. *Sci Total Environ* 2007;**373**:68–76.

154 Chien LC, Gao CS, Lin HH. Hair mercury concentration and fish consumption: risk and perceptions of risk among women of childbearing age. *Environ Res* 2010;**110**:123–129.

155 Hsiao HW, Ullrich SM, Tanton TW. Burdens of mercury in residents of Temirtau, Kazakhstan I: hair mercury concentrations and factors of elevated hair mercury levels. *Sci Total Environ* 2011;**409**:2272–2280.

156 Rees JR, Sturup S, Chen C, Folt C, Karagas MR. Toenail mercury and dietary fish consumption. *J Expo Sci Environ Epidemiol* 2007;**17**:25–30.

157 Mozaffarian D, Shi P, Morris S *et al*. Mercury exposure and risk of cardiovascular disease in two U.S. cohorts. *N Engl J Med* 2011;**364**:1116–1125.

158 Schulz C, Conrad A, Becker K, Kolossa-Gehring M, Seiwert M, Seifert B. Twenty years of the German Environmental Survey (GerES). Human biomonitoring: temporal and spatial (West Germany/East Germany) differences in population exposure. *Int J Hyg Environ Health* 2007;**210**:271–297.

159 Frery N, Vandentorren S, Etchevers A, Fillol C. Highlights of recent studies and future plans for the French human biomonitoring (HBM) programme. *Int J Hyg Environ Health* 2012;**215**:127–132.

160 Perharic L, Vracko P. Development of national human biomonitoring programme in Slovenia. *Int J Hyg Environ Health* 2012;**215**:180–184.

161 Cerna M, Krsková A, Cejchanová M, Spěváčková V. Human biomonitoring in the Czech Republic: an overview. *Int J Hyg Environ Health* 2012;**215**:109–119.

162 Berman T, Amitai Y, Almog S, Richter ED. Human biomonitoring in Israel: past, present, future. *Int J Hyg Environ Health* 2012;**215**:138–141.

163 Schoeters G, Den Hond E, Colles A *et al*. Concept of the Flemish human biomonitoring programme. *Int J Hyg Environ Health* 2012;**215**:102–108.

164 Viso AC, Casteleyn L, Biot P, Eilstein D. Human biomonitoring programmes and activities in the European Union. *J Epidemiol Community Health* 2009;**63**:623–624.

165 European Commission. Environment and Health Action Plan 2004–2010. Available at http://europa.eu/legislation_summaries/public_health/health_determinants_environment/l28145_en.htm.

166 Joas R, Casteleyn L, Biot P *et al*. Harmonised human biomonitoring in Europe: activities towards an EU HBM framework. *Int J Hyg Environ Health* 2012;**215**:172–175.

167 Llop S, Ballester F, Estarlich M *et al*. Urinary 1-hydroxypyrene, air pollution exposure and associated life style factors in pregnant women. *Sci Total Environ* 2008;**407**:97–104.

168 Kock HM, Wittassek M, Brüning T, Angerer J, Heudorf U. Exposure to phthalates in 5–6 years old primary school starters in Germany: a human biomonitoing study and cumulative risk assessment. *Int J Hyg Environ Health* 2011;**214**:188–195.

169 Llorca M, Farré M, Picó Y, Teijón ML, Alvarez JG, Barceló D. Infant exposure of perfluorinated compounds: levels in breast milk and commercial baby food. *Environ Int* 2010;**36**:584–592.

170 Pino A, Amato A, Alimonti A, Mattei D, Bocca B. Human biomonitoring for metals in Italian urban adolescents: data from Latium Region. *Int J Hyg Environ Health* 2012;**215**:185–190.

171 Gil F, Capitán-Vallvey LF, De Santiago E *et al*. Heavy metal concentrations in the general population of Andalusia, South of Spain: a comparison with the population within the area of influence of Aznalcollar mine spill (SW Spain). *Sci Total Environ* 2006;**372**:49–57.

172 Batariova A, Spevácková V, Benes B, Cejchanová M, Smíd J, Cerná M. Blood and urine levels of Pb, Cd and Hg in the general population of the Czech Republic and proposed reference values. *Int J Hyg Environ Health* 2006;**209**:359–366.

173 Castaño A, Sánchez-Rodríguez JE, Cañas A *et al*. Mercury, lead and cadmium levels in the urine of 170 Spanish adults: a pilot human biomonitoring study. *Int J Hyg Environ Health* 2012;**215**:191–195.

174 Schroijen C, Baeyens W, Schoeters G *et al*. Internal exposure to pollutants measured in blood and urine of Flemish adolescents in function of area of residence. *Chemosphere* 2008;**71**:1317–1325.

175 Levy LS, Jones K, Cocker J, Assem FL, Capleton AC. Background levels of key biomarkers of chemical exposure within the UK general population: pilot study. *Int J Hyg Environ Health* 2007;**210**:387–391.

176 Kuno R, Roquetti MH, Becker K, Seiwert M, Gouveia N. Reference values for lead, cadmium and mercury in the blood of adults from the metropolitan area of Sao Paulo, Brazil. *Int J Hyg Environ Health* 2013;**216**:243–249.

177 Bocca B, Madeddu R, Asara Y, Tolu P, Marchal JA, Forte G. Assessment of reference ranges for blood Cu, Mn, Se and Zn in a selected Italian population. *J Trace Elem Med Biol* 2011;**25**:19–26.

178 Puklova V, Krsková A, Cerná M *et al*. The mercury burden of the Czech population: an integrated approach. *Int J Hyg Environ Health* 2010;**213**:243–251.

179 Schulz C, Angerer J, Ewers U, Kolossa-Gehring M. The German Human Biomonitoring Commission. *Int J Hyg Environ Health* 2007;**210**:373–382.

180 Schulz C, Wilhelm M, Heudorf U, Kolossa-Gehring M and Human Biomonitoring Commission of the German Federal Environment Agency. Update of the reference and HBM values derived by the German Human Biomonitoring Commission. *Int J Hyg Environ Health* 2011;**215**: 26–35.

181 Bevan R, Jones K, Cocker J, Assem FL, Levy LS. Reference ranges for key biomarkers of chemical exposure within the UK population. *Int J Hyg Environ Health* 2013;**216**: 170–174.

182 Forte G, Madeddu R, Tolu P, Asara Y, Marchal JA, Bocca B. Reference intervals for blood Cd and Pb in the general population of Sardinia (Italy). *Int J Hyg Environ Health* 2011;**214**:102–109.

183 Hoet P, Jacquerye C, Deumer G, Lison D, Haufroid V. Reference values and upper reference limits for 26 trace elements in the urine of adults living in Belgium. *Clin Chem Lab Med* 2013;**51**:839–849.

184 Aylward LL, Kirman CR, Schoeny R, Portier CJ, Hays SM. Evaluation of biomonitoring data from the CDC national exposure report in a risk assessment context: perspectives across chemicals. *Environ Health Perspect* 2013;**121**:287–294.

185 Albertini R, Angerer J, Boogaard PJ *et al*. The use of biomonitoring data in exposure and human health risk assessments. *Environ Health Perspect* 2006;**43**:119–153.

186 Barr DB, Angerer J. Potential uses of biomonitoring data: a case study using the organophosphorus pesticides chlorpyrifos and malathion. *Environ Health Perspect* 2006;**114**:1763–1769.

187 Calafat AM, McKee RH. Integrating biomonitoring exposure data into the risk assessment process: phthalates (diethyl phthalate and di[2-ethylhexyl] phthalate) as a case study. *Environ Health Perspect* 2006;**114**:1783–1789.

188 Hays SM, Aylward LL. Using Biomonitoring Equivalents to interpret human biomonitoring data in a public health risk context. *J Appl Toxicol* 2009;**29**:275–288.

189 Angerer J, Aylward LL, Hays SM, Heinzow B, Wilhelm M. Human biomonitoring assessment values: approaches and data requirements. *Int J Hyg Environ Health* 2011;**214**: 348–360.

190 Hays SM, Aylward LL, LaKind JS *et al*. Guidelines for the derivation of Biomonitoring Equivalents: report from the Biomonitoring Equivalents Expert Workshop. *Regul Toxicol Pharmacol* 2008;**51**:S4–S15.

191 Centers for Disease Control and Prevention. National Report on Human Exposure to Environmental Chemicals. Available at http://www.cdc.gov/exposurereport/ (accessed December 2013).

192 WHO/FAO. Codex Alimentarius. International Food Standards. Available at http://www.codexalimentarius.org/about-codex/en/ (accessed November 2013).

193 European Commission. Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF (accessed November 2014).

194 European Commission. Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. Available at http://ec.europa.eu/environment/water/water-drink/legislation_en.html.

195 European Commission. Commission directive 2003/40/EC of 16 May establishing the list, concentration limits and labelling requirements for the constituents of natural mineral waters and the conditions for using ozone-enriched air for the treatment of natural mineral waters and spring waters. Available at http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32003L0040

196 European Commission. Council Directive of 15 October 1984 on the approximation of the laws of the Member States relating to ceramic articles intended to come into contact with foodstuffs. Available at http://europa.eu/legislation_summaries/consumers/product_labelling_and_packaging/l21300_en.htm.

197 European Commission. Health and Consumers. Food safety in Europe. Available at http://ec.europa.eu/food/food/index_en.htm (accessed December 2013).

198 Directorate-General Health and Consumer Protection. Reports on tasks for scientific cooperation. Report of experts participating in Task 3.2.11, March 2004. Assessment of the dietary exposure to arsenic, cadmium, lead and mercury of the population of the EU Member States. Available at http://ec.europa.eu/food/food/chemicalsafety/contaminants/scoop_3-2-11_heavy_metals_report_en.pdf.

199 Directorate-General Health and Consumer Protection. Opinion of the Scientific Committee on Food on acute risks posed by tin in canned foods, January 2002. Available at http://ec.europa.eu/food/fs/sc/scf/out110_en.pdf.

200 Codex Alimentarius Commission. *Procedural Manual*, 17th edn. WHO/FAO, Rome, 2007.

201 Codex Alimentarius. Codex general standard for contaminants and toxins in food and feed. Codex Standard 193-1995. Available at http://www.codexalimentarius.org/download/standards/17/CXS_193e.pdf.

202 Codex Alimentarius. *Understanding the Codex Alimentarius*, 3rd edn. FAO, Rome, 2006. Available at ftp://ftp.fao.org/codex/publications/understanding/understanding_EN.pdf.

203 European Union. Regulation (EC) No. 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2004R0882:20060525:EN:PDF.

204 Food Standards Agency. Multi-Annual National Control Plan for the United Kingdom. Available at http://www.food.gov.uk/enforcement/regulation/europeleg/feedandfood/ncpuk#.Up7XACeAgUU (accessed December 2013).

205 AECOSAN. Plan Nacional de Control Oficial de la Cadena Alimentaria. Available at http://www.aesan.msc.es/AESAN/web/control_oficial/seccion/plan_plurianual_control_oficial.shtml (accessed December 2013).
206 Federal Agency for the Safety of the Food Chain. Multi Annual National Control Plan (MANCP). Available at http://www.afsca.be/about/mancp/ (accessed December 2013).
207 European Food Safety Authority. Available at http://www.efsa.europa.eu/ (accessed November 2013).
208 European Commission. Commission Regulation (EC) No. 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead,cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs. Available at http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32007R0333.
209 European Union. Regulation (EC) No. 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:031:0001:0024:EN:PDF.

CHAPTER 5
The oligoelements

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Abstract: The role of trace elements in human nutrition is well recognized by public health agencies, nutritionists and researchers from various areas of knowledge. Humans require several inorganic elements as components of the diet. Some of the inorganic elements are required in extremely small quantities, only micrograms per day, while other elements may be needed in higher amounts. A lack, or an insufficient amount, of a nutrient can result in a deficiency disease which can be life-threatening in extreme cases. The essential nutrients are widely distributed in foods and most people can obtain sufficient amounts by consuming a varied diet. This chapter draws attention to some of the important and unique aspects of the oligoelements, addressed within the context of trace element intake, from deficiency to toxicity. A notable feature is the large number (17) of nutrients discussed: arsenic, boron, chromium, cobalt, copper, fluoride, iodine, iron, magnesium, manganese, nickel, molybdenum, selenium, sulfur, tin, vanadium and zinc. Also, considerable attention is given to the interaction between some of these nutrients, their physiological role (functions) and the enzymes containing these elements. Trace elements recently found to be essential are discussed, along with new ways of conceptualizing the application of nutrient requirements.

Keywords: oligoelements, physiological functions, deficiencies and toxicities

5.1 Considerations

The human body is composed of elements which can be roughly divided into abundant elements and trace elements. Abundant elements consist of the major elemental constituents of tissues. Other semi-major elements are involved in functions of the living organism, such as the maintenance of osmotic pressure and membrane potential (potassium, sodium, etc.). Figure 5.1 shows the elemental composition of the average adult human body. Almost 99% of the mass of the human body is made up of the six elements oxygen, carbon, hydrogen, nitrogen, calcium and phosphorus. The other elements comprise only about 0.6% and are referred to as the oligoelements: arsenic, boron, chromium, cobalt, copper, fluoride, iodine, iron, magnesium, manganese, molybdenum, nickel, selenium, sulfur, tin, vanadium and zinc, which are thought to be necessary for life or play a role in promoting health (e.g., fluorine, which hardens dental enamel but seems to have no other function).

Oligoelements or trace elements are terms which have been applied to a group of elements present in biological systems that are essential to humans and animals in small concentrations. The term 'oligo' comes from the Greek word meaning little. Nutritionists and biochemists refer to the elements which are present in very small amounts, relative to the major constituents of tissues, as trace elements, and these are as important as the major nutrients, being required for proper growth and development [1].

In 1990, the Expert Consultation of World Health Organization (WHO)/Food and Agricultural Organization (FAO)/International Atomic Energy Agency (IAEA) defined the essentiality of a trace element as follows:

> An element is considered essential to an organism when reduction of its exposure below a certain limit results consistently in a reduction in a physiologically important function, or when the element is an integral part of an organic structure performing a vital function in the organism [2].

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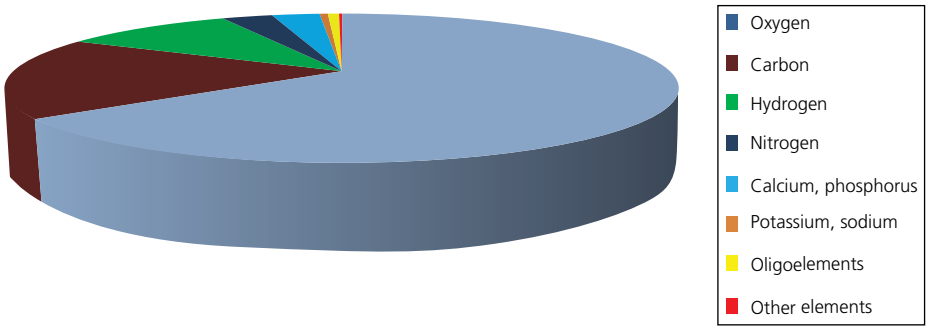


Figure 5.1 Elements in the human body (mass percent). (See insert for colour representation of the figure.)

In this case, a concentration lower than the optimum may lead to poor performance of the organism, causing structural and physiological abnormalities, while a higher concentration may also have deleterious, even lethal, effects [3, 4]. For example, humans with imbalances of iron, zinc, copper and selenium show behavioural changes such as attention and memory deficits, anxiety and depression. In old age, an excess of these trace elements combined with enhanced oxidative processes promotes the development of Lewy bodies [5–7] and amyloid β plaque [8–10], and defects in the huntingtin gene [7, 11]. These factors are also associated with the neurodegenerative pathologies of Parkinson's [8], Alzheimer's [12] and Huntington's [13] diseases, respectively.

In the past, the trace elements were examined in isolation without considering the interaction between nutrients and other components in the diet. It is now known that they participate in various metabolic functions in the body, especially in the formation of enzymes vital to several biochemical processes carried out by cells. Trace elements are required for the transportation of oxygen, for muscle contraction, nerve impulse conduction and various vital functions. For example, oligoelements such as zinc, selenium and copper have a significant influence on the functioning of the immune system [14]. Furthermore, these elements, together with the iron, perform structural and catalytic functions in proteins and enzymes involved in structural, metabolic and antioxidant functions [15, 16] and also participate in the synthesis of neurotransmitters, specifically catecholamines.

Recent investigations focusing on the interaction between trace elements have involved research on the essential elements selenium and iodine. There is evidence that selenium acts as a component of the enzyme responsible for converting thyroxine to triiodothyronine (T_3); thus it is possible that the systemic utilization of iodine is impaired in subjects who are deficient in selenium [17, 18].

5.2 Importance of oligoelements

In this context, trace elements are considered to have four main known roles in living organisms.

1. In close association with enzymes, some trace elements form an integral part of the catalytic centres at which the biochemical reactions occur. Working in concert with a protein, and frequently with other organic coenzymes, the trace element attracts substrate molecules and facilitates their conversion to a specific end product.
2. Some trace elements donate or accept electrons in reduction or oxidation reactions. These redox reactions are of primary importance in the generation and utilization of metabolic energy through the 'burning' of foods in cells. Chemical transformations of molecules frequently involve redox reactions.
3. Some trace elements, especially iron, bind, transport and release oxygen in the body.
4. Some trace elements have structural roles, imparting stability and three-dimensional structures to important biological molecules.

Although trace elements play key roles in a variety of processes necessary for life, the occurrence of overt simple or uncomplicated deficiencies of any of the trace elements is probably relatively uncommon because of the powerful homeostatic mechanisms which the human body possesses. However, there are situations which may make a trace element nutritionally significant: (i) inborn

errors of metabolism that affect absorption, retention or excretion; (ii) alterations in metabolism and/or biochemistry as a secondary consequence to malnutrition, disease, injury or stress; (iii) marginal deficiencies (slight deviation from the optimal intake of an essential nutrient) induced by various dietary manipulations or by direct or indirect interaction with another nutrient or drug; and (iv) the enhanced requirement for a trace element caused by a sudden or severe change in the system requiring that element. These details can be summarized by the statement that the insufficient intake of a specific trace element would become obvious only when the body is stressed in some way that enhances the need for, or interferes with the utilization of, that element.

The next section addresses the 17 oligoelements which have some function in the body's performance: arsenic, boron, chromium, cobalt, copper, fluoride, iodine, iron, magnesium, manganese, molybdenum, nickel, selenium, sulfur, tin, vanadium and zinc. These are acquired through a balanced and diversified diet [14].

5.3 The oligoelements

5.3.1 Arsenic

There is evidence that arsenic is present in the body in pentavalent and trivalent oxidation states and that it interacts strongly with sulfhydryl groups of organic molecules. It affects several enzymes, causing damage in several cell systems. Because of their similar properties, arsenate can substitute for phosphate and other phosphate intermediates in several biochemical reactions. At the cellular level, arsenate depletes ATP in human erythrocytes, interrupting the production of energy. Hughes *et al*. [19] reported that human erythrocytes died within a few hours after arsenic exposure, presumably because of the loss of both ATP and plasma membrane integrity. A dose of 140 mg of inorganic trivalent arsenic can cause the death of an adult human by adversely affecting cell respiration. There is evidence that arsenic may induce the metabolism of metallothionein [20]. However, no studies to determine the nutritional importance of arsenic in humans have been published in the literature, although some research has indicated that arsenic is an essential nutrient for rats, chickens and pigs. For example, Roxarsone (4-hydroxy-3-nitrobenzene arsonic acid) is widely used in poultry production as a feed additive to increase weight gain and improve feed efficiency [21]. It is known that arsenic compounds have been widely used in medicine for the treatment of a variety of illnesses, for example syphilis, trypanosomiasis, malaria, asthma and psoriasis [19].

5.3.2 Boron

Boron is a trace mineral essential to human health and has metabolic functions. According to Nielsen [22], experiments have demonstrated that boron affects the metabolism of macrominerals, through changes in the hormones involved in this metabolism. This is due to the fact that boron acts on the parathyroid glands to regulate calcium, magnesium and phosphorus balance [23]. These experiments also showed that boron affects brain functioning, psychomotor performance and the response to estrogen ingestion [22]. The acceptable range of boron intake according to the WHO is 1–13 mg/day for adults [24]. Antibiotics such as boromycin, aplasmomycins, borophycin and tartrolons are among the best-known natural boron-containing compounds. Attempts are underway to incorporate boron into different biologically active molecules, particularly for medicinal application, for example boron neutron capture in brain tumour therapy. Some boron-containing biomolecules appear to act as signalling molecules that interact with cell surfaces. In general, boron is found in the form of boric acid, $B(OH_3)$, at physiological pH. Boron has a high absorption rate, which can reach around 90%. Most mineral intake is hydrolysed resulting in boric acid which, as a neutral component, is easily absorbed and transported by the enterocytes. During boron transport in the body, it is likely that $B(OH)_3$ is weakly bound to substances containing *cis*-hydroxyl groups.

5.3.3 Chromium

Chromium can exist in several oxidation states, principally as metallic (Cr^0), trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) chromium. The hexavalent chromium form is considered to have no nutritional value and is carcinogenic since, on contact, it can cause lipid peroxidation, increased oxidative stress, genome instability [25], DNA damage and cell death [26]. Trivalent chromium is a stable and biologically active form of chromium and is found in various types of foods, including egg yolk, whole-grain products, high-bran breakfast cereals, coffee, nuts, green beans, broccoli, meat, brewer's yeast, and some brands of wine and beer. Chromium is also present in many dietary supplements. Data have shown

that in humans chromium plays an important role in carbohydrate, lipid and glucose metabolism, being beneficial for patients with type 2 diabetes [27]. According to Kiefer [28],

> chromium acts like a kind of doorman. While insulin escorts glucose to 'doors' in the cell membrane, chromium (probably in combination with protein fragments) rings the doorbell, and perhaps holds the door open by activating receptors, while insulin ushers its precious cargo into the cell.

In this context, chromium is believed to form an integral component of the so-called glucose tolerance factor, a proposed metal–protein complex sometimes referred to as chromodulin.

5.3.4 Cobalt

Cobalt is an essential oligoelement that forms part of the structure of vitamin B_{12} and is responsible for the production of red blood cells. It is essential for the normal functioning of all cells, particularly those of bone marrow, the nervous system and the gastrointestinal tract [29]. In situations of cobalt deficiency, there is no formation of vitamin B_{12} and the synthesis of coenzyme B_{12}, which is essential for the conversion of methylmalonyl-CoA to succinyl-S-S-CoA, cannot occur. Subsequently, the conversion of propionyl-S-CoA to succinate will not be triggered, leading to the non-production of glucose in the Krebs cycle [30]. Cobalt in excess can cause dysfunction of the thyroid gland, dermatitis, cardiomyopathy, hepatotoxicity, nephrotoxicity and polycythaemia. High concentrations of cobalt are found in fish (0.01 mg/kg), nuts (0.09 mg/kg), fresh green leafy vegetables (0.009 mg/kg) and cereals (0.01 mg/kg), and most of the cobalt ingested is in the inorganic form. The mean population intake of cobalt is 0.012 mg/day. The gastrointestinal absorption of cobalt is dose-dependent, this element being almost completely absorbed in very low doses, whereas larger doses are less well absorbed. Nutritional factors also influence cobalt absorption, for example it is reduced in the presence of amino acids and increases in cases of iron deficiency.

5.3.5 Copper

Copper has many properties that make it an essential component in the body. It is responsible for the mobilization of iron for haemoglobin synthesis and stimulates the immune system, increasing the number of defence cells and preventing the emergence of diseases. Copper is also essential for collagen formation, which ensures greater bone strength and stiffness [31]. Copper aids the participation of enzymes in various biological reactions, including superoxide dismutase, amino acid oxidase, cytochrome c oxidase and dopamine β-hydroxylase ceruloplasmin, among others. Copper deficiency alters the metabolism of cholesterol and the cholesterol hydroxylase enzyme, increasing the level of serum cholesterol [32]. The involvement of copper in skeletal metabolism, the immune system and helping in reducing the risk of cardiovascular disease is a triad of major importance in current discussions concerning this mineral and human nutrition. Copper is widely distributed in foods and its most important sources are liver, mussels, oysters, whole grains, nuts and chocolate. The main food sources provide between 300 and 2000 μg per 100 g of mineral food [29].

5.3.6 Fluoride

The fluoride ion is a crucial element in human nutrition. It is mainly found in bones and teeth, owing to its great affinity for calcium phosphate, and around 99% of the fluoride present in the body is in the hard tissue [33]. For the prevention of fluoride deficiencies, several countries use water fluoridation. The incorporation of 0.7–1.2 mg/L of fluoride in drinking water has promoted a decrease in dental caries of up to 50% [34]. However, the fluoridation of water is much discussed, due to possible damage to the health of the population. Higher concentrations can cause damage to the skeletal system (skeletal fluorosis), interference with kidney function and impair learning and memory. Water is the major source of fluoride intake for an adult, because most foods are low in fluoride, exceptions being marine fish, clam, lobster, crab, shrimp and some teas [35–37]. The absorption of fluoride from foods is around 50–80% compared with the essentially complete absorption from drinking water. Dietary fluoride obtained from food and water is efficiently absorbed from both the stomach and small intestine without regulation; thus, if ingested in large quantities, the body is subjected to fluoride intoxication [33].

5.3.7 Iodine

Iodine is an essential micronutrient for humans and other animals. The only known function of iodine in the human body is that it is the fundamental component of the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4).

These hormones are involved in the maintenance of metabolic rate, cellular metabolism and integrity of connective tissue. Thyroid hormones are necessary for the development of the nervous system. These hormones are very important for the functioning of various organs such as the heart, liver, kidney, ovary and others. Over 75% of the iodine in the human body is present in the thyroid gland and the remainder is distributed throughout the body [38]. In general, iodine intake from water is estimated to be less than 0.03 mg/day. The main foods which are rich in iodine are of marine origin (oysters, clams and other shellfish and saltwater fish). The content of iodine in raw food is reduced by cooking. Iodine, as iodide, is present in multivitamin and mineral supplements (providing up to 0.49 mg/day of iodine) and is a component of kelp products. It is also present in licensed medicines, topical antiseptics and radiographic contrast agents. Levels in cereals and grains vary depending on the iodine content of the soil. Iodine interacts with selenium and possibly with vanadium [39].

5.3.8 Iron

Iron is a vital element for living organisms. The role of iron in the body is limited almost exclusively to transporting oxygen in the blood through the haemoglobin present in red blood cells and the formation of haemoglobin and myoglobin. Iron also plays a vital role in energy production as a constituent of various enzymes including catalase, peroxidase and cytochrome. Many other enzymes also contain or require iron for their biological function [29, 32]. Total body iron averages approximately 3800 mg in men and 2300 mg in women. Iron is stored mainly in the liver within the iron storage proteins ferritin and haemosiderin. Small amounts of ferritin are also present in the plasma, although this differs from tissue ferritin since it has a low iron content, even in iron-overloaded individuals [40]. Interactions may occur between iron and other metals such as copper, manganese, zinc and chromium. Studies in rats have shown that iron supplementation impairs the absorption of zinc, raising concerns that iron supplements may have an adverse effect on zinc nutrition in humans. However, the magnitude of this effect is less apparent in human studies than in rats. In general, calcium inhibits the absorption of iron, which may be relevant in relation to the consumption of iron supplements with milk or calcium-rich foods [41, 42]. Dietary sources rich in iron include liver, meat, beans, nuts, dried fruits, poultry, fish, whole grains or enriched cereals, soybean flour and most dark green leafy vegetables.

5.3.9 Magnesium

Magnesium is an essential mineral that is needed for a broad variety of physiological functions [15]. It is essential for calcium and vitamin C absorption, as well as aiding the metabolism of phosphorus, sodium and potassium. Magnesium converts blood glucose into energy and it acts together with calcium to maintain the proper functioning of muscles (calcium stimulates contraction and magnesium relaxation). There is increasing interest in the role of magnesium in clinical nutrition and physiology, as this mineral affects many cellular functions, including the transport of potassium (K^+) and calcium (Ca^{2+}) ions, and modulates signal transduction, energy metabolism and cell proliferation [26]. Magnesium is required as a cofactor for many enzyme systems. It is required for protein synthesis and for both anaerobic and aerobic energy generation and for glycolysis, either indirectly as a part of the magnesium-ATP complex or directly as an enzyme activator. Magnesium plays a multifunctional role in cell metabolism (particularly in relation to key phosphorylation sites) and has a critical role in cell division. It has been suggested that magnesium is necessary for the maintenance of an adequate supply of nucleotides for the synthesis of RNA and DNA. Magnesium regulates the movement of potassium in myocardial cells and is also known to act as a calcium channel blocker. Magnesium is an important element in the metabolism and/or action of vitamin D, and is essential for the synthesis and secretion of parathyroid hormone. Magnesium deficiency can cause fatigue, depression, insomnia, muscle spasms, heart failure, tachycardia, arrhythmia, anorexia, nausea and vomiting [43]. An excess of magnesium can cause respiratory problems, muscle weakness, heart rhythm disorders, hypotension and inhibition of bone calcification.

5.3.10 Manganese

Like many other trace elements, manganese functions in the body are complex and still under investigation by the medical and public health communities. Manganese is a component of a number of enzymes and activates a range of others. Glycosyltransferases, essential enzymes for the synthesis of proteoglycans necessary for the formation of cartilage and bone, are specifically activated by manganese [29, 42]. Manganese is present in foods, particularly

green vegetables (2 mg/kg), nuts (14.9 mg/kg), bread (8 mg/kg) and other cereals (6.81 mg/kg). Tea is a rich source of manganese, containing 2.71 mg/kg and is the largest contributor to manganese intake [44]. It is present in several licensed medicines, in combination with other substances, used for the prevention and treatment of nutrient deficiencies and other related conditions. Manganese is also present in a number of multivitamin and/or mineral food supplements at levels of up to 10 mg. Manganese and iron compete for absorption sites. Calcium, phosphorus and magnesium may also interfere with manganese absorption [29].

5.3.11 Molybdenum

Molybdenum is a mineral that is present in small amounts in the human body. The basis of the importance of molybdenum lies in its role in metalloenzymes, participating in reactions as a cofactor. All the molybdoenzymes are oxidoreductases that exploit the variable valence state of molybdenum. The molybdenum in molybdoenzymes is inserted as part of a prosthetic group known as the 'molybdenum cofactor'. In humans, xanthine oxidase and sulfite oxidase are important molybdoenzymes. It is recognized that molybdenum interacts with copper and sulfates in living organisms, but the mechanism of this interaction has not been elucidated. The presence of dietary copper and sulfate affects the amount of molybdenum absorbed and retained by the body [45, 46]. In general, foodstuffs from above-ground plant material, such as legumes, leafy vegetables and cauliflower, contain relatively high concentrations of molybdenum compared with food from tubers or animals. The highest levels of molybdenum are found in nuts (0.96 mg/kg fresh weight), canned vegetables (0.31 mg/kg fresh weight) and cereals (0.23 mg/kg fresh weight) [47].

5.3.12 Nickel

Nickel has various biological functions in the body. It plays important roles as a catalytic centre in redox and non-redox enzymes, where it has important ramifications in terms of human health (e.g. urease), energy science (e.g. hydrogenase) and the environment (e.g. carbon monoxide dehydrogenases) [48]. Its accumulation in the environment may represent a serious hazard to human health. Among the known health-related effects of nickel are skin allergies, lung fibrosis, variable degrees of kidney and cardiovascular system poisoning and stimulation of neoplastic transformation. The mechanism of the latter effect is not known and is the subject of detailed investigation. Nickel is present in a variety of foods particularly pulses, oats (0.18 mg/kg in miscellaneous cereals) and nuts (1.77 mg/kg). Lower levels of nickel are present in drinking water. Nickel is present in a few multimineral food supplements at levels of approximately 0.005 mg per daily dose. The average intake of nickel in the UK is 0.13 mg/day from food and 0.02–0.04 mg/day from drinking water. In humans, nickel influences iron absorption and metabolism, and may be an essential component of the haemopoietic process.

5.3.13 Selenium

Selenium is of fundamental importance to human health. It is well recognized that dietary selenium is important for a healthy immune response. It is an essential component of several major metabolic pathways. There is also evidence that selenium has a protective effect against some forms of cancer [49–51]. The biologically active form of selenium is selenocysteine. Selenocysteine is incorporated into selenoproteins, of which over 30 have been identified. The selenoproteins include the glutathione peroxidases (which protect against oxidative damage), the iodothyronine deiodinases (involved in the production of triiodothyronine from thyroxine), selenoprotein Pb (involved in antioxidant and transport functions) and the thioredoxin reductases (involved in the maintenance of the intracellular redox state) [51, 52]. Since selenium is an essential component of the enzyme tetraiodothyronine 5′-deiodinase 1 (which is involved in iodine metabolism), there is a complex interaction between selenium and iodine. Severe selenium deficiency may increase the hypothyroid stress caused by iodine deficiency. However, supplementation with selenium alone may aggravate iodine deficiency by accelerating iodine loss. This interaction is likely to be significant only in cases of severe deficiency. Selenium interacts with other metals and ascorbic acid. Selenium status affects the metabolism and thus the toxicities of some xenobiotics [53]. Selenium is present in foods particularly fish (0.32 mg/kg), offal (0.42 mg/kg), brazil nuts (0.25 mg/kg), eggs (0.16 mg/kg) and cereals (0.02 mg/kg). In foods, selenium is generally present as the amino acid derivatives selenomethionine and selenocysteine. Selenium is present in several licensed medicines both alone and in combination with other substances, and

is present in a number of food supplements at doses of up to 0.3 mg per daily dose. Selenium compounds are readily absorbed from the small intestine. The extent to which it is absorbed is dependent on the nature of the compound, with soluble selenate and selenomethionine being the most readily absorbed [51].

5.3.14 Sulfur

Sulfur is a chemical element essential for all living organisms and is an important constituent of many amino acids. Sulfur is the sixth most abundant macromineral in breast milk and the third most abundant mineral based on percentage of total body weight. The amino acids cysteine, methionine, homocysteine and taurine contain sulfur that forms disulfide bonds between peptides. These bonds are of great importance in the formation of the spatial structure of proteins. It is a constituent of some vitamins, participating in the synthesis of collagen; it neutralizes toxic chemicals in the liver and helps in the secretion of bile [15, 26]. It is found in vegetables such as asparagus, garlic, leek and onions and in fish, cheese and egg yolk. Unlike the inorganic form, the sulfur present in food is not toxic and its excess is eliminated in urine. Its deficiency retards growth. Dietary supplementation with sulfur-containing amino acids and proteins may be recommended for vegan athletes, children or patients with HIV, because of an increased risk of sulfur amino acid deficiency in these groups. The oxidized (inorganic) form of sulfur is sulfate. Dietary sulfate is poorly absorbed. Instead sulfate is produced in the body primarily by oxidation of the sulfur-containing amino acids. There is no recommended dietary allowance (RDA) for sulfur or its derivatives [40].

5.3.15 Tin

Tin is rarely found as the metallic element in nature and is usually found combined with other substances, most commonly as the dioxide (SnO_2). Organotin compounds can be synthesized and are highly toxic, but these are not found in food, beverages or food supplements. Here, tin refers to ionic tin, except where specific tin compounds are mentioned. The occurrence of tin in various organisms from different locations is highly dependent on the existence of local mineral sources of tin. Although not commonly present in fresh water, it occurs at a concentration of around 0.003 mg/kg in sea water [26, 29]. The presence of tin in fresh food of both vegetable and animal origin is highly dependent on the concentration of tin in the soil where the food is produced. Canning may result in dissolution of the tin lining of the can, particularly if the products are acidic. The regulatory limit for total tin in canned products is 200 mg/kg. The major dietary sources of tin are fruit products and canned vegetables. Stannous chloride, $SnCl_2$, is a permitted food additive (E512). Tin is present in some multivitamin and mineral food supplements at levels of up to 0.01 mg per daily dose recommended by the manufacturer. No medicinal products containing tin have been licensed [21]. There is no proven biological function for tin. It has been suggested that, because of its coordination chemistry, it may contribute to macromolecular structure and functioning at the active site of metalloenzymes. In humans, the ingestion of tin has been shown to lower the net retention of zinc. It can also alter the excretion of selenium in faeces and urine, but without affecting the overall balance. In rats the absorption of copper has been reported to be decreased by the ingestion of tin. The gastrointestinal absorption of tin is low. The solubility and bioavailability of inorganic tin compounds varies and is dependent on the oxidation state.

5.3.16 Vanadium

Vanadium occurs in different forms, as the –1, 0, +2, +3, +4 and +5 oxidation states. The predominant forms in biological materials are vanadate and vanadyl. Beverages, fats, oils, fresh fruits and vegetables contain low levels of vanadium, whereas whole grains, seafood, meats and dairy products contain higher amounts (0.005–0.03 mg/kg). A few foods, including spinach, parsley, mushrooms and oysters, contain relatively high amounts of vanadium (0.10 mg/kg). Vanadium is present in a number of multivitamin/mineral dietary supplements at levels of approximately 0.025 mg/day. There are no licensed medicines containing vanadium [54]. No specific function has been identified for vanadium in higher animals. *In vitro* and animal studies suggest that vanadium may function as an oxidation–reduction catalyst, and may regulate the sodium–potassium adenosine triphosphatase enzyme, although this has not been proven [55]. Although no specific data have been published, it is possible that vanadium interferes with the storage and metabolism of iron, because absorbed vanadium is bound to transferrin. Intestinal absorption of vanadium is low (<5%) and the mechanism of absorption has not been defined [55].

5.3.17 Zinc

Zinc is an essential trace element for humans, animals and plants. It is vital for many biological functions and plays a crucial role in more than 300 enzymes in the human body [56]. Meat and cereal products are rich in zinc. Zinc salts are also widely available in food supplements. Zinc is present in a number of licensed medicines both alone and in combination with other ingredients, and it is used for the treatment and prevention of deficiency. The maximum daily dose of products available from a pharmacist is 150 mg, and of products available on the General Sales List in the UK is 5.0 mg [40, 54]. Zinc is an essential constituent of more than 200 metalloenzymes. Zinc plays a key role in the synthesis and stabilization of genetic material and is necessary for cell division and the synthesis and degradation of carbohydrates, lipids and proteins [40, 56]. Zinc and copper are mutually antagonistic, each interfering with the gastrointestinal uptake of the other, thus potentially leading to an imbalance. Similarly, zinc and iron compete for absorption sites. Excess zinc may also decrease magnesium and calcium uptake and, conversely, high levels of calcium in the diet can decrease zinc absorption. Zinc salts reduce the bioavailability of fluoroquinoline antibiotics [29, 40]. The absorption of zinc salts from food is approximately 20–40%, being higher from fish and meat but lower from cereals, where the phytate content impairs absorption. The absorption of zinc salts is dependent on their solubility. Zinc is absorbed both by passive diffusion and an unknown membrane carrier process that requires energy [56].

5.4 The functional specificity of oligoelements

The oligoelements are in most cases essential trace elements present at very low levels and function to activate the catalytic sites of enzymes and to act as cofactors. However, the details of the specific biochemical behaviour of some trace elements are still not well understood. Copper, iron and zinc are micronutrients present in relatively high levels in the human body. Copper and iron are transported, used and stored bound to specific proteins such as ceruloplasmin, ferritin and transferrin, which prevent or minimize the reactions catalysed by these minerals. Furthermore, iron, copper and molybdenum play an important biological role in the redox process by interacting with donor atoms of electrons such as nitrogen, sulfur and oxygen, whereas other trace elements zinc and nickel carry out their activity bound to specific substrates [57]. The functional specificity of oligoelements is enhanced by specific carrier and storage proteins, such as transferrin and ferritin for iron and ceruloplasmin for copper [58]. These proteins recognize and bind with oligoelements in a specific manner, ensuring their reserves and transporting them to specific sites in the organism.

Selenium and zinc are also involved in cellular defence mechanisms and it has been demonstrated that the antioxidant enzymes, such as glutathione peroxidase, antagonize mineral-dependent carcinogenic processes. Increased oxidative stress, due to the production of excessive amounts of free radicals along with the effects of chronic inflammation, plays a major role in the initiation and progression of a host of disease states [59]. Details of the specific biochemical behaviour of some trace elements are shown in Figure 5.2. The characteristics of trace element deficiency, especially in adults, may be explained by a reduction in antioxidant actions of enzymes such as glutathione reductase.

A subject which has recently attracted considerable attention is the reduction in the antioxidant potential thought to underlie the development of numerous lifestyle disease conditions. The proper selection of food to meet our daily needs and healthy eating habits can be achieved by adapting the amount and quality of food in order to maintain health, prevent disease and prolong life. In this context, nutritional recommendations have

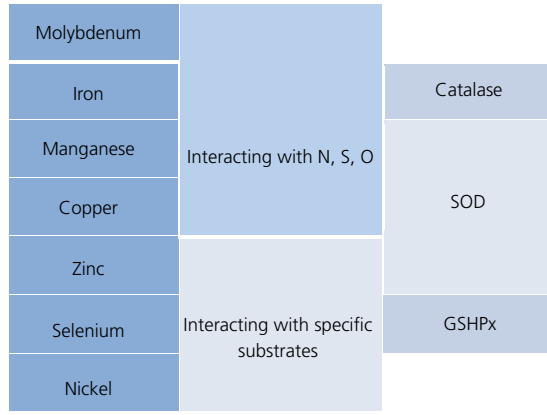


Figure 5.2 Details of the specific biochemical behaviour of some trace elements. SOD, superoxide dismutase; GSHPx, glutathione peroxidase.

Table 5.1 Recomended daily dietary intakes of trace elements.

Element	Quantity	Intake per day
Arsenic	Provisional tolerable intake	2 μg/kg body weight
Chromium	Estimated safe and adequate intake	50–200 μg
Cobalt	Recommended dietary allowance	3 μg vitamin B_{12}*
Copper	Estimated safe and adequate intake	2–3 mg
	Maximum acceptable daily load	0.5 mg/kg body weight†
Fluoride	Estimated safe and adequate allowance	1.5–4.0 μg
Iodine	Recommended dietary allowance	150 μg
	Maximum tolerable intake	17 μg/kg body weight
Iron	Recommended dietary allowance	1.0 or 18 mg (age/sex dependent)
	Maximum acceptable daily load	0.8 mg/kg body weight
Magnesium	Recommended dietary allowance	300 mg (female), 350 mg (male)
Manganese	Estimated safe and adequate intake	2.5–5.0 mg
Molybdenum	Estimated safe and adequate intake	0.15–0.5 mg
Selenium	Estimated safe and adequate intake	50–200 μg
Tin	Maximum acceptable daily load	2 mg/kg body weight†
Zinc	Recommended dietary allowance	15 mg
	Maximum acceptable daily load	1.0 mg/kg body weight†

*Expressed as weight of vitamin B_{12}, which contains about 4% weight of cobalt.
†Provisional value.

been established through RDAs to provide guidance regarding the amount of nutrients needed for a balanced daily diet [60]. Risk assessment of essential trace elements examines high intakes resulting in toxicity and low intakes resulting in nutritional deficiencies. Recommended daily intakes are presented in Table 5.1.

Rice *et al.* [61] showed that the American population is experiencing an epidemic of obesity due to poor eating habits in terms of nutrient and energy intake. This obesity [62] is directly linked to the intake of oligoelements and it can be controlled with a healthy and balanced diet combined with physical activity. Furthermore, this combination offers an important approach to reducing the risk of cardiovascular disease [60], type 2 diabetes [63], kidney disease and even a predisposition to cancer [60] (see Table 5.2 for further details). Besides these diseases, anaemia can also be acquired or worsened by a poor diet often lacking in iron. This condition is characterized by a reduced haemoglobin concentration impairing the proper functioning of the blood (i.e. transport of oxygen from the lungs to all cells of the body), besides lowering the production of erythrocytes [60]. Studies have also demonstrated the association of trace elements with other conditions that are not directly related to food, such as osteoporosis (F, Zn, Cu, B, Mn and Fe), maintenance of the thyroid gland (Se and I) and the menopause (Fe, Cr, Zn and Mn).

5.5 Nutritional aspects, deficiencies and excess of oligoelements

Essential trace elements of the human body include zinc, copper, selenium, chromium, cobalt, iodine, manganese and molybdenum. Although these elements account for only 0.02% of total body weight, they play significant roles, for example as active centres of enzymes or as trace bioactive substances. A major outcome of trace element deficiencies is reduced activity of the enzymes concerned. However, since each trace element is related to many enzymes, deficiency of a single trace element is often not associated with a specific clinical manifestation, but rather manifests as a combination of various symptoms. Given that trace elements are present in very small amounts and the absence of specific clinical features associated with their deficiency, it is often difficult for clinicians to identify the deficiency of a particular trace element.

Table 5.3 lists the enzymes containing trace elements, and summarizes the physiological functions of trace elements and the characteristics of their deficiency and their excess.

Table 5.2 Oligoelements and their potential effects in relation to certain diseases.

	Obesity	Cancer	Cardiovascular disease	Kidney disease
Copper	—	Maintenance of DNA with Zn and Cu. Cofactor of superoxide dismutase	Increase in level due to degradation of ceruloplasmin	—
Iron	—	Binds to proteins (ferritin). Forms reactive oxygen species, triggering oxidative stress. Promotes lipid peroxidation, mutagenesis and DNA cleavage	Catalyses lipid peroxidation. Disturbs the integrity of the endothelial membrane and its functioning. Associated with development of atherosclerosis	—
Zinc	Functional and structural relationship with insulin	Important for the maintenance of conformation, stability and activity of over 200 metalloenzymes (superoxide dismutase). Its deficiency causes damage to the enzymes	Protects against myocardial damage caused by free radicals. Catalyses the dismutation of O_2 to H_2O_2	Deficiency causes testicular atrophy, depression, immune deficiency, growth retardation and abnormalities in relation to taste and smell
Selenium	—	Modulates antioxidant capacity. Metabolizes carcinogens. Modulates immune function. Promotes cell proliferation and inhibits induction of apoptosis	Increases the ability of the enzyme glutathione peroxidase to combat the oxidative modification of lipids. Reduces platelet aggregation	Enhances the activity of glutathione peroxidase and has cardioprotection and immunostimulatory effects. Supplementation may be beneficial
Manganese	Insulin sensitivity. Oxidation of glucose in patients with diabetes mellitus	—	Essential for normal myocardial contractility	Increased levels. Phosphate binder
Vanadium	Inhibits tyrosine phosphatase. Activates various components of insulin signalling pathway	Modulates the expression of inducible nitric oxide synthase and p53. Induces apoptosis. Alkylation prevents DNA damage by chemical carcinogens	Controls hypertension	—
Nickel	—	—	Affects the electrical properties of the heart (together with Co)	Seems to have some role in enzymatic reactions. Muscle degeneration of the kidneys

Source: adapted from Wada [64].

5.6 Conclusions

This chapter provides an overview of the interaction between trace elements acting as nutrients and their physiological role. Also, enzymes containing trace elements are discussed. The studies cited show that increasing amounts of data have been collected to examine the importance of the association of trace elements with various forms of nutritional, metabolic, hormonal or physiological stress in animals and humans. These studies indicate that in certain situations a trace element is of nutritional significance. Thus, many of the elements described in this review should be considered as desirable inclusions in nutrient databanks.

It is clear from the preceding discussion that, except for a few major trace elements, only sparse data of uncertain reliability are available to evaluate the dietary content of trace elements emerging as important in human nutrition. The data on certain trace elements, notably boron, chromium and selenium, are likely to be

Table 5.3 Trace elements: functions and symptoms of their deficiency and excess.

Trace element	Physiological functions	Symptoms of deficiency	Symptoms of excess
Arsenic	Methionine metabolism	Studies in animals show that arsenic is essential for growth, development and reproduction, possibly because of its role in the metabolism of methionine, an amino acid involved in growth	Headaches, confusion, severe diarrhea, and drowsiness. Arsenic is related to heart disease, cancer, cerebrovascular diseases, chronic lower respiratory diseases and diabetes
Boron	Metabolism of calcium and other major minerals	The signs of boron deficiency in animals vary in nature and severity according to the dietary content of aluminium, calcium, magnesium and potassium	Scanty hair, anaemia and seizure disorders
Chromium	Essential to normal carbohydrate, lipid and protein metabolism. Acts as a cofactor of insulin	Glucose intolerance, hyperinsulinaemia, neuropathy, increased intraocular pressure	Pathological anatomical changes in the lungs, kidneys and liver
Cobalt	Integral part of vitamin B_{12}, which is essential for fatty acid metabolism	Anaemia	Cardiomyopathy. Features of chronic toxicity include effects on the heart, thyroid and possibly the kidney
Copper	Role in the electron transport chain	Anaemia, neutropenia, and reduction in the catalytic activity of enzymes employing copper as a cofactor	Disorders in the expression of copper transporters
Fluoride	Constituent of bone collagen and glycosaminoglycans of the vascular system, the skin and other tissues	Susceptibility to dental caries	Long-term exposure to high levels of fluoride leads to dental destruction, radiculopathy, myelopathy and peripheral neuropathy and severe forms of skeletal deformity
Iodine	Essential constituent of the thyroid hormones thyroxine and T_3	Hypothyroidism, weakness, cold intolerance, weight gain, thinning hair and goiter (thyroid enlargement)	Thyroiditis, goiter, hypo- or hyper-thyroidism, thyroid papillary cancer and dermatoses (iodermia)
Iron	Production of red blood cells and formation of haemoglobin	Hypochromic microcytic anaemia, pallor, fatigue and decreased work performance	Haemosiderosis, haemochromatosis, accumulation in liver, heart and some endocrine tissues. Iron toxicity can be fatal
Magnesium	Energy metabolism and nucleic acid synthesis, physiological calcium antagonist	Hypomagnesaemia, neuromuscular, cardiac or nervous disorders	Hypermagnesaemia, hypotension, cutaneous flushing, nausea and vomiting, neuromuscular dysfunction, ranging from drowsiness to respiratory depression, hypotonia, areflexia and coma in severe cases
Manganese	Cofactor for enzymes involved in protein and energy metabolism	Osteoporosis, epilepsy, atherosclerosis, impaired wound healing, and increased susceptibility to cancer	Fatigue, myalgia, headache
Molybdenum	Enzyme cofactor	High blood methionine, low blood and urinary uric acid concentrations	Reduces the utilization of copper. Anaemia, cardiac hypertrophy
Nickel	Propionic acid metabolism	Depressed growth and reproductive performance. Affects distribution and proper functioning of other nutrients including calcium, iron, zinc and vitamin B_{12}	Dermatitis, hypernickelaemia

(*Continued*)

Table 5.3 (Continued)

Trace element	Physiological functions	Symptoms of deficiency	Symptoms of excess
Selenium	Cofactor of antioxidant enzymes	Deleterious effects on the central nervous system	Selenosis
Sulfur	Metabolism and action of methionine and taurine and oxidative metabolism of cysteine	Low-sulfur proteins and abnormal processing of sulfur-containing amino acids	Sulfur dioxide, a major air pollutant, may adversely affect animal and human health by causing bronchitis, bronchoconstriction and increased pulmonary resistance
Tin	Function in the tertiary structure of proteins	Poor growth, decreased efficiency of food utilization, alopecia and changes in the mineral concentration in various organs	Organotin compounds are appreciably toxic and attack the central nervous system. The resulting myelinopathies and spongy degeneration of the brain lead to limb incoordination and ataxia, and may result in complete paralysis and death
Vanadium	Role in the regulation of Na^+/K^+-ATPase, phosphoryl-transfer enzymes, adenylate cyclase and protein kinases	Skeletal deformations	Depressed growth, elevated organ vanadium content, diarrhea, depressed food intake and death. Signs of excessive vanadium intake in humans include gastrointestinal disturbances
Zinc	Essential constituent of metalloenzymes. Synthesis of carbohydrates, lipids and proteins	Growth retardation, mental retardation, dermatitis, hair loss. Susceptibility to infections	Abdominal pain, nausea and vomiting. Prolonged use of high doses of zinc can result in hypocupraemia (deficiency of copper)

Source: adapted from Wada [64].

in great demand by nutrient databank users in the near future. Although some progress has been made in recognizing the importance of trace elements in nutrition to the point that some are included in major nutrient databases, a major challenge lies ahead since more trace element data are needed. Nutrient databank managers are becoming increasingly aware that trace elements other than copper, iron and zinc are important in human nutrition and these include arsenic, boron, chromium, cobalt, fluoride, iodine, magnesium, manganese, nickel, molybdenum, selenium, sulfur, tin and vanadium.

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Abbreviations

ATP: Adenosine triphosphate
DNA: Deoxyribonucleic acid
E512: Stannous chloride additive
FAO: Food and Agricultural Organization
GSH-Px: Glutathione peroxidase
HIV: human immunodeficiency virus
IAEA: International Atomic Energy Agency
RDA: Recommended dietary allowance
RNA: Ribonucleic acid
T_3: Triiodothyronine
T_4: Thyroxine
WHO: World Health Organization

References

1 Gao S, Jin Y, Unverzagt FW. Trace element levels and cognitive function in rural elderly Chinese. *J Gerontol A Biol Sci Med Sci* 2008;**63**:635–641.
2 World Health Organization. *Trace Elements in Human Nutrition: report of a joint FAO/WHO/IAEA Expert Consultation*. WHO Technical Report Series. WHO, Geneva, 1995.

3 World Health Organization. *Diet, nutrition, and the prevention of chronic diseases. Report of a WHO Study Group*. WHO Technical Report Series No. 797. WHO, Geneva, 1990.

4 Nielsen FH. New essential trace elements for the life sciences. *Biol Trace Elem Res* 1990;**27**:599–611.

5 Aguirre P, Mena N, Tapia V, Arredondo M, Núñez MT. Iron homeostasis in neuronal cells: a role for IREG1. *BMC Neurosci* 2005;**6**:3–9.

6 Dexter DT, Carayon A, Javoy-Agid F *et al*. Alterations in the levels of iron, ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglia. *Brain* 1991;**114**:1953–1975.

7 Thomas B, von Coelln R, Mandir AS *et al*. MPTP and DSP-4 susceptibility of substantia nigra and locus coeruleus catecholaminergic neurons in mice is independent of parkin activity. *Neurobiol Dis* 2007;**26**:312–322.

8 Gutteridge JM. Iron and oxygen radicals in brain. *Ann Neurol* 1992;**32**:S16–S21.

9 Herrera-Rivero M, Hernández-Aguilar ME, Manzo J, Aranda-Abreu GE. Enfermedad de Alzheimer: inmunidad y diagnostic. *Rev Neurol* 2010;**51**:153–164.

10 Koh JY. Endogenous zinc in neurological diseases. *J Clin Neurol* 2005;**1**:121–133.

11 van Roon-Mom WMC, Pepers BA, Hoen PA *et al*. Mutant huntingtin activates Nrf2-responsive genes and impairs dopamine synthesis in a PC12 model of Huntington's disease. *BMC Mol Biol* 2008;**9**:84–93.

12 Ben-Shachar D, Eshel G, Finberg JP, Youdim MB. The iron chelator desferrioxamine (Desferal) retards 6-hydroxydopamine-induced degeneration of nigrostriatal dopamine neurons. *J Neurochem* 1991;**56**:1441–1444.

13 Rosas HD, Salat DH, Lee SY *et al*. Cerebral cortex and the clinical expression of Huntington's disease: complexity and heterogeneity. *Brain* 2008;**131**:1057–1068.

14 Al-Bader A, Christenson JT, Simonet F, Abul H, Dashti H, Schmuziger M. Inflammatory response and oligo-element alterations following cardiopulmonary bypass in patients undergoing coronary artery bypass grafting. *Cardiovasc Surg* 1998;**6**:406–414.

15 World Health Organization. *Trace Elements in Human Nutrition and Health*. WHO, Geneva, 1996.

16 Halliwell B, Gutteridge JMC. Biologically relevant metal ion-dependent hydroxyl radical generation. An update. *FEBS Lett* 1992;**307**:108–116.

17 Arthur JR, Beckett GJ. Selenium deficiency and thyroid hormone metabolism. In: Wendel A (ed.) *Selenium in Biology and Medicine*. Springer, Berlin, 1989, pp. 90–95.

18 Arthur JR, Nicol F, Beckett GJ. Hepatic iodothyronine 5′-deiodinase: the role of selenium. *Biochem J* 1990;**272**:537–540.

19 Hughes MF, Beck BD, Chen Y, Lewis AS, Thomas DJ. Arsenic exposure and toxicology: a historical perspective. *Toxicol Sci* 2011;**123**:305–332.

20 Chen CL, Whanger PD. Interaction of selenium and arsenic with metallothionein: effect of vitamin B_{12}. *J Inorg Biochem* 1994;**54**:267–276.

21 US Food and Drug Administration. Questions and answers regarding 3-Nitro (Roxarsone). Available at http://www.fda.gov/AnimalVeterinary/SafetyHealth/ProductSafetyInformation/ucm258313.htm(2011)

22 Nielsen FH. Boron in human and animal nutrition. *Plant Soil* 1997;**193**:199–208.

23 Hunt CD. Dietary boron: progress in establishing essential role in human physiology. *J Trace Elem Med Biol* 2012;**26**:157–160.

24 Korkmaz M. Boron: environmental exposure and human health. In: Nriagu JO (ed.) *Encyclopedia of Environmental Health*, Vol. **1**. Elsevier, Burlington, MA, 2011, pp. 442–445.

25 Wise SS, Holmes AL, Wise JP. Hexavalent chromium-induced DNA damage and repair mechanisms. *Rev Environ Health* 2008;**23**:39–57.

26 Cozzolino SMF. *Biodisponibilidade de Nutrientes*, 4th edn. Ed. Manole, Barueri, SP, Brazil, 2012.

27 Anderson RA. Chromium as an essential nutrient for humans. *Regul Toxicol Pharmacol* 1997;**26**:S35–S41.

28 Kiefer D. Chromium: an element essential to health. *Life Extension* 2004;**10**:60–67.

29 Fraga CG. Relevance, essentiality and toxicity of trace elements in human health. *Mol Aspects Med* 2005;**26**:235–244.

30 Rucker RB, Fascetti AJ, Keen CL. Trace minerals. In: Kaneko JJ, Harvey JW, Bruss ML (eds) *Clinical Biochemistry of Domestic Animals*, 6th edn. Academic Press, Burlington, MA, 2008, pp. 663–693.

31 Torres-Vega A, Pliego-Rivero BF, Otero-Ojeda GA, Gómez-Oliván LM, Vieyra-Reyes P. Limbic system pathologies associated with deficiencies and excesses of the trace elements iron, zinc, copper, and selenium. *Nutr Rev* 2012;**70**:679–692.

32 Arredondo M, Núñez MT. Iron and copper metabolism. *Mol Aspects Med* 2005;**26**:313–327.

33 Cerklewski FL. Fluoride bioavailability: nutritional and clinical aspects. *Nutr Res* 1997;**17**:907–929.

34 Queste A, Lacombe M, Hellmeier W *et al*. High concentrations of fluoride and boron in drinking water wells in the Muenster region: results of a preliminary investigation. *Int J Hyg Environ Health* 2001;**203**:221–224.

35 Bhatnagar A, Kumar E, Sillanpää M. Fluoride removal from water by adsorption: a review. *Chem Eng J* 2011;**171**:811–840.

36 Quock RL, Gao JX, Chan JT. Tea fluoride concentration and the pediatric patient. *Food Chem* 2012;**130**:615–617.

37 Camargo JA. Fluoride toxicity to aquatic organisms: a review. *Chemosphere* 2003;**50**:251–264.

38 Melse-Boonstra A, Jaiswal N. Iodine deficiency in pregnancy, infancy and childhood and its consequences for brain development. *Best Pract Res Clin Endocrinol Metab* 2010;**24**:29–38.

39 Abraham GE. The history of iodine in medicine. Part 1: from discovery to essentiality. *Original Internist* 2006;**13**:29–36.

40 Goldhaber SB. Trace element risk assessment: essentiality vs. toxicity. *Regul Toxicol Pharmacol* 2003;**38**:232–242.
41 Bury NR, Boyle D, Cooper CA. Iron. In: Wood CM, Farrell AP, Brauner CJ (eds) *Fish Physiology*, Vol. **31**, Part A. Springer Science and Business Media, New York, 2011, pp. 201–251.
42 Erikson KM, Syversen T, Aschner JL, Aschner M. Interactions between excessive manganese exposures and dietary iron deficiency in neurodegeneration. *Environ Toxicol Pharmacol* 2005;**19**:415–421.
43 Harris ED. Cofactors: inorganic. In: Caballero B, Allen L, Prentice A (eds) *Encyclopedia of Human Nutrition*, 3rd edn. Elsevier, Amsterdam, 2013, pp. 357–365.
44 Keen CL, Ensunsa JL, Lönnerdal B, Zidenberg-Cherr S. Manganese. In: Caballero B, Allen L, Prentice A (eds) *Encyclopedia of Human Nutrition*, 2nd edn. Elsevier, Amsterdam, 2005, pp. 217–225.
45 Majumdar A, Sarkar S. Bioinorganic chemistry of molybdenum and tungsten enzymes: a structural–functional modeling approach. *Coordin Chem Rev* 2011;**255**:1039–1054.
46 Zhang Y, Rump S, Gladyshev VN. Comparative genomics and evolution of molybdenum utilization. *Coordin Chem Rev* 2011;**255**:1206–1217.
47 Vazquez-Gonzalez JF, Bermejo-Barrera P, Bermejo-Martinez F. Determination of traces of molybdenum in foods by electrothermal atomic absorption spectrometry. *Anal Chim Acta* 1989;**219**:79–87.
48 Martin-Diaconescu V, Maroney MJ. Nickel bioinorganic systems. In: Reedijk J, Poeppelmeier K (eds) *Comprehensive Inorganic Chemistry II*, 2nd edn, Vol. **3**. Elsevier, Amsterdam, 2013, pp. 295–322.
49 Navarro-Alarcon M, Cabrera-Vique C. Selenium in food and the human body: a review. *Sci Total Environ* 2008;**400**: 115–141.
50 Hartikainen H. Biogeochemistry of selenium and its impact on food chain quality and human health. *J Trace Elem Med Biol* 2005;**18**:309–318.
51 Pedrero Z, Madrid Y. Novel approaches for selenium speciation in foodstuffs and biological specimens: a review. *Anal Chim Acta* 2009;**634**:135–152.
52 Stockler-Pinto MB, Mafra D, Farage NE, Boaventura GT, Cozzolino SMF. Effect of Brazil nut supplementation on the blood levels of selenium and glutathione peroxidase in hemodialysis patients. *Nutrition* 2010;**26**:1065–1069.
53 Yao Y, Pei F, Kang P. Selenium, iodine, and the relation with Kashin–Beck disease. *Nutrition* 2011;**27**:1095–1100.
54 Vincent JB, Neggers Y. Roles of chromium(III), vanadium and zinc in sports nutrition. In: Bagchi D, Nair S, Sen CK (eds) *Nutrition and Enhanced Sports Performance*. Academic Press, San Diego, CA, 2013, pp. 447–454.
55 Mukherjee B, Patra B, Mahapatra S, Banerjee P, Tiwari A, Chatterjee M. Vanadium: an element of atypical biological significance. *Toxicol Lett* 2004;**150**:135–143.
56 Prasad AS. Discovery of human zinc deficiency: 50 years later. *J Trace Elem Med Biol* 2012;**26**:66–69.
57 Takagi Y, Okada T. Metabolism of trace elements. *Jpn J Clin Med* 2001;**59**:401–412.
58 de Silva DM, Aust SD. Ferritin and ceruloplasmin in oxidative damage: review and recent findings. *Can J Physiol Pharmacol* 1993;**71**:715–720.
59 Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine*, 4th edn. Oxford University Press, New York, 2007.
60 Liao CM, Lin TL, Chen SC. A Weibull-PBPK model for assessing risk of arsenic-induced skin lesions in children. *Sci Total Environ* 2008;**392**:203–217.
61 Rice BH, Quan EE, Miller GD. Meeting and exceeding dairy recommendations: effects of dairy consumption on nutrient intakes and risk of chronic disease. *Nutr Rev* 2013;**71**: 209–223.
62 Lind L, Lithell H, Hvarfner A, Pollare T, Ljunghall S. On the relationships between mineral metabolism, obesity and fat distribution. *Eur J Clin Invest* 1993;**23**:307–310.
63 Sharma S, Prasad R, Choudhary AM, Jain S, Goyalb S, Agarwal V. Beneficial effect of chromium supplementation on glucose, HbA1C and lipid variables in individuals with newly onset type-2 diabetes. *J Trace Elem Med Biol* 2011;**25**:149–153.
64 Wada O. What are trace elements? Their deficiency and excess states. *Jpn Med Assoc J* 2004;**47**:351–358.

CHAPTER 6
The toxic elements

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Abstract: Toxic elements in food are defined as elements which can be present in food in amounts that can be potentially hazardous to human health. These elements in food originate from several different sources among which the most frequently studied are agricultural production of plants and animals, industrial or household processing, and migration from packaging during storage of final products. All metals are probably toxic if ingested in sufficient amounts. Although all elements can interact within the human body, it is possible to differentiate between elements considered to be essential and those that cause toxicological symptoms at extremely low levels and which have no known beneficial physiological functions. The most dangerous metals we are likely to meet in our food are beryllium, cadmium, lead and mercury.

Keywords: beryllium, cadmium, lead, mercury, toxic element, food

6.1 Toxic metals in foods

Toxic elements in food are defined as those present in food in amounts that can be potentially hazardous to human health. Toxic elements in food originate from several different sources among which the most frequently studied are agricultural production of plants and animals, industrial or household processing, and migration from packaging during storage of final products [1, 2].

It is not always possible to distinguish clearly between metals in this category and others that play essential roles in human metabolism and body function. All metals are probably toxic if ingested in sufficient amounts. Sometimes the margin between toxicity and sufficiency is very small, as is the case for example with selenium. It is difficult to consider toxicity of a single metal in isolation from other metals and indeed from all the other components of a food. Under normal conditions all metals are capable of interacting with other metals in the body if consumed together. For example, the physiological effects, including its toxicity, of cadmium are closely related to the amount of zinc also present. Likewise the function of iron in cells is affected by both copper and cobalt, and to some extent also by molybdenum and zinc. Several other similar interactions between metals in the body are known to occur.

Despite the above reservations, it is possible to differentiate between elements that are known with certainty to be essential and those which cause severe toxic symptoms at low concentrations and have no known beneficial functions. Mercury, cadmium and lead are usually considered to qualify for inclusion among the toxic metals, though there are indications that cadmium and even lead may one day be shown to be essential elements. Although all elements can interact within the human body, it is possible to differentiate between elements which are considered essential and those that cause toxicological symptoms at extremely low levels and which have no known beneficial physiological functions. The main toxic elements that are representative of the second group are mercury, lead, cadmium and arsenic. These toxic elements could contaminate agricultural soils, water supplies and the environment and consequently find their way into the human food chain [3].

The term ‘heavy metals’ is often used to describe the group of toxic metals. It is a descriptive rather than a

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scientific classification. Strictly speaking it should only be used for those elements with an atomic weight of 200 or above, such as mercury (201), thallium (204), lead (207) and bismuth (209). In practice it is usually used to refer to metals of a high specific gravity which have a strong attraction to biological tissues and which are slowly eliminated from biological systems. However, a typical listing of heavy metals will often be found to include arsenic, beryllium, boron, selenium and other metals and metalloids, as well as mercury, cadmium and lead. This is probably a convenience and related more to the metals' toxicity rather than to their specific gravity. A US official document in 1972 defined heavy metals as 'common metallic impurities that are colored by H_2S: Ag, As, Bi, Cd, Cu, Hg, Pb, Sb and Sn'. This distinction based on chemical properties has been adopted by regulatory authorities in several other countries.

The predominant route of entrance of toxic elements into the food chain arises during agricultural plant cultivation, via absorption of macronutrients and micronutrients from the soil by plants. Soils can be naturally rich in some highly toxic elements. Some of the contaminants, especially soluble ones, are present in soil solution in high concentration. For example, cadmium content in soils originating from crystal rocks was determined to range from 1 to 90,000 μg/kg [3–5], while soils overlying some sedimentary rocks contained several hundred milligrams of arsenic per kilogram [6]. Besides natural occurrence, elevated concentrations of inorganic contaminants in soil can also be a result of human activities. It was established that elevated concentrations of arsenic in soil can be the consequence of coal burning in power stations, while cadmium contamination of rice in Japan was linked to the contaminated fields [7]. Soils can also be polluted by other industrial activities such as ore melting or battery production. As one of the most studied pollutants, lead enters the food chain as a result of combustion of lead-containing petrol additives, smelting and other anthropogenic activities [8]. Regardless of the route by which lead finds its way into the soil, it mainly occurs in the upper layers of soil profiles due to its intensive sorption by soil [9, 10]. Bearing in mind anthropogenic sources of environmental pollution, intensive agriculture itself can significantly contribute to soil pollution. For example, arsenic is used as a constituent of some insecticides, herbicides and defoliants while mercury is introduced into the soil as a constituent of fungicides, fertilizers, limestone, natural gypsum and manure [11]. Avoidance of soil pollution by some inorganic contaminants, for example cadmium, is almost impossible to achieve, since cadmium is present in practically all phosphate fertilizers as an impurity.

The availability of inorganic contaminants in the soil to food plants depends on many factors, including soil properties such as pH, amount of clay, amount and type of organic compounds in soil, redox potential and textural class, and the presence of microorganisms. By influencing the factors which determine availability, agricultural practice can affect the available/unavailable ratio of toxic metals.

The uptake of toxic metals by terrestrial plants is often considered as the entrance point of many inorganic food contaminants into the food chain and thus is the subject of many studies oriented to different metals, concentrations, species, genotypes and translocation rates [12, 13]. Alternatively, toxic elements can enter the food chain from different environmental compartments, for example from freshwater plants that are also able to take up toxic metals from rivers and canals [14], or through food animals via feed additives containing dangerous elements like arsenic. Small localized incidents occur quite frequently, for example consumption of seeds treated with mercuric fungicides [15], and can also be considered a means of entry of inorganic contaminants into the food chain. Figure 6.1 shows a pathway for the entry of toxic metals into food.

Where inorganic contaminants are taken up by food plants from soil, the main fraction of the toxic elements remains in the roots and the plant organs that were in direct contact with the soil. On the other hand, depending on the type of plant, fruits can be the most important edible parts and thus when considering the contamination of food plants by inorganic contaminants, the focus has to be on the transport of inorganic contaminants to edible parts of plants. Some contaminants such as arsenic are toxic to plants at low concentrations and their accumulation in roots causes plant injury, preventing further transport to above-ground parts [6].

In animal food products, one of the most important factors that influences the content of toxic metals is the lifespan of the animal. Animals with a long lifespan, such as horses, older cattle and game, can accumulate inorganic contaminants. When exposed to feed contaminated with toxic metals, long-lived animals accumulate

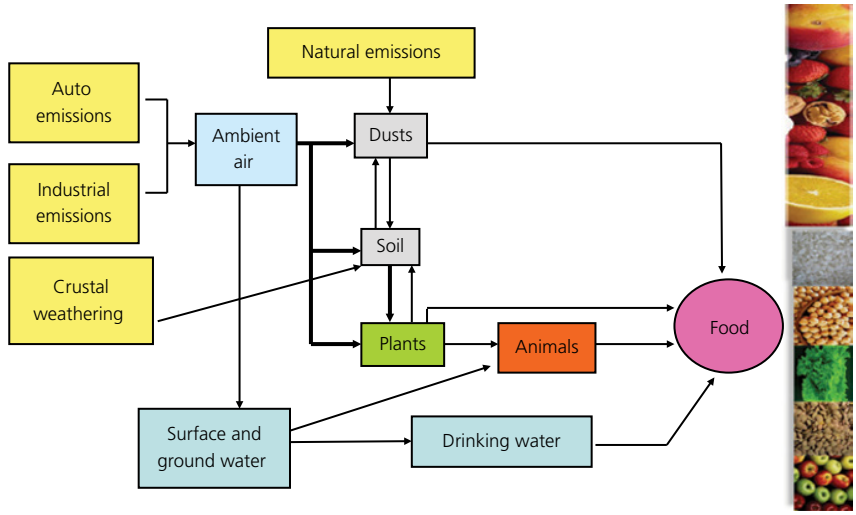


Figure 6.1 Pathways of metals from the environment to food. (See insert for colour representation of the figure.)

metals in blood, bones and meat. The edible organs where the highest concentrations of metals are expected are kidney and liver [16]. Despite the fact that game is not usually part of a regular daily diet, it is important to mention that it can be contaminated with lead, cadmium and other contaminants [17]. In comparison, the toxic metal content in meat from intensively produced animals, especially ones with a short lifespan, represents a low risk [18].

The most dangerous metals likely encountered in our food and which cause severe toxic symptoms at low concentrations and have no known beneficial functions are beryllium, cadmium, lead and mercury [1].

6.2 Beryllium

6.2.1 General information

Beryllium is the 44th most abundant element in the earth's crust, its average concentration being estimated at 2–5.0 mg/kg. This metal is a naturally occurring element that is present in a variety of materials including rocks, soil, coal, volcanic dust and oil [19]. It is found at concentrations ranging from 1 to 15 ppm in rocks at the earth's surface and at lower concentrations in soil. Because of its special properties of light weight, heat resistance and hardness, beryllium is used for specialized applications in electronics fabrication and as a component of systems used in the aerospace, defence and nuclear power industries [20, 21].

It is present in air, soil and water as the result of a number of natural and human activities. The concentrations in groundwater are usually very low. Beryllium is found at very low levels in many foods (fruits, vegetables, meats and shellfish). The concentrations in air are normally very low. Almost all airborne beryllium is due to oil and coal combustion. Coal ash, incinerator ash and industrial wastes are important human sources of concentrated beryllium that can pollute soil and groundwater if not disposed of properly.

Beryllium compounds are commercially mined, and the beryllium is purified for use in nuclear weapons and reactors, aircraft and space vehicle structures, instruments, X-ray machines, and mirrors. Beryllium ores are used to make speciality ceramics for electrical and high-technology applications. Beryllium alloys are used in automobiles, computers, sports equipment (golf clubs and bicycle frames), and dental bridges.

In air, compounds of this element are present mostly as fine dust particles. The dust eventually settles over land and water. Rain and snow aid in the removal of beryllium from air. Sufficiently small particles may remain airborne for about 10 days. The beryllium content of the earth's crust is 2.6 ppm and of soil is 6 ppm, and it can pass into the plants grown on it provided it is in a soluble form. Typical levels in plants vary between

1 and 40 ppb, too low to affect animals which eat these plants. Approximately 50 beryllium minerals occur in nature, and more than half of these minerals are silicates. A major portion of beryllium in soil does not dissolve in water but remains bound to soil, so it is not very likely to move deeper into the ground and enter groundwater. In the environment, chemical reactions can change the water-soluble beryllium compounds into insoluble forms. In some cases, water-insoluble compounds can change to soluble forms. Exposure to water-soluble forms in the environment will generally pose a greater threat to human health than water-insoluble forms.

Most of the beryllium in water settles in the material on the bottom. Beryllium compounds remain in ocean water for a few hundred years before settling to the bottom of the ocean.

6.2.2 Environmental considerations

The general population can be exposed to low levels of this element by breathing air, eating food or drinking water that contains beryllium [22, 23]. It is naturally found at low levels in some foods. The intake of beryllium for most people will be very small. If the average concentration of beryllium in air is assumed to be less than 0.03 ng/m^3, and if a normal adult inhales approximately 20 m^3 of air per day, then the inhalation exposure would be approximately 0.6 ng of beryllium per day. This value may be somewhat higher for persons living near the sources of beryllium emission.

For drinking water, it is reasonable to assume that in highly industrialized countries, the contribution to the overall beryllium intake is of the order of 0.4–1 μg/day. Thus, compared with food, drinking water will contribute very little to the total intake of this metal.

The most important way that people are exposed to beryllium is by breathing in vapours or dust. Studies have found that ingestion of beryllium results in less than 1% of it being absorbed. Absorption through the skin was found to be negligible. Therefore inhalation is the most important exposure pathway.

Occupational exposure occurs at places where the chemical is mined, processed and converted into metal, alloys and other chemicals. Workers engaged in machining metals containing beryllium, in recycling beryllium from scrap alloys, or in using beryllium products may also be exposed to higher levels. People who live near hazardous landfill sites that contain high concentrations of beryllium may be exposed to higher than normal levels.

The metal occurs naturally in tobaccos and can be inhaled from cigarette smoke. Beryllium metal and metal alloys may be found in consumer products such as electronic devices (e.g. televisions, calculators and personal computers).

6.2.3 Toxic effects

Beryllium is not an element that is crucial for humans; in fact it is one of the most toxic chemicals known. It can enter the body via air, food or water, but does not enter the body from skin contact with the metal unless the skin is scraped or cut and beryllium particles become imbedded in the wound. Only a small amount enters the body on contact with a beryllium salt dissolved in water. When inhaled by humans it can damage the lungs and cause pneumonia.

The most common toxic effect is called berylliosis, a dangerous and persistent lung disorder that can also damage other organs such as the heart. In about 20% of all cases, people die of this disease. Breathing in air containing high levels of beryllium can cause an inflammatory response in the lung called acute beryllium disease. Symptoms include nasal irritation, sore throat, weight loss, fatigue and reduced breathing capacity. Skin exposure to concentrated beryllium can result in an allergic skin response. Nodules composed of immune cells known as granulomas can form. Skin exposure can also cause contact dermatitis, an inflammatory response, with symptoms of redness, irritation and itching.

Beryllium can also cause allergic reactions in people who are hypersensitive to this chemical. These reactions can be very intense and can even cause a person to become seriously ill, a condition known as chronic beryllium disease (CBD) [24–26]. The symptoms are weakness, tiredness and breathing problems. Some people that suffer from CBD will develop anorexia and blueness of hands and feet. Occasionally, patients can be in such a serious condition that CBD causes their death. In addition to causing berylliosis and CBD, beryllium can also increase the chances of cancer development and DNA damage.

The US federal government has developed regulations and recommendations to protect public health. Regulations can be enforced by law. Federal agencies that establish regulations for toxic substances include the Environmental Protection Agency (EPA) [27], the

Occupational Safety and Health Administration (OSHA) [28], and the Food and Drug Administration (FDA) [29]. Recommendations provide valuable guidelines to protect public health but cannot be enforced by law. Federal organizations that develop recommendations for toxic substances include the Agency for Toxic Substances and Disease Registry (ATSDR) [23] and the National Institute for Occupational Safety and Health (NIOSH) [30].

6.2.4 Beryllium in foods

Beryllium is commonly found as a trace element in foods because it enters the air, water and soil as a result of natural processes and human activities. It occurs naturally in the environment in small amounts, but human activities have added beryllium to the environment through production of metal and combustion of coal and oil.

Beryllium exists in air as very small dust particles. It enters waterways during weathering of soils and rocks. Industrial emissions add beryllium to air, and wastewater disposals add beryllium to water. It usually settles in sediment. Beryllium is not likely to move deeper into the soil and dissolve within groundwater. In water, chemicals react with beryllium, causing it to become insoluble. This is fortunate because the water-insoluble form of beryllium causes much less harm to organisms than the water-soluble form.

Beryllium is not accumulated in the bodies of fish. However, some fruits and vegetables such as kidney beans and pears may contain significant levels of beryllium. These levels can enter animals that eat them, but luckily most animals excrete it quickly through urine and faeces.

At present, there are no indications that beryllium levels in food and drinking water pose a serious health risk [31]. There is no evidence of this element being transformed or biomagnified within food chains. Data concerning beryllium content of foods are meagre. In limited studies of beryllium in Australian and German foods, the levels in the former were generally low and ranged from 0.01 to 0.1 ppm, while those in German foods were slightly higher. Beryllium levels in food samples range from less than 1 µg/kg to approximately 20 µg/kg, with few exceptions. Levels have been reported as 0.08 ppm in polished rice, 0.12 ppm in bread, 0.17 ppm in potatoes, 0.24 ppm in tomatoes, and 0.33 ppm in lettuce, all expressed on a dry weight basis. The beryllium level in cows' milk has been reported as 0.02 ppm in ash. Beryllium may be accumulated in some plants, being found at concentrations as high as 3 ppm in birch, aspen and willow. The average daily beryllium intake has been estimated as about 20 µg/day, mostly from foods [32]. Table 6.1 shows the beryllium content of various types of foods [23, 31, 33].

Table 6.1 Beryllium contents in various fresh foods, fruits and fruit juices.

Product	*N*	Concentration (µg/kg fresh weight)	
		Mean	Range
Apple juice	4	22.5	ND to 43.6
Bananas, pulp	400	4.2	ND to 18
Beans	3	0.07	ND to 0.07
Cabbage	1	0.2	—
Cabbage	95	0.091	ND to 0.50
Cane sugar			
Brown	—	30	—
Demerara	—	6	—
Refined	—	2	—
Granulated	—	0.2	—
Carrots, raw	—	<25	—
Citrus fruit			
Ruby red grapefruit	1	1.3	—
Lime	1	ND	—
Tangerine	1	0.8	—
Clams, hardshell	31	2 ± 3	—
Clams, softshell	10	<2	—
Crabs	6	15	10–20
Crisp bread	—	112*	—
Coriander	—	34*	—
Corn, field	—	<25	—
Dill	—	59*	—
Egg plant (aubergine)	—	26*	—
Fish, whole			
Mullet	8	11	1.6–19
Blackfish	4	11	3.7–18
Fish, filleted			
Mullet	2	1.5	ND to 2.6
Blackfish	1	0.4	—
Garden pea	—	109*	—
Green pepper	—	42*	—
Grape cultivars	3	4.4	<0.1–7
Hen eggs, yolk	1	0.2	—

(Continued)

Table 6.1 (Continued)

Product	*N*	Concentration (µg/kg fresh weight)	
		Mean	Range
Hen eggs, yolk and white	1	0.06	—
Honey			
Eucalyptus	—	0.18 ± 0.09	—
Robinia	—	0.38 ± 0.02	—
Lemon products			
CA lemon	1	17.4	—
Bottled lemon	1	17.0	—
Lemonade	1	55.3	—
Lettuce	—	16*	—
Meat	3	4	4–5
Milk	100	0.2	ND to 0.7
Mushrooms	1	1.6	—
Mushrooms, European wild	1303	9	<5–36
Orange juice	5	2.8	ND to 2.8
Oysters	59	0.6	0.2–5.4
Oysters, east coast USA	93	<2	—
Oysters, west coast USA	40	<2	—
Papaya (pulp)	1	37.3	—
Parsley	—	77*	—
Peanuts, kernels	2	0.5	0.3–0.8
Pears	—	65*	—
Potatoes	41	0.4–0.6	0.2–1.4
Red currant	1	1.1	—
Rice	3	4	3–5
Rice, peeled	—	72*	—
Stone fruit (pulp)			
Apricot	1	<0.1	—
Peach	1	ND	—
Plum	1	1.6	—
Prune	1	3.6	—
Sour cherry	1	1.5	—
Tomato sauce	2	42.4	39.8–45.0
Tomatoes	—	17*	—
Tropical fruit			
Banana	1	1.5	—
Kiwi	1	3	—
Mango	1	4.5	—
Pineapple	1	<0.1	—
Vegetable marrow (pumpkin)	—	20*	—

Source: data from Vaessen & Szteke [31].

6.3 Cadmium

6.3.1 General information

Cadmium is a relatively rare element and is not found in the pure state in nature but mainly in association with zinc, lead and copper ores in the earth's crust at concentrations between 0.1 and 1 mg/kg [34]. It is also a natural constituent of ocean water, with average levels between <5 and 110 ng/L, with higher levels reported near coastal areas and in marine phosphates and phosphorites. Natural emissions of cadmium can result from volcanic emissions, forest fires, generation of sea salt aerosols, weathering of rocks or other natural phenomena [35]. Anthropogenic activities have further contributed to background levels of cadmium in soil, water and living organisms. This metal is not degradable in nature and will, once released to the environment, stay in circulation. Cadmium and cadmium compounds, compared with other heavy metals, are relatively water soluble. They are therefore also more mobile in soil, generally more bioavailable and tend to bioaccumulate. Factors affecting bioaccumulation include the physicochemical form of cadmium, the presence of other metals, pH, salinity and temperature of aquatic environments, season, cation-exchange capacity of soils, and the species of organism or plant.

Cadmium has a limited number of applications but is used for a variety of consumer and industrial materials. Its principal uses are as follows. Cadmium hydroxide is one of the two electrode materials in Ni–Cd batteries, which have extensive applications in the railroad and aircraft industry for starting and emergency power and in consumer applications such as cordless power tools, cellular telephones, portable computers, portable household appliances and toys. Cadmium sulfide and cadmium sulfoselenide are utilized as bright yellow to deep red pigments in plastics, ceramics, glasses, enamels and artists colours. Because cadmium is used as a pigment, it is often found in plastic toys and food containers. Organic cadmium salts are used as stabilizers for polyvinylchloride (PVC). Cadmium coatings are utilized on steel, aluminium and certain other non-ferrous metal fasteners and moving parts to provide the best available combination of corrosion resistance and low coefficient of friction. In addition, cadmium coatings exhibit excellent plating characteristics on a wide variety of substrates. It is also used as a component of various alloys. Other minor uses include cadmium

telluride and cadmium sulfide in solar cells, and other semiconducting cadmium compounds in a variety of electronic applications.

Both natural processes and anthropogenic activities contribute to cadmium contamination of the environment and consequently of the food chain [36, 37], especially cereals and vegetables. Thus diet is the main route of exposure to this metal in the non-smoking population, representing more than 90% compared with other routes of exposure (inhalation, skin contact) [38]. Although the quantities of cadmium in food are relatively low and absorption in the gastrointestinal tract is reduced, daily exposure for a long period and the long half-life in the body leads to significant accumulation of this toxic metal.

As cadmium in food is primarily due to the technological use of this metal, there are certainly possibilities for reducing the exposure by source-directed measures [39]. Cadmium has already been removed or substituted in a number of food contact materials, and efforts to reduce its concentration in sewage sludge and in phosphate fertilizers are underway. Such purification or substitution efforts, however, are sometimes difficult as they may be costly. However, several new applications of cadmium batteries such as in electric and hybrid electric vehicles, remote area power storage systems, and solar cells could become significant cadmium markets.

6.3.2 Environmental considerations

Cadmium is released into the environment via non-ferrous metal mining and refining, manufacture and application of phosphate and sewage fertilizers, fossil fuel combustion, and waste incineration and disposal. As stated above, cadmium emissions to the environment are normally transported continually between the three main environmental compartments: air, water and soils.

Three distinct categories may be recognized with respect to cadmium-in-air concentrations: cadmium in ambient air, cadmium air levels in occupational exposure situations, and cadmium in air from the smoking of tobacco. All three categories may affect human cadmium intake and human health, but the levels and the transfer mechanisms to humans are very different. Whereas cadmium from occupational environments and cadmium from cigarette smoke are transferred directly to humans, cadmium in ambient air is generally deposited onto waters or soils and then eventually transferred to plants and animals, and finally enters the human body through the food chain [40–42].

Total emission to air from natural sources is estimated at about 150–2600 tonnes per year [36]. Emissions from industrial activities have been found to exceed those of natural origin by an order of magnitude [43]. However, available data indicate that anthropogenic emissions of cadmium have decreased by an average of about 50% from 1990 to 2003 in developed countries due to advances in pollution control technology. The dominant sources of atmospheric emission will vary depending on the region or country considered. The major source of cadmium emission to the air is non-ferrous metal production. In countries with extensive waste incineration the pattern may be significantly different. In Denmark, for example, waste incineration accounts for 50% of total air emission and combustion of oil products accounts for 35% of the total [44]. In the USA, combustion of coal and oil is assumed to be responsible for around 76% of total emission, whereas waste incineration accounts for around 7% of the total [45]. Atmospheric cadmium is in the form of suspended particulate matter, which may consist of very small particles (<1 μm) derived from sea spray, industrial emissions, combustion of fossil fuels, or the erosion of soils. The main cadmium compounds found in air are cadmium oxide, chloride and sulfate, and these compounds are expected to undergo minimal transformation in the atmosphere. In processes that involve extremely high temperature (e.g. the iron and steel industries), the metal can volatilize and be emitted as vapour. Cadmium present in the air may be transported from a hundred to a few thousand kilometres and have a typical atmospheric residence time of about 1–10 days before deposition occurs by wet or dry processes [43] onto surface soils and water, which can result in elevated cadmium levels even in remote locations [46, 47]. Except for those who live near cadmium-emitting industries, inhalation of cadmium in the ambient air is not a major source of exposure.

Cadmium concentrations in uncontaminated soil range from 0.01 to 2.7 mg/kg, the main factor determining the cadmium content of soil being the chemical composition of the parent rock. Higher levels of this element may be found in soil near industrial areas or hazardous waste sites, and soils near roads may contain high levels of cadmium from car exhaust. The use of cadmium-containing fertilizers and sewage sludge is most quoted as the primary reason for the increase in the cadmium content of soils over the last 20 or 30 years in Europe [36].

Cadmium in soils must be classified in three areas: agricultural soils, non-agricultural soils and controlled landfills. The metal in controlled landfills is virtually immobile, and is unlikely to have any effect on human health; that in non-agricultural soils will generally not affect human health as it does not enter the food chain. In agricultural soils, the element is likewise relatively immobile under normal conditions, but could become more mobile under certain conditions such as increased soil acidity, probably as a result of the increase in the exchangeable portion of cadmium. The major factors governing cadmium mobility in soils are speciation, pH, soluble organic matter content, hydrous metal oxide content, presence of organic and inorganic ligands, and competition from other metals [45]. Some studies have concluded that soil type also affects uptake of cadmium by plants. Thus, for soil with the same total content, the metal has been found to be more soluble and more plant-available in sandy soil than in clay soil [48]. Similarly, its mobility and bioavailability are higher in non-calcareous than in calcareous soils [49]. Because of its high rates of soil-to-plant transfer, cadmium is a contaminant found in most human foodstuffs [50, 51]. Leafy vegetables such as lettuce and spinach and certain staples such as potatoes and grain foods exhibit relatively high values of 30–150 ppb [52]. However, the concentration of cadmium in soils is not the primary determinant of cadmium in plants. Cadmium is much less mobile in soils than in air and water.

Cadmium in surface water and groundwater may be natural origin from the rocks and soil or from human activities, including industry, domestic wastewater, agricultural discharge, mine runoff, solid waste disposal and atmospheric deposition [53]. Much of the metal entering fresh waters from industrial sources may be rapidly adsorbed by particulate matter, and thus sediment may be a significant sink for cadmium emitted to the aquatic environment [38]. Partitioning of cadmium between the adsorbed-in-sediment state and dissolved-in-water state is therefore an important factor in whether cadmium emitted to waters is or is not available to enter the food chain and affect human health. From a practical analytical consideration, cadmium in aqueous systems can be classified as dissolved (soluble, filterable), particulate (nonfilterable, suspended) and sediment species. The uptake of cadmium by organisms occurs chiefly in the aqueous phase, the filter-feeding organisms being exceptions. Cadmium in water can contaminate shellfish and crustaceans.

Cadmium exists in water as the hydrated ion, as inorganic complexes such as carbonates, hydroxides, chlorides or sulfates, or as organic complexes with humic acids [45]. The relative proportions of the different physicochemical forms depend on the environmental conditions and this is an important factor in the toxicity and the uptake and bioaccumulation of cadmium by both aquatic and land-based plants and animals. For example, cadmium uptake by *Mytilus edulis* increases when cadmium is complexed with EDTA, humic acid or alginic acid, whereas the oyster *Crassostrea virginica* accumulates significantly less cadmium when it is complexed. There is general agreement that in sea water cadmium is present primarily as a variety of chloride complexes, whereas in fresh water the dominant forms depending on pH are the free hydrated cadmium ion and/or cadmium carbonate.

The average cadmium content in the world's oceans has variously been reported as low as under 5 ng/L [38] to as high as 110 ng/L but increased concentrations of 80–250 ng/L have been reported in French and Norwegian coastal zones. Concentrations measured in European rivers generally vary between 10 and 100 ng/L [54]. Estimates of the residence time in ocean water range from 7000 to 250,000 years. This indicates that cadmium may be accumulated and transported in significant amounts over long distances in the ocean. Cadmium concentrates in fresh water and marine animals to concentrations hundreds to thousands of times higher than in the water [43]. Reported bioconcentration factors range from 113 to 18,000 for invertebrates, from 3 to 4190 for freshwater aquatic organisms, and from 5 to 3160 for saltwater aquatic organisms [36]. Bivalve molluscs and crustaceans are filter feeders that accumulate metals from the aquatic environment independent of environmental pollution, and contaminated waters could further increase the content of metals [55]. Cadmium content of some Pacific oysters was found to be 13.5 mg/kg dry weight, whereas twofold higher cadmium content was reported for some New Zeland bluff oysters [56]. The purple sea urchin is a well-known bioindicator used in metal pollution monitoring along the Mediterranean and Atlantic coasts [57, 58]. In estuarine coastal systems and particulate matter the residence time of cadmium has been estimated as a relatively low 1–2 years. Cadmium in the water accumulated more rapidly in the sediments than in living organisms.

6.3.3 Toxic effects

Cadmium has been reviewed by the International Register of Potentially Toxic Chemicals of the United Nations Environment Programme. As a result, it has been described as 'one of the most dangerous trace elements in the food, and the environment of man' and it has been included on the list of chemical substances and processes considered to be potentially dangerous at the global level [59]. In addition, the International Agency for Research on Cancer (IARC) has classified cadmium as a category 1 carcinogen [60]. This element might constitute a serious health hazard for large groups of the population [61]: because it is relatively poorly absorbed into the body, the uptake of cadmium in the human digestive tract usually does not exceed 5–10% of its concentration in food (the absorption rate of cadmium via dust that settles in the respiratory tract is higher), but once absorbed is slowly excreted, with a biological half-life estimated to be 10–30 years [62], and accumulates in the kidney causing renal damage. The absorption of cadmium would be increased in iron-deficient subjects, whereas the presence in food of divalent or trivalent cations such as calcium, zinc or magnesium reduces its absorption [43]. The toxicity of cadmium is mainly due to its free form. Other factors, including age [63] and gender may also influence cadmium uptake or retention.

Cadmium exposure has been associated with nephrotoxicity, osteoporosis, neurotoxicity, carcinogenicity, teratogenicity and endocrine and reproductive effects [36]. Whatever the route of exposure, the metal is transported in blood attached to haemoglobin or metallothionein before being distributed throughout the body. It accumulates mainly in the liver and the kidneys. Cadmium is stored in the body mainly in complexed forms with metallothionein. Environmental exposure to cadmium is associated with renal tubular dysfunction [64]. The most severe form of food-borne chronic cadmium intoxication is itai-itai disease [65], which combines bone disorders and renal effects and is characterized by extreme pain. The disease was mainly diagnosed in a limited area in Japan that had been polluted. Cadmium concentrations found in locally grown rice were 10 times higher than in other areas [66]. Today, cadmium levels in food are monitored by health authorities and permitted levels in foodstuffs are regulated by legislation in many countries.

6.3.4 Cadmium in foods

In 1972, the Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA) established a provisional tolerable weekly intake (PTWI) for cadmium of 400–500 μg per person per week (approximately 7–8 μg/kg body weight per week and 60–70 μg/day for a 60-kg person) [67]. The position of the JEFCA was based on an oral absorption rate of 5% and a daily excretion rate of 0.005% of total body burden. In 1988 the FAO/WHO set and confirmed a PTWI of 7 μg/kg body weight. In 1992, the PTWI for cadmium was refined and subsequently expressed in terms of cadmium intake per kilogram of body weight. This refinement also recognized that the model PTWI for cadmium did not include a safety factor. The Committee warned that there was only a relatively small margin between exposure from the normal diet and the exposure that would produce adverse effects. Despite this narrow safety factor, the PTWI for cadmium was retained at 7 μg/kg body weight, corresponding to 1 μg/kg body weight daily. Thus, for a 70-kg person, the tolerable daily intake is 70 μg of cadmium. In 1995, the European Commission's Scientific Committee for Food (SCF) confirmed the PTWI of 7 μg/kg body weight per week. The Committee stressed the importance of foodstuffs as the main contributor to non-occupational human exposure in non-smokers and recommended that efforts should be undertaken to reduce dietary exposure. In 2009 and subsequently confirmed in 2011, the Panel on Contaminants in the Food Chain of the European Food Safety Authority (CONTAM Panel of EFSA) issued an opinion in which they recommended that the PTWI should be reduced to a tolerable weekly intake (TWI) of 2.5 μg/kg body weight in order to ensure a high level of protection to consumers, including subgroups of the population such as children, vegetarians or people living in highly contaminated areas [36, 68]. In light of the recommended lowering of the PTWI, it is important to better identify major dietary sources of cadmium exposure.

The average dietary exposure to cadmium for adults across European countries was estimated to be 1.9–3.0 μg/kg body weight per week, while high-exposure adults have estimates in the range 2.5–3.9 μg/kg body weight per week by the CONTAM Panel [36]. According to this report, the highest dietary exposure to cadmium

is from cereals and cereal products (daily exposure 4.189 µg) and vegetables, nuts and pulses (daily exposure 3.667 µg), followed by edible offals and offal products (daily exposure 3.3031 µg), while daily exposure to cadmium from fish and seafood is assessed to be 1.662 µg. The CONTAM Panel noted that subgroups of the population, such as vegetarians, children, smokers and people living in highly contaminated areas, may exceed the TWI by about twofold. For this reason, the Panel concluded that exposure to cadmium at the population level should be reduced [69].

Maximum levels (MLs) for heavy metals in foodstuffs have been set by Commission Regulation No. 1881/2006 [70] of 19 December 2006, the framework EU legislation which sets maximum levels for chemical contaminants in foodstuffs, as amended by Regulation No. 420/2011 of 29 April 2011. These Regulations establish MLs for cadmium in a wide range of foodstuffs including milk, meat, fish, cereals, vegetables, fruit and fruit juices. The limits laid down in the Regulations are reproduced in Table 6.2 [36]. However, these levels continue to be constantly reviewed by the Commission. An updated scientific basis is therefore of great importance.

A number of studies have reviewed cadmium content in a range of different foods. In a collaborative effort under the Scientific Cooperation (SCOOP) initiative involving 13 EU Member States, cadmium concentrations ranged from non-detected in many foods to a high mean of 1.20 mg/kg for cephalopods [71]. For the 64th meeting of the JECFA, average concentrations of cadmium were reported from Australia, Canada, Germany, Japan, New Zealand and the USA including some aggregated data from a further four countries or regions [72]. Cadmium is principally present in cereals, potatoes, root vegetables, fruit, meat and offal. The highest concentrations of cadmium were measured by FASFC in offal (liver and kidney) of game and horse [73, 74]. High levels of cadmium in European countries and reported by EFSA [69] were found in algal formulations, cocoa-based products, crustaceans, edible offal, fungi, oilseeds, seaweeds and water molluscs (Table 6.3). Areas with elevated levels may show higher cadmium concentrations in locally produced food, for example, for rice, Japan reported higher values than the general average, 0.061 compared with 0.017 mg/kg, respectively [72]. The most significant contributors to cadmium in the diet are cereals and vegetables, including potatoes. Meat and especially liver and kidney follow in importance [71].

Table 6.2 Cadmium maximum levels (mg/kg wet weight) in foodstuffs.

1	Meat (excluding offal) of bovine animals, sheep, pig, and poultry	0.050
2	Horsemeat, excluding offal	0.20
3	Liver of bovine animals, sheep, pig, poultry, and horse	0.50
4	Kidney of bovine animals, sheep, pig, poultry, and horse	1.0
5	Muscle meat of fish, excluding species listed in 6 and 7	0.050
6	Bonito (*Sarda sarda*), common two-banded seabream (*Diplodus vulgaris*), eel (*Anguilla anguilla*), grey mullet (*Mugil labrosus labrosus*), horse mackerel or scad (*Trachurus* spp.), louvar or luvar (*Luvarus imperialis*), mackerel (*Scomber* spp.), sardine (*Sardina pilchardus*), sardinops (*Sardinops* spp.), tuna (*Thunnus* spp., *Euthynnus* spp., *Katsuwonus pelamis*), wedge sole (*Dicologoglossa cuneata*)	0.10
7	Muscle meat of bullet tuna (*Auxis* spp.)	0.20
8	Muscle meat of anchovy (*Engraulis* spp.) and swordfish (*Xiphias gladius*)	0.30
9	Crustaceans: muscle meat from appendages and abdomen. In case of crabs and crab-like crustaceans (Brachyura and Anomura), muscle meat from appendages	0.50
10	Bivalve molluscs	1.0
11	Cephalopods (without viscera)	1.0
12	Cereals, excluding bran, germ, wheat, and rice	0.10
13	Bran, germ, wheat, and rice	0.20
14	Soybeans	0.20
15	Vegetables and fruit, excluding leaf vegetables, fresh herbs, leafy brassica, fungi, stem vegetables, root vegetables, and tuber vegetables and seaweed potatoes	0.050
16	Stem vegetables, root and tuber vegetables, excluding celeriac. For potatoes the maximum level applies to peeled potatoes	0.10
17	Leaf vegetables, fresh herbs, leafy brassica, celeriac and the following fungi: *Agaricus bisporus* (common mushroom), *Pleurotus ostreatus* (oyster mushroom), *Lentinula edodes* (shiitake mushroom)	0.20
18	Fungi, excluding those listed in point 17	1.0
19	Food supplements excluding food supplements listed in point 20	1.0
20	Food supplements consisting exclusively or mainly of dried seaweed products derived from seaweed or of dried bivalve	3.0

The foods mentioned cover more than 80% of intake for the normal consumer. Molluscs and crustaceans constitute only a small part of the diet, so these commodities do not contribute much to the cadmium intake

for the average consumer, though both products may contain high levels of cadmium. Certain wild mushrooms may also contain high levels; however, these foodstuffs are minor contributors to the overall intake of cadmium, as they are eaten in relatively small amounts. Cadmium occurrence levels in food reported by EFSA [69] are shown in Table 6.3.

6.4 Lead

6.4.1 General information

Lead is a non-essential, toxic and bioaccumulating metal. Lead is a naturally occurring heavy metal found in the small amounts in the earth's crust [75, 76]. However, nowadays the most important sources of lead in our environment are due to anthropogenic activities such as burning fossil fuels, mining and various manufacturing. Major sources of lead in ambient air come from leaded gasoline and industries such as manufacture of storage batteries and ore smelting. Cigarette smoke is contaminated with lead due to lead arsenate insecticides sprayed on tobacco.

The general population is exposed to lead from air and food in roughly equal proportions. Earlier, lead in foodstuff originated from pots used for cooking and storage, and lead acetate was previously used to sweeten port wine. During the last century, lead emissions to ambient air have further polluted the environment, with over 50% of lead emissions originating from petrol. Over the last few decades, however, lead emissions in developed countries have decreased markedly due to the introduction of unleaded petrol.

In 2004 lead was reclassified by the IARC Working Group from a 'possible' to a 'probable' human carcinogen.

6.4.2 Environmental considerations

Lead is a toxic metal that has been known to humankind for thousands of years. It has been used in hundreds of ways, from pewter to water pipes. Only recently, however, has attention been focused on lead as a major source of ill health affecting millions of persons. Some 30 important health conditions are linked to lead, many affecting children and the unborn [77]. Lead is found in the earth's crust and is increasing with time due to the disintegration of radioactive elements such as uranium changing to lead.

Lead is widely distributed in the environment. For example, lead contamination has even been found in the Arctic ice caps and is increasing each decade. The routes of environmental exposure to lead resulting in its absorption into the body are inhalation (30–50% of inhaled dose absorbed into the bloodstream), ingestion (8–15% of ingested dose absorbed into the bloodstream) and, to a limited extent, dermal contact. Lead is released to the environment from both natural and anthropogenic sources; however, most exposure results from anthropogenic sources (e.g. mining, smelting, industrial uses) [78]. Lead exists in various inorganic and organic forms, which affect its environmental fate, transport and bioavailability. Regardless of the form, however, lead is not degraded and remains available for exposure. In the mid 1980s, combustion of leaded gasoline contributed about 90% of all anthropogenic lead emissions, but the percentage decreased sharply through the late 1990s as a result of the phase-out of leaded gasoline.

Lead exposure can occur through food and water, as well as in the workplace, by means of hobbies and through exposure to lead-contaminated soil and air. The main route of exposure to lead for the general adult population currently is oral exposure from food and drinking water, followed by inhalation [79, 80]. Inhalation is an important route of exposure for individuals living in the vicinity of point sources of lead.

6.4.3 Toxic effects

Absorption of ingested lead may constitute a serious risk to public health. The main target for lead toxicity is the nervous system in both adults and children. The symptoms of acute lead poisoning are headache, irritability, abdominal pain and various symptoms related to the nervous system. Lead encephalopathy is characterized by sleeplessness and restlessness. Children may be affected by behavioural disturbances and learning and concentration difficulties. In severe cases of lead encephalopathy, the affected person may suffer from acute psychosis, confusion and reduced consciousness. Some chronic effects of lead poisoning are colic, constipation and anaemia. It may also induce increased blood pressure and cardiovascular disease in adults. Symptoms of lead toxicity may appear years after exposure, or even after exposure has ceased, as a result of the sudden release of stored lead due to illness, alcoholism, stress or other changes in metabolism.

A combination of factors contributes to the prevalence of lead toxicity today [81]. In addition to its wide distribution in our environment, lead absorption is

Table 6.3 Summary of the total cadmium occurrence data by food group.

Food category	*N*	Mean (µg/kg)			Food category	*N*	Mean (µg/kg)			Food category	*N*	Mean (µg/kg)		
		LB	MB	UB			LB	MB	UB			LB	MB	UB
Grains and products	280	35.1	38.1	41.0	Legumes dried	2,267	19.6	20.5	21.3	Fish roe	112	49.0	50.5	51.9
Grains for humans	9,297	32.5	33.4	34.3	Tree nuts	1,368	34.2	36.2	38.1	Crustaceans	2,194	128	132	136
Grain milling products	3,388	23.9	24.8	25.7	Oilseeds	3,496	371	371	371	Water molluscs	3,866	316	317	319
Bread and rolls	2,078	14.6	15.2	15.7	**Fruit and fruit products**	116	7.09	7.99	8.90	Frogs' legs	97	204	207	209
Pasta (raw)	614	21.2	21.6	22.1	Citrus fruits	647	1.16	2.61	4.06	**Milk and dairy products**	60	4.98	6.26	7.53
Breakfast cereals	678	19.6	20.4	21.2	Pome fruits	1,505	3.61	5.04	6.46	Liquid milk	3,196	0.49	1.05	1.61
Fine bakery wares	1,417	15.1	15.9	16.6	Stone fruits	470	1.06	3.53	6.01	*Goat milk*	55	16.3	17.2	18.1
Vegetables and products	840	16.7	17.3	17.9	Berries and small fruits	1,055	2.59	3.89	5.19	Milk-based beverages	17	8.06	8.77	9.47
Root vegetables	2,091	24.4	46.3	68.3	Olives for oil production	50	0.00	0.50	1.00	Concentrated milk	455	4.29	7.64	11.0
Bulb vegetables	777	12.1	12.7	13.2	Miscellaneous fruits	528	2.92	4.19	5.46	Whey beverages	10	3.93	4.62	5.30
Fruiting vegetables	2,694	6.02	7.27	8.53	Dried fruits	403	2.25	4.71	7.18	Cream and products	651	0.72	8.90	17.1
Brassica vegetables	1,952	5.39	6.43	7.47	Jam and spreads	192	2.87	5.86	8.85	Fermented products	893	1.26	4.84	8.41
Leaf vegetables	3,414	35.6	36.4	37.1	Other fruit products	602	3.17	4.46	5.74	Lactose	4	0.00	5.73	11.5
Legume vegetables	233	2.89	4.39	5.89	**Meat and edible offal**	908	8.30	11.3	15.5	Cheese	2,872	3.24	10.8	18.3
Stem vegetables (fresh)	732	23.9	24.9	25.9	Livestock meat	15,462	7.60	11.3	15.0	Milk and imitates	60	8.93	11.1	13.3
Chicory roots	65	25.4	25.5	25.5	*Horse meat*	1,402	359	362	366	**Eggs and products**	25	1.68	4.37	7.06
Seaweeds	202	1,122	1,122	1,122	Poultry	4,821	3.21	7.99	12.8	Eggs, fresh	1,183	1.27	3.33	5.39
Tea and infusions	6,044	55.1	55.3	55.5	Game mammals	2,479	3.04	4.37	5.69	Eggs, powder	10	0.00	10.4	20.8
Cocoa powder	732	183	183	183	Game birds	680	1.78	4.70	7.63	**Sugar and confectionery**	156	11.7	15.6	19.6
Coffee beans	4,065	11.0	12.0	13.0	Mixed meat	184	1.23	2.74	4.25	Sugars	361	1.97	3.13	4.30
Coffee imitates (solid)	210	13.0	13.3	13.6	Edible offal	18,296	315	317	319	Sugar substitutes	2	18.0	18.0	18.0
Vegetable products	349	10.8	11.2	11.5	Preserved meat	416	2.61	7.37	12.1	Chocolate	1,286	80.1	80.9	81.8
Fungi, cultivated	1,926	134	135	136	Sausages	2,663	6.19	15.9	25.6	*Bitter chocolate*	30	123	123	123
Fungi, wild, edible	791	344	344	345	Meat specialities	88	2.64	4.68	6.72	*Bitter-sweet chocolate*	58	135	135	135
Starchy roots and tubers					Pâtés and terrines	310	4.86	11.3	17.7	*Milk chocolate*	184	18.7	19.6	20.6
Potatoes and products	2,280	21.0	21.7	22.4	Meat imitates	311	22.2	22.9	23.6	Confectionery (non-chocolate)	395	5.60	7.15	8.70
Other roots and tubers	852	14.0	14.1	14.2	**Fish and seafood**	1,294	175	176	177	Dessert sauces	14	14.8	14.9	14.9
Legumes, nuts, oilseeds	14	27.7	30.6	33.5	Fish meat	11,106	22.6	26.0	29.5	Molasses and other syrups	178	2.64	3.28	3.91
Legumes no pods	118	3.21	4.49	5.77	Fish products	869	17.3	19.0	20.7	Honey	2,269	3.61	9.01	14.4
Fats and oils	10	0.00	2.27	4.53	Wine-like drinks	113	0.84	1.16	1.48	Ready-to-eat meal for children	1,350	4.34	5.69	7.03
Animal fat	1,090	2.41	3.90	5.40	Liqueur	26	5.40	6.04	6.68	Dairy products for children	19	4.55	6.70	8.96
Fish oil	30	17.3	20.6	23.9	Spirits	30	1.22	1.48	1.74	Juice and herbal tea for children	44	2.96	4.19	5.42

Vegetable fat	163	5.96	6.53	7.10	Alcoholic mixed drinks	120	1.22	1.48	1.74	**Special nutritional products**	56	13.4	16.6	19.8
Vegetable oil	636	4.14	4.96	5.78	**Drinking water**	2,288	0.34	3.37	6.41	Food for weight reduction	27	24.6	24.9	25.3
Fats of mixed origin	225.0	0	30.0	35.0	Tap water	16,618	0.07	0.21	0.35	Dietary supplements	1,351	72.6	74.6	76.6
Margarine and similar	55	1.82	4.57	7.33	Bottled water	2,380	0.20	0.42	0.65	*Algal formulations*	413	1,514	1,515	1,515
Fruit and vegetable juices	118	2.49	2.94	3.39	Water ice	1	0.00	0.25	0.50	Food for sports people	31	44.3	48.6	52.9
Fruit juice	1,944	2.16	3.51	4.85	**Herbs, spices and condiments**	46	54.3	54.6	54.9	Dietetic foods for diabetics	183	18.6	19.4	20.2
Concentrated fruit juice	9	0.44	1.04	1.64	Herbs	554	42.4	43.9	45.4	Food for medical management	20	22.5	23.0	23.4
Fruit nectar	169	0.48	2.13	3.79	Spices	881	87.5	90.1	92.8	**Composite food**	80	4.32	11.7	19.0
Mixed fruit juice	62	1.31	3.15	4.98	Herb and spice mixtures	142	54.5	61.1	67.7	Cereal-based dishes	66	11.3	13.9	16.6
Powdered fruit juice	25	2.24	2.54	2.84	Seasoning or extracts	183	17.9	19.7	21.5	Rice-based meals	21	57.1	58.7	60.4
Vegetable juice	218	8.90	9.61	10.3	Condiment	303	11.3	13.0	14.7	Potato-based dishes	2	11.0	11.0	11.0
Mixed vegetable juice	8	4.75	7.88	11.0	Dressing	87	1.97	3.50	5.03	Beans-based meals	16	1.44	14.1	26.7
Mixed fruit/vegetable juice	14	3.59	5.49	7.38	Chutney and pickles	8	10.1	10.1	10.1	Meat-based meals	502	5.59	7.11	8.63
Non-alcoholic beverages	44	44.1	44.5	44.9	Savoury sauces	64	8.14	9.32	10.5	Fish and seafood meals	113	23.7	24.5	25.3
Soft drinks	972	0.98	1.53	2.07	Flavourings or essences	91	12.4	16.9	21.5	Vegetable-based meals	7	0.67	12.9	25.2
Tea (infusion)	1,511	0.92	0.92	0.93	Baking ingredients	161	11.7	13.2	14.7	Egg-based meal	8,281	1.27	3.33	5.39
Coffee (beverage)	813	0.61	0.66	0.72	**Food for children**	45	14.2	14.5	14.8	Mushroom-based meals	791	344	344	345
Coffee imitates beverage	35	0.72	0.74	0.75	Infant formulae powder	542	2.43	3.67	4.91	Ready-to-eat soups	223	8.20	10.2	12.1
Cocoa beverage	2,196	2.75	2.75	2.85	Infant formulae liquid	40	3.03	3.70	4.38	Prepared salads	109	13.9	23.2	32.5
Alcoholic beverages	105	0.78	1.14	1.51	Follow-on formula powder	512	3.56	4.19	4.82	**Snacks, desserts, other foods**	1	0.00	10.0	20.0
Beer and similar	1,150	0.69	1.77	2.85	Follow-on formula liquid	58	1.00	1.20	1.40	Snack food	587	26.8	27.0	27.2
Wine	2,604	0.55	1.24	1.93	Cereal-based food for children	1,647	11.9	12.3	12.6	Ices and desserts	659	3.62	4.11	4.59
Fortified and liqueur wines	34	0.35	0.53	0.71						Other non-classifiable foods	256	19.0	28.0	37.0

N, number of samples; LB, lower bound; MB, middle bound; UB, upper bound.

Source: European Food Safety Authority [69].

enhanced by a deficiency in the diet of vital trace elements such as manganese, zinc, copper, chromium, calcium and magnesium. Such deficiencies are common due to refined food diets and poor eating habits.

Lead is a highly poisonous metal (regardless if inhaled or swallowed), affecting almost every organ and system in the body. Chronic exposure to lead at relatively low levels can result in damage to the kidneys and liver, and to the reproductive, cardiovascular, immune, haematopoietic, nervous and gastrointestinal systems. Short-term exposure to high amounts of lead can cause gastrointestinal distress, anaemia, encephalopathy and death. The most critical effect of low-level lead exposure is reduced cognitive and intellectual development in children [82].

Lead has no known preferred function in the body. Lead can substitute for calcium in the bones and thus delay or possibly prevent osteoporosis. However, calcium is the preferred element and lead causes other metabolic problems when it replaces calcium or another preferred mineral [83].

6.4.4 Lead in foods

Lead contamination of food arises from numerous sources, including air and soil [84, 85]. Atmospheric lead from industrial pollution or leaded gasoline can contaminate food through deposition on agricultural crop plants. Soil lead arising from lead-containing ordnance stored on former munitions sites and from ammunition used in rifle or military firing, atmospheric deposition, or inappropriate application of pesticides, fertilizers or sewage sludge can contaminate agricultural crop plants through uptake or through deposition of the soil on plant surfaces. Contaminated plants and soil are in turn a source of contamination of livestock.

Water is also a source of lead contamination of food. Surface water sources can be contaminated through runoff (drainage), atmospheric deposition and, on a local level, by leaching of lead from game shot or fishing sinkers. Contaminated surface waters are a potential source of contamination of aquatic food animals. For drinking water and water for food preparation, the use of lead pipes or lead-containing fixtures in water distribution systems is a primary source of contamination.

Lead contamination of food can also arise from food processing, food handling and food packaging. Sources of lead in food processing areas include lead paint and lead-containing equipment, such as piping and lead-soldered machinery. In the packaging area, lead-soldered cans have been identified as a very important source of lead contamination of food. Other packaging items that are potential sources of lead contamination include coloured plastic bags and wrapping papers, cardboard containers that contain lead or are coloured with lead-containing dyes, lead foil capsules on wine bottles, and lead-glazed ceramic, lead crystal or lead-containing metal vessels used for packaging or storing foods. The toxic effects of lead in food have been reviewed several times by the JECFA [86, 87] and by the FDA [88], SCOOP [89] and EFSA [90].

Table 6.4 shows lead content of various types of foods [90].

6.5 Mercury

6.5.1 General information

Mercury occurs naturally in the earth's biogeochemical system and is one of the 'ancient' metals; in classical times, it was known by the Greeks as *hydrargyrum* (liquid silver or quicksilver) due to its aspect and liquid state. The name 'Mercury' was given by the Romans to the swift messenger of the gods. Mercury is not widely distributed in the environment. Its average concentration in the earth's crust is about 0.5 mg/kg and there are only a limited number of places where it occurs in concentrated form suitable for extraction. These are found in the so-called 'mercuriferous belt', a chain of volcanic areas that stretches along the Mid-Atlantic Ridge, the Mediterranean, South and East Asia and the Pacific Ring [1]. In these areas, mercury is present as cinnabar ore (HgS), which has a characteristic red colour. In this belt are found the ancient mines of Europe and the Western world at Idria in the former Yugoslavia, Almaden in Spain and New Almaden in California. Mercury was used by the alchemists in their attempts to transmute base metals into gold; since then, centuries of human activities, such as mining and fossil fuel burning, have been mobilizing increasing amounts of the element in the atmosphere, ocean and terrestrial system [91]. Today mercury is widely used in industry, mainly because of its chemical properties. It serves as a catalyst in a variety of industrial and laboratory reactions. Its high electrical conductivity makes the liquid metal valuable also to the electrical industry. About one-quarter of total production is employed in the manufacture of

Table 6.4 Lead content from different foods.

Category	*N*	%LC	Mean (µg/kg)			Category	*N*	%LC	Mean (µg/kg)			Category	*N*	%LC	Mean (µg/kg)		
			LB	MB	UB				LB	MB	UB				LB	MB	UB
Grains and grain-based products						Coffee imitates (solid)	16	13	223	224	224	Berries and small fruits	1,919	60	12	15	17
Grains for human consumption	4,936	48	27	31	35	Vegetable products	427	27	30	30	31	Miscellaneous fruits	1,264	70	8	11	15
Grain milling products	1,842	54	26	29	33	Fungi, cultivated	1,722	50	53	57	60	Dried fruits	285	44	23	25	28
Bread and rolls	1,192	44	25	29	33	Fungi, wild, edible	786	23	478	491	503	Jam, marmalade and other fruit spreads	147	51	16	17	19
Pasta (raw)	381	51	21	24	27	**Starchy roots and tubers**						Other fruit products (excluding beverages)	716	39	20	23	25
Breakfast cereals	789	60	19	25	30	Potatoes and potato products	1,370	56	16	19	22	**Meat and edible offal**					
Fine bakery wares	675	52	21	25	28	Other starchy roots and tubers	984	43	16	17	18	Livestock meat	18,193	78	11	16	20
Vegetables and vegetable products						**Legumes, nuts and oilseeds**						Poultry	4,674	79	10	15	20
Root vegetables	2,350	40	18	19	21	Legumes, beans, green, without pods	610	62	13	16	20	Game mammals	3,532	51	963	966	969
Bulb vegetables	799	58	29	31	33	Legumes, beans, dried	1,395	39	30	34	38	Game birds	596	51	264	267	270
Fruiting vegetables	2,891	71	7	11	14	Tree nuts	983	58	26	33	39	Mixed meat	162	81	3	5	7
Brassica vegetables	1,977	62	10	13	16	Oilseeds	1,036	38	43	46	48	Edible offal, farmed animals	20,571	58	36	41	46
Leaf vegetables	3,122	44	37	41	44	Other seeds	19	53	80	87	93	Preserved meat	470	41	21	23	25
Legume vegetables	77	49	22	26	30	**Fruit and fruit products**						Sausages	1,810	67	11	17	22
Stem vegetables (fresh)	1,267	60	17	21	25	Citrus fruits	548	62	9	12	14	Meat specialties	47	30	14	14	15
Sea weeds	66	27	2,663	2,677	2,692	Pome fruits	1,195	60	10	12	15	Pastes, pâtés and terrines	292	33	32	34	35
Tea and herbs for infusions (solid)	473	42	353	355	358	Stone fruits	826	65	9	12	16	Meat imitates	24	29	42	42	43

(Continued)

Table 6.4 (Continued)

Category	*N*	%LC	Mean (µg/kg)			Category	*N*	%LC	Mean (µg/kg)			Category	*N*	%LC	Mean (µg/kg)		
			LB	MB	UB				LB	MB	UB				LB	MB	UB
Fish and other seafood						**Eggs and egg products**						Fats of mixed origin	1	0	—	50	50
Fish meat	9,433	68	16	22	28	Eggs, fresh	1,194	74	8	11	14	Margarine and similar products	110	80	6	10	13
Fish products	170	45	23	26	28	Eggs, powder	9	78	13	14	15	**Fruit and vegetable juices**					
Crustaceans	2,135	74	18	33	48	**Sugar and confectionery**						Fruit juice	1,538	57	7	9	11
Water molluscs	3,478	36	171	182	192	Sugars	181	66	12	13	14	Concentrated fruit juice	81	17	44	44	45
Amphibians, reptiles, snails, insects	31	32	41	44	47	Sugar substitutes	33	61	25	28	31	Fruit nectar	112	50	7	9	11
Milk and dairy products						Chocolate (cocoa) products	723	19	53	55	57	Mixed fruit juice	64	31	8	9	9
Liquid milk	4,043	76	3	4	5	Confectionery (non-chocolate)	398	60	32	37	43	Dehydrated/ powdered fruit juice	31	55	21	24	26
Milk-based beverages	27	48	27	29	31	Dessert sauces	31	35	30	31	31	Vegetable juice	252	69	6	8	10
Concentrated milk	296	44	22	23	24	Molasses and other syrups	100	58	9	9	10	Mixed vegetable juice	2	0	—	10	10
Whey and whey products	8	38	12	12	13	Honey	1,924	54	32	36	41	Mixed fruit and vegetable juice	11	0	—	12	12
Cream and cream products	268	69	6	8	10	**Animal and vegetable fats and oils**						**Non-alcoholic beverages**					
Fermented milk products	455	64	14	17	20	Animal fat	566	63	14	16	18	Soft drinks	680	63	13	14	16
Milk derivatives	455	64	14	17	20	Fish oil	1	0	—	8	8	Tea (infusion)	764	1	—	12	12
Cheese	1,262	56	17	21	25	Vegetable fat	114	41	23	23	24	Coffee (beverage)	32	28	—	4	4
Milk and milk product imitates	52	62	12	15	17	Vegetable oil	924	65	20	23	26	Coffee imitates beverage	32	28	—	4	4
												Cocoa beverage	4	50	8	10	12
Alcoholic beverages						**Herbs, spices and condiments**						Infant formulae, liquid	253	77	0.5	1	1
Beer and beer-like beverage	894	76	9	12	14	Herbs	518	25	276	278	281	Follow-on formulae, powder	37	78	2	3	4

Wine	2,302	22	23	25	27	Spices	935	24	280	285	291	Follow-on formulae, liquid	37	78	0.2	0.3	0.5
Fortified and liqueur wines	18	17	—	15	15	Herb and spice mixtures	132	20	293	297	301	Cereal-based food	294	55	9	13	17
Wine-like drinks	139	23	19	19	20	Seasoning or extracts	149	46	282	292	302	Ready-to-eat meal	969	62	7	14	20
Liqueur	22	64	27	28	30	Condiment	280	28	52	28	56	Yoghurt, cheese and milk-based dessert	26	27	—	4	4
Spirits	88	48	9	11	12	Dressing	63	57	24	30	37	Fruit juice and herbal tea	33	33	6	7	7
Alcoholic mixed drinks	3	67	8	9	10	Chutney and pickles	10	40	16	17	19	**Products for special nutritional use**					
Drinking water						Savoury sauces	45	42	46	53	59	Food for weight reduction	16	75	613	632	651
Tap water	4,118	38	5	6	6	Flavourings or essences	43	49	85	92	99	Dietary supplements	2,489	30	695	701	706
Bottled water	2,594	72	0.2	1	1	Baking ingredients	161	37	46	47	47	Food for sports people	130	30	482	490	499
Water ice (for consumption)	7,119	52	—	4	4	**Food for infants and small children**						Dietetic food for diabetics	91	35	28	29	29
						Infant formulae, powder	253	77	4	6	8	Medical food	87	7	10	7	13

N, number of samples; LC%, left-censored proportion; LB, lower bound; MB, middle bound; UB, upper bound.
Source: European Food Safety Authority [91].

scientific and electrical instruments and another quarter as electrodes in the chloralkali industry. The remainder is used in the production of paints and pigments, as well as in agrochemicals and in medicinal and pharmaceutical products and other specialist items. Organic mercury was formerly used in quantity as a fungicidal dressing for seeds of wheat and other cereals, but this application has now been banned [1]. Mercury is considered one of the most dangerous of all the metals likely to be found in food. It causes severe toxic symptoms at low concentrations and has no known beneficial functions; even cadmium and lead may be shown to be essential elements [92]. In the form of methylmercury, it is a potent neurotoxin that affects human and wildlife development and health [93]. Methylmercury accumulates through multiple levels in the aquatic food chain. Humans are exposed mainly through consumption of fish contaminated by methylmercury, particularly those who consume high levels of fish [93].

6.5.2 Environmental considerations

The natural biogeochemical cycle of mercury involves atmospheric transport, deposition to land and ocean, and revolatilization. The ultimate sink of mercury is burial to the deep-ocean sediments, which occurs very slowly [94]. The overall lifetime of mercury in the combined atmosphere–ocean–terrestrial system against transfer to the sediments is 3000 years [95]. The vapour arises naturally from emissions from volcanoes as well as by volatilization from soil [96]. It has been calculated that natural degassing of the earth's crust and oceans releases up to 150,000 tonnes of mercury vapour into the atmosphere annually. It is also produced by combustion of fossil fuels, and from mines, metal refineries, waste incinerators and crematoria. These human activities are estimated to produce about 10,000 tonnes of mercury vapour each year [97]. Atmospheric mercury is oxidized to soluble divalent mercury and returns slowly to earth in rainwater. In surface waters and sediments the divalent mercury can be reduced again, or methylated by microorganisms to form methylmercury. Although both inorganic mercury and methylmercury species may be assimilated by biota at the lowest levels of food chains (bacteria, archaea, phytoplankton and other algae), only methylmercury is biomagnified within food chains and thus presents the key exposure risk to humans [98].

The concept of mercury bioavailability includes the availability of inorganic mercury species to microbial populations responsible for mercury methylation and the transfer of methylmercury in prey items to predators within food webs. Methylmercury concentrations increase over time (bioaccumulation) in an animal, and increase by up to an order of magnitude or more at each successive trophic level (biomagnification) [99]. The methylmercury bioaccumulation step from water to phytoplankton and other seston represents the largest single increase for methylmercury concentrations in aquatic ecosystems, and can be 10^4 or greater [100, 101]. Methylmercury may initially enter food webs either through consumption by heterotrophic feeders of the methylmercury-containing microbial populations responsible for its formation, or by release of dissolved methylmercury into water followed by its assimilation by phytoplankton and algae. Diet is the main source of methylmercury for higher-order consumers (e.g. invertebrates, fish, mammals). In general, trophic interactions (predator–prey relationships) and the initial mercury concentration at the base of food chains may be most influential in determining the pathway and transfer rate of methylmercury into higher trophic level species [100]. Methylmercury is efficiently assimilated into tissues and is slowly eliminated from the body, so it strongly bioaccumulates in organisms. The growth rates of consumer animals also affect their accumulation of methylmercury. Species with slower growth rates tend to have higher methylmercury concentrations because less biomass is produced per unit of mercury consumed, in contrast with species with faster growth rates which will have lower methylmercury concentrations, the so-called 'biodilution effect' [99]. Biomagnification factors for methylmercury concentrations at successively higher trophic levels range from 4 to 10 [102]. Because only methylmercury is biomagnified, the fraction of methylmercury increases progressively with trophic level, typically reaching over 90% of total mercury in the muscle tissues of predatory fish and other high trophic level species [103]. Overall, muscle tissue is a better indicator of dietary mercury sources and the processes of mercury biomagnification driven by food web structure, whereas liver mercury best reflects age-related accumulation [99].

Mercury has been regulated domestically in industrialized countries since at least the 1950s and internationally since the 1970s [104, 105]. The United Nations Conference on the Human Environment in Stockholm in 1972 initiated action on high-priority pollutants in

the marine environment, including mercury [106]. Numerous early international policies on mercury were developed in the context of regional water quality cooperation on hazardous substances. Recent efforts by the United Nations Environment Programme (UNEP) to address mercury have been the most comprehensive international activities to date. In February 2009, the UNEP Governing Council established a mandate to begin negotiations on a global, legally binding mercury treaty. This decision follows several years of voluntary activities under a mercury programme, in which interested countries and other parties established partnerships on topics such as artisanal gold mining, transport and fate research, coal combustion, and waste management [94]. Reductions in mercury emissions will not necessarily result in immediate reductions in methylmercury exposure. In some cases, such as certain ocean basins, concentrations may continue to rise despite cuts in emissions because they are not yet at steady state with respect to deposition [94].

6.5.3 Toxic effects

Humans are exposed mainly through consumption of fish contaminated by methylmercury, particularly those who consume high levels of fish. High-profile mercury poisoning incidents drew scientific and policy attention to the risks of methylmercury. In the 1950s, in Minamata, Japan, people who ate fish contaminated by high levels of methylmercury experienced neurological damage such as visual, auditory and sensory disturbances, numbness and difficulty walking [107, 108]. Those exposed *in utero* experienced more serious effects, including mental retardation, cerebral palsy, deafness and blindness [107]. The Japanese tragedy, which was followed by other incidents of mercury poisoning also caused by industrial pollution elsewhere in Japan, caused considerable concern in other countries where fish is consumed in quantity and industrial pollution occurs. Several well-fished lakes in Sweden and Canada, near mercury-using wood pulp factories, were known to be heavily polluted. Levels of methylmercury of up to 10 mg/kg were found in the fish [109]. As a result, fishing was banned in several of these lakes and efforts were made to clean up the pollution.

Although fish is the major source of methylmercury in the diet, intoxication has also occurred on a large scale as a result of ingestion of methylmercury from non-fish sources. In Iraq, at the end of the 1960s and again in 1971, people were exposed to high levels of ethylmercury and methylmercury by eating bread made with grain treated with alkylmercury compounds used as antifungal agents for dressing cereal seeds. Health effects included numbness, problems with vision, speech and hearing, as well as deaths in adults and more serious neurological effects in the offspring of exposed pregnant women [110, 111]. Similar incidents were reported subsequently from Pakistan and Guatemala [112]. The application of alkylmercury compounds as antifungal agents was banned.

In general, mercury uptake by plants is very low and mercury residues are independent of soil mercury concentration, most of the mercury found in foliage originating from atmospheric deposition [5, 113]. Mercury concentrations in plants are very low, being very close to or below the detection limits in most analysed samples. Thus it is assumed that mercury exposure in farm animals is very low. Data from biomonitoring studies in livestock from relatively unpolluted areas [114] indicate that total mercury concentrations in meat and meat products are generally below 10–20 µg/kg fresh weight, which is below the quantification limit (generally 1–5 µg/kg fresh weight) in many liver and muscle samples. Based on these data, mercury residues in meat products do not appear to pose a relevant risk for human health; in fact the EU has not established a maximum mercury concentration for meat or meat products [114]. Organ meat may sometimes contain high levels of mercury as a result of industrial pollution of water or soil by use of sewage sludge as an agricultural top dressing. A range of 1.0–136 µg/kg in kidneys and 7.0–14.0 µg/kg in livers of cattle in the Netherlands has been attributed to the latter cause [115].

Recent epidemiological studies have linked exposure to methylmercury in pregnant women to neurological and developmental effects in their offspring. Studies in the Faroe Islands [116] and New Zealand [117] showed correlations between maternal methylmercury exposure and developmental deficits in offspring, whereas a study in the Sheychelles found no association [118]. Cardiovascular effects, especially in adult males, have also been linked to methylmercury exposure [119, 120].

6.5.4 Mercury in food

Health authorities and food legislators in most countries have responded to the toxic effects of mercury, especially organic mercury, by introducing strict controls on

Table 6.5 Summary of total mercury occurrence data by food group.

Category	*N*	%LC	Mean (µg/kg)			Category	*N*	%LC	Mean (µg/kg)			Category	*N*	%LC	Mean (µg/kg)		
			LB	MB	UB				LB	MB	UB				LB	MB	UB
Grains and grain-based products	4,545	60	0.9	2.0	3.1	Cocoa beans and cocoa products	126	56	1.7	3.7	5.7	Berries and small fruits	358	87	0.1	1.5	2.9
Grains for human consumption	2,680	52	1.0	2.1	3.2	Coffee beans and coffee products (solid)	298	49	1.4	1.7	1.9	Miscellaneous fruits	149	89	0.1	1.0	1.9
Grain milling products	671	65	0.6	1.6	2.6	Coffee imitates (solid)	13	46	0.7	0.9	1.1	Dried fruits	33	73	0.2	1.7	3.2
Bread and rolls	596	75	0.7	1.7	2.7	Vegetable products	139	55	13	13	13	Jam, marmalade and other fruit spreads	57	44	3.3	5.6	7.8
Pasta (raw)	81	77	0.5	2.2	4.0	Fungi, cultivated	508	32	9.1	10	11	Other fruit products	126	75	0.2	1.3	2.5
Breakfast cereals	230	82	0.5	3.1	5.6	Fungi, wild, edible	165	19	105	106	107	**Meat and meat products**	10,304	56	1.9	2.7	3.5
Fine bakery wares	287	73	0.5	1.7	2.9	**Starchy roots and tubers**	1,234	75	0.2	0.8	1.4	Meat and meat products	23	61	0.7	1.3	1.9
Vegetables and vegetable products	4,299	62	6.0	7.0	7.8	Potatoes and potatoes products	421	92	0.1	1.1	2.1	Livestock meat	3,078	66	0.8	1.7	2.5
Vegetable and vegetable products	103	47	0.5	1.0	1.4	Other starchy roots and tubers	813	67	0.3	0.6	1.0	Poultry	1,450	66	1.2	2.3	3.5
Root vegetables	724	71	0.4	1.5	2.6	**Legumes, nuts and oilseeds**	1,311	51	2.3	2.8	3.3	Game mammals	1,613	54	2.4	3.3	4.3
Bulb vegetables	325	76	0.1	1.2	2.3	Legumes, beans, green, without pods	102	75	0.4	1.0	1.5	Game birds	376	81	0.6	2.0	3.4
Fruiting vegetables	669	70	0.2	0.9	1.6	Legumes, beans, dried	483	53	1.4	1.8	2.2	Mixed meat	382	46	0.9	1.1	1.3
Brassica vegetables	481	61	0.4	0.8	1.3	Tree nuts	170	65	2.6	3.8	4.9	Edible offal, farmed animals	2,453	38	3.1	3.6	4.1
Leaf vegetables	339	83	0.5	2.1	3.8	Oilseeds	556	39	3.2	3.7	4.2	Edible offal, game animals	259	30	11	11	12
Legume vegetables	13	46	0.3	0.6	0.9	**Fruit and fruit products**	1,368	74	0.3	1.2	2.1	Preserved meat	174	65	1.0	2.9	4.9
Stem vegetables (fresh)	246	91	0.1	1.5	2.9	Fruit and fruit products	3	33	0.1	0.2	0.2	Sausages	364	63	0.8	1.4	1.9
Sugar plants	65	22	0.7	0.7	0.8	Citrus fruits	150	69	0.1	0.6	1.1	Meat specialities	27	33	0.9	0.9	1.0
Seaweeds	1	100	0	2.5	5.0	Pome fruits	349	63	0.2	0.6	0.9	Pastes, pâtés and terrines	96	33	1.3	1.4	1.5
Tea and herbs for infusions (solid)	85	68	6.0	7.7	9.5	Stone fruits	143	72	0.1	1.0	1.9	Meat imitates	9	56	1.2	1.4	1.6
Milk and dairy products	3,345	64	0.9	1.5	2.1	Sugar substitutes	2	50	0.2	0.2	0.3	**Non-alcoholic beverages**	699	46	3.4	4.0	4.5
Milk and dairy products	32	97	0.0	6.7	13	Chocolate (cocoa) products	314	60	1.4	2.1	3.2	Non-alcoholic beverages	17	47	0.1	3.7	7.3
Liquid milk	1,624	74	0.2	0.7	1.1	Confectionery (non-chocolate)	280	73	0.5	4.3	8.1	Soft drinks	301	71	0.0	0.8	1.6

Milk based beverages	3	33	2.9	3.0	3.0	Dessert sauces	11	45	1.1	1.4	1.6	Tea (infusion)	369	20	6.4	6.6	6.8
Concentrated milk	96	55	0.8	1.1	1.3	Molasses and other syrups	52	60	0.2	0.3	0.5	Coffee (beverage)	12	33	1.9	2.0	2.2
Whey and whey products	2	100	0	0.2	0.3	Honey	892	64	0.5	2.7	4.8	**Alcoholic beverages**	652	79	0.1	0.4	0.7
Cream and cream products	140	60	0.5	0.7	0.9	**Animal and vegetable fats and oils**	835	61	1.1	1.6	2.0	Beer and beer-like beverage	256	79	0.1	0.4	0.8
Fermented milk products	323	67	0.4	2.1	3.8	Animal and vegetable fats and oils	3	0	3.6	3.6	3.6	Wine	359	77	0.1	0.4	0.7
Cheese	1,095	49	2.0	2.4	2.8	Animal fat	396	52	1.1	1.3	1.5	Fortified and liqueur wines	2	50	0.1	0.1	0.1
Milk and milk product imitates	30	90	2.0	3.6	5.3	Fish oil	103	99	0.2	1.5	2.9	Wine-like drinks	16	88	0.1	0.4	0.7
Eggs and egg products	798	58	0.6	1.2	1.8	Vegetable fat	36	75	0.9	1.1	1.3	Spirits	19	95	0.0	0.6	1.2
Eggs, fresh	790	58	0.6	1.2	1.8	Vegetable oil	268	56	1.5	2.1	2.6	**Drinking water**	1,637	90	0.0	0.1	0.2
Eggs, powder	8	88	0.4	1.8	3.2	Margarine and similar products	29	72	0.6	0.7	0.8	Drinking water	73	99	0.0	0.1	0.2
Sugar and confectionery	1,617	73	0.6	2.6	4.7	**Fruit and vegetable juices**	651	89	0.1	3.2	6.2	Tap water	22	77	0.1	0.2	0.2
Sugar and confectionery	15	93	0.1	1.0	1.0	Fruit and vegetable juices	44	89	0.1	0.3	0.5	Well water	422	76	0.0	0.1	0.2
Sugars	51	82	0.1	0.3	0.4	Fruit juice	416	63	0.1	2.9	5.7	Bottled water	1,120	95	0.0	0.1	0.2
Herbs, spices and condiments	529	47	3.1	4.3	5.5	Infant formulae, liquid	1	100	0	0.2	0.4	**Composite food**	304	41	16	18	19
Herbs, spices and condiments	3	67	27	32	37	Follow-on formulae, powder	128	86	0.7	2.7	4.7	Composite food	83	66	2.8	4.6	6.4
Herbs	34	62	13	15	17	Cereal-based food for infants and young children	102	90	0.2	1.3	2.4	Cereal-based dishes	15	13	6.9	9.7	12

(Continued)

Table 6.5 (Continued)

Category	*N*	%LC	Mean (µg/kg)			Category	*N*	%LC	Mean (µg/kg)			Category	*N*	%LC	Mean (µg/kg)		
			LB	MB	UB				LB	MB	UB				LB	MB	UB
Spices	174	37	3.7	5.3	6.8	Ready-to-eat meal for infants and young children	228	77	0.1	1.0	1.9	Potato-based dishes	2	0	1.3	1.3	1.3
Herb and spice mixtures	38	66	2.3	7.3	12	Yoghurt, cheese and milk-based dessert for infants and young children	8	100	0	0.1	0.1	Beans-based meals	5	100	0	0.2	0.3
Seasoning or extracts	69	61	1.4	1.9	2.3	Fruit juice and herbal tea for infants and young children	1	0	6.0	6.0	6.0	Meat-based meals	37	35	4.6	6.6	8.6
Condiment	54	61	0.8	0.9	1.0	**Products for special nutritional use**	1,608	68	96	99	102	Fish and seafood-based meals	84	4	42	42	43
Dressing	22	45	2.1	2.2	2.2	Products for special nutritional use	82	52	1.0	2.6	4.2	Vegetable-based meals	3	67	1.9	3.7	5.5
Chutney and pickles	3	0	0.9	0.9	0.9	Food for weight reduction	15	80	0.6	2.0	3.5	Ready to eat soups	33	73	11	11	12
Savoury sauces	5	60	0.1	0.2	0.3	Dietary supplements(b)	1,233	66	123	126	129	Prepared salads	42	7	15	15	15
Flavourings or essences	8	50	0.7	4.0	7.2	Food for sports people	168	57	19	22	25	Snacks, desserts, and other foods	451	54	1.2	1.5	1.9
Baking ingredients	119	33	1.8	1.9	2.1	Dietetic food for diabetics	51	96	0.3	0.8	1.3	**Snacks, desserts, and other foods**	1	100	0	0.1	0.1
Food for infants and small children	834	63	0.6	1.6	2.5	Medical food	59	95	0.2	1.7	3.3	Snack food	248	58	0.7	0.9	1.2
Food for infants and small children	222	11	1.1	1.2	1.3							Ices and desserts	135	43	0.8	0.8	0.9
Infant formulae, powder	144	79	1.0	2.2	3.5							Other foods	31	68	8.3	10	12

N, number of samples; %LC: percentage of left-censored data; LB, lower bound; MB, middle bound; UB, upper bound.
Source: European Food Safety Authority [125].

Table 6.6 Statistical description of concentrations of total mercury for the groups 'Fish and other seafood' and 'Fish meat'.

Category	*N*	%LC	Mean (µg/kg)			Category	*N*	%LC	Mean (µg/kg)		
			LB	MB	UB				LB	MB	UB
Fish and other seafood	21,539	12	131	133	136	Jack mackerel	3	0	127	127	127
Fish and other seafood, unspecified	1,968	3	100	100	101	John Dory	6	0	302	302	302
Fish meat	13,737	7	177	178	180	Lizardfish	2	0	611	611	611
Fish products	241	8	37	38	38	Luvarus	1	0	590	590	590
Fish offal	158	58	12	19	26	Mackerel	1,348	5	106	108	109
Crustaceans	1,478	21	43	47	50	Meagre	2	50	145	170	195
Molluscs	3,926	26	31	36	41	Perch	423	0	165	165	165
Amphibians, reptiles, snails, insects	31	48	19	20	21	Pike	267	0	394	394	394
Fish meat						Plaice	194	2	64	64	65
Anchovy	110	33	73	83	92	Ray	32	3	229	229	230
Angler fish	61	30	186	195	204	Redfish	221	0	189	189	189
Babel	10	0	211	211	211	Roach	17	0	122	122	122
Barracuda	1	0	340	340	340	Salmon, trout	1,741	7	31	33	35
Bass	78	10	199	203	206	Sardine and pilchard	399	18	32	38	44
Bonito	25	8	580	583	586	Scorpion fish	1	0	422	422	422
Bream	253	11	224	225	226	Sea bass	10	0	300	300	300
Capelin	11	82	2.0	5.0	8.0	Sea catfish, wolf-fish	67	54	103	109	114
Carp	338	5	55	55	55	Shad	1	0	173	173	173
Char	8	0	32	32	32	Shark	272	11	688	691	695
Cod, whiting	1,308	18	91	94	96	Smelt	2	0	325	325	325
Dentex	3	0	2,019	2,019	2,019	Sole	49	24	69	77	84
Eel	487	2	177	178	178	Sprat	107	1	21	21	21
Flounder	23	17	85	91	97	Sturgeon	4	50	40	52	65
Garfish	3	0	590	590	590	Swordfish	264	5	1,210	1,212	1,214
Grenadier	3	0	104	104	104	Tuna	849	5	286	290	291
Grey mullet	52	23	152	159	167	Turbot	4	0	62	62	62
Grouper	2	0	195	195	195	Weever	11	0	763	763	763
Gurnard	4	25	103	109	116	Whitefish	37	16	77	85	93
Hake	131	16	130	136	142	Wrasse	12	0	511	511	511
Halibut	1,713	0	209	209	209	Fish meat, unspecified, as reported*	1,502	10	279	280	280
Herring	1,272	0	36	36	36	Fish meat, overall results†	12,235	10	164	166	168

N, number of samples; % LC, percentage of left-censored data; LB, lower bound; MB, middle bound; UB, upper bound.

*Data described as reported.

†Data calculated on overall concentrations of individual specified fish species excluding fish meat unspecified and such used for exposure calculation.

Source: European Food Safety Authority [125].

Table 6.7 Statistical description of concentrations of methylmercury for the food groups 'Fish and other seafood' and 'Fish meat'.

Category	*N*	%LC	Mean (µg/kg)			Category	*N*	%LC	Mean (µg/kg)		
			LB	MB	UB				LB	MB	UB
Fish and other seafood						Hake	11	64	32	64	96
Fish meat	969	6	131	135	139	Halibut	61	0	127	127	127
Fish products	33	12	39	39	40	Herring	39	0	30	30	30
Fish offal	4	100	23	23	23	Mackerel	122	9	123	127	132
Crustaceans	42	48	70	102	134	Perch	2	0	56	56	56
Molluscs	35	57	15	61	107	Salmon and trout	28	50	13	38	63
Fish meat						Sardine and pilchard	16	88	14	58	102
Anchovy	5	80	22	62	102	Sea catfish, wolf-fish	1	0	121	121	121
Angler fish	3	33	173	190	206	Shark	4	0	1,520	1,520	1,520
Bass	5	60	31	61	91	Smelt	1	0	73	73	73
Bream	4	50	61	86	111	Sole	4	0	0	50	100
Carp	33	21	13	13	13	Sprat	25	0	8	8	8
Cod and whiting	183	4	19	19	20	Swordfish	10	0	819	819	819
Eel	8	0	172	172	172	Tuna	125	2	220	221	221
Flounder	45	0	66	66	66	Fish meat, unspecified	226	1	225	225	225
Grey mullet	8	88	18	62	106						

N, number of samples; % LC, percentage of left-censored data; LB, lower bound; MB, middle bound; UB, upper bound.
Source: European Food Safety Authority [125].

levels of the element permitted in foods such as fish. In Australia, for instance, there is a maximum permitted level of 0.5 mg/kg fresh weight in most fish and molluscs, with the exception of certain larger predators, including shark and tuna, which are permitted to contain 1.0 mg/kg [121]. The European countries follow a European Commission decision, which establishes a limit of 0.5 mg/kg in edible parts of fresh fish, with a higher limit of 1.0 mg/kg for tuna, shark, swordfish and halibut [1]. In the USA, the FDA has set a limit of 1 mg/kg for methylmercury in fresh fish [122], although the EPA believe that the limit should be lower. The agency has proposed that intake of mercury should be reduced to not more than 0.1 μg/kg body weight per day [123]. This is considerably less than the JEFCA PTWI of 4 μg/kg body weight per week. In this sense, following a request from the European Commission, the EFSA CONTAM Panel was asked to deliver a scientific opinion on the risks to human health related to the presence of inorganic mercury and methylmercury in food. The Panel was asked to consider new developments regarding the toxicity of inorganic mercury and methylmercury since the last opinion of the EFSA on 24 February 2004 and to evaluate whether the PTWIs established by the JECFA of 1.6 μg/kg body weight for methylmercury and 4 μg/kg body weight for inorganic mercury were considered appropriate. In response EFSA has received a total of 59,820 results from testing for the presence of mercury in food from 20 European countries. The data reported represent the period from 2002 to 2011, although the call for data was originally limited to the period from 2006 to 2011. A total number of 59,650 results were described with sufficient detail to be used in the statistical analysis of the respective food groups. Overall, 58,730 (98.2%) of the analytical results were reported for total mercury, 1087 (1.8%) for methylmercury and only three samples were reported for inorganic mercury. Data on methylmercury were provided by four countries: Germany (788 results), Spain (206 results), Czech Republic (90 results) and Slovakia (three results). The data provided were sampled in the period 2002–2011, with only 55 results covering the period before 2004 [124]. The results of this study are summarised in Table 6.5. Statistical results of concentrations of total mercury and methylmercury for the groups 'Fish and other seafood' and 'Fish meat'are given in **Tables** 6.6 and 6.7. The mercury content varied widely among different fish species, depending on size and feeding habits, and as expected was higher in predatory fish. This is in line with the results from other studies showing a higher mercury concentration in older predatory fish species [125]. The highest mean concentrations were found in swordfish (MB mean 1212 μg/kg) and shark (MB mean 691 μg/kg). Similarly to total mercury, the highest methylmercury concentration was reported in 'Fish meat' (MB mean 135 μg/kg), followed by 'Crustaceans' (MB mean 102 μg/kg); with the highest mean concentrations for swordfish and shark too.

In this same report [124], studies of food processing reported that mercury, when present in food, is stable and resistant to the effects generally encountered during processing. The WHO [125] stated that methylmercury in fish is bound to tissue protein rather than with fatty deposits, so trimming and skinning of fish does not reduce the mercury content of the fillet portion. Moreover, the mercury concentration in fish is not changed when cooked. However, because some moisture is usually lost during cooking, mercury concentrations are often slightly higher in cooked fish than in raw wet tissue. In addition, some preparation methods, such as deep frying, can actually increase the weight of the fish, potentially resulting in slightly lower concentrations of mercury. However, the total amount of mercury in fish remains relatively unchanged after cooking, and the slight changes in mercury concentrations due to cooking methods are relatively insignificant and generally do not need to be considered when estimating dietary exposures.

Abbreviations

ATSDR: Agency for Toxic Substances and Disease Registry
CBD: Chronic beryllium disease
COMTAM: Contaminants in the Food Chain of the European Food Safety Authority
EDTA: Ethylendiaminetetraacetic acid
EFSA: European Food Safety Authority
EPA: Environmental Protection Agency
EU: European Union
FAO: Food and Agriculture Organization
FASFC: Federal Agency for the Safety of the Food Chain
FDA: Food and Drug Administration
IARC: International Agency for Research on Cancer
JECFA: Joint Expert Committee on Food Additives

MB: Middle bound
ML: Maximum level
NIOSH: National Institute for Occupational Safety and Health
OSHA: Occupational Safety and Health Administration
PTWI: Provisional tolerable weekly intake
PVC: Polyvinylchloride
SCF: Scientific Committee for Food
SCOOP: Scientific Cooperation
TWI: Tolerable weekly intake
UNEP: United Nations Environment Programme
WHO: World Health Organization

References

1 Reilly C. *Metal Contamination of Food*. Wiley-Blackwell, Melbourne, 2002.
2 Deshpande SS. *Handbook of Food Toxicology*. Marcel Dekker, New York, 2002.
3 Page AL, Bingham FT, Chang AC. Cadmium. In: Lepp NW (ed.) *Effect of Heavy Metal Pollution on Plants. Vol. 1. Effects of Trace Metals on Plant Function*. Applied Science, London, 1981, pp. 77–109.
4 Cook PJ, Freney RJ. Sources of cadmium in agriculture. In: Simpson J, Curnow B (eds) *Cadmium Accumulations in Australian Agriculture*. Australian Government Publishing Service, Canberra, 1988.
5 Kabata-Pendias A, Pendias H. *Trace Elements in Soils and Plants*, 3rd edn. CRC Press, Boca Raton, FL, 2001.
6 Peterson PJ, Benson LM, Sieve Z. Metalloids. In: Lepp NW (ed.) *Effect of Heavy Metal Pollution on Plants. Vol. 1. Effects of Trace Metals on Plant Function*. Applied Science, London, 1981, pp. 279–342.
7 Asami T. Cadmium pollution of soils and human health in Japan. In: Låg J (ed.) *Human and Animal Health in Relation to Circulation Processes of Selenium and Cadmium*. Norwegian Academy of Science and Letters, Oslo, 1991, pp. 115–125.
8 Alloway BJ. Cadmium. In: Alloway BJ (ed.) *Heavy Metals in Soils*. Blackie and Son, Glasgow, 1990.
9 Cartwright BC, Merry RH, Tiller KG. Heavy metal contamination of soils around a lead smelter at Port Pirie, South Australia. *Aust J Soil Res* 1977;**15**:69–81.
10 Gulson BL, Tiller KG, Mizon KJ, Merry RH. Use of lead isotopes in soils to identify the source of lead contamination. *Environ Sci Technol* 1981;**15**:691–696.
11 Andersson A. Mercury in soil. In: Nriagu JO (ed.) *The Biogeochemistry of Mercury in the Environment*. Elsevier, Amsterdam, 1979, pp. 79–102.
12 Kevresan S, Cirin-Novta V, Kuhajda K *et al*. Alleviation of cadmium toxicity by naphtenate treatment. *Biol Plant* 2004;**48**:453–455.
13 Kevresan S, Petrovic N. Effect of different cadmium concentrations on cadmium retranslocation into the reproductive organs in pea of different ages. *Cent Eur J Occup Environ Med* 2003;**9**:322–326.
14 Pajevic S, Vuckovic M, Stankovic Z, Krstic B, Kevresan Z, Radulovic S. The content of some macronutients and heavy metals in aquatic macrophytes of three ecosystems connected to the Danube in Yugoslavia. *Arch Hydrobiol* 2002;Suppl. **141**(1–2):73–83.
15 Bakir F, Damlujy S, Amin-Zaki L *et al*. Methyl mercury poisoning in Iraq. An Interuniversity Report. *Science* 1973;**181**: 230–241.
16 Sharma RP, Street JC, Shupe L. Translocation of lead and cadmium from feed to edible tissues of swine. *J Food Safety* 1982;**4**:151–163.
17 Tojagic S, Kevresan Z, Jokanovic M. Some quality indicators in vineyard snail meat (Helix pomatia). *Vet Glas* 2004;**58**:157–161.
18 Pavlovic A, Kevresan Z, Kelemen-Masic D, Mandlic A. *Micro and toxic elements in skin meat and gills of grass carp (Ctenopharyngodon idella)*. In: Proceedings of the 8th Symposium of Analytical and Environmental Problems, Szeged, Hungary, October 2001, pp. 279–342.
19 Alloway BJ, Aires DC. *Chemical Principles of Environmental Pollution*, 2nd edn. Blackie Academic and Professional, London, 1997, p. 218.
20 Manahan SE. *Environmental Chemistry*, 8th edn. CRC Press, Boca raton, FL, 2005, p. 289.
21 Kreiss K, Day GA, Schuler CR. Beryllium: a modern industrial hazard. *Annu Rev Public Health* 2007;**28**:259–277.
22 Jakubowski M, Palczynski C. Beryllium. In: Nordberg GF, Fowler BA, Nordberg M, Friberg L (eds) *Handbook on the Toxicology of Metals*, 3rd edn. Academic Press, Burlington, MA, 2007, pp. 415–431.
23 Agency for Toxic Substances and Disease Registry. Toxicological profile for beryllium, September 2002. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=185&tid=33
24 Tinkle SS, Antonini JM, Rich BA *et al*. Skin as a route of exposure and sensitization in chronic beryllium disease. *Environ Health Perspect* 2003;**119**:1202–1208.
25 Wambach PF, Laul JC. Beryllium health effects, exposure limits and regulatory requirments. *J Chem Health Safety* 2008;July/August:5–12.
26 Kolanz ME. Introduction to beryllium: uses, regulatory history, and disease. *Appl Occup Environ Hyg* 2001;**16**:559–567.
27 Environmental Protection Agency. Health assessment document for beryllium. EPA/600/8-84/026F. Office of Health and Environmental Assessment, Environmental Protection Agency, Washington, DC, 1987.
28 Occupational Safety and Health Administration. http://www.osha.gov.
29 Food and Drug Administration. http://www.fda.gov.
30 National Institute for Occupational Safety and Health. Beryllium and beryllium compounds. NIOSH pocket guide to chemical hazards. Available at http://www.cdc.gov/niosh/npg/npgd0054.html.

31 Vaessen HAMG, Szteke B. Beryllium in food and drinking water: a summary of available knowledge. *Food Addit Contam* 2000;**17**:149–159.
32 Hazardous Substance Data Bank. Beryllium and beryllium compounds. Available at http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~CwUFUx:2.
33 Barnes KW. Trace metal determinations in fruit, juice, and juice products using an axially viewed plasma. *Atom Spectrosc* 1997;**18**:84–101.
34 Agence Francaise de Sécurité Sanitarie des Aliments. Avis de l'Agence francaise de sécurité sanitarie des aliments relatif á une demande d'appuiscientifique et technique concernant la contamination de denrés alimentaires d'origine animales par du cadmium provenant de la contamination de suppléments minéraux (sulfate de zinc) entrant dans la composition des aliments pour animaux. Afssa Saisine No. 2006-SA-0057, 2006.
35 L'Institut National de l'Environnement Industriel et des Risques (INERIS). Le cadmium et ses derivés Fiche de données toxicologiques et environnementales des substances chimiques. DRC-01-25590-00DF249.doc, Version No. 2–3, Février 2005.
36 European Food Safety Authority. Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on cadmium in food. *EFSA J* 2009;**980**:1–139.
37 Nordberg GF, Nogawa K, Nordberg M, Friberg LT. Cadmium. In: Nordberg GF, Fowler BA, Nordberg M, Friberg LT (eds) *Handbook on the Toxicology of Metals*, 3rd edn. Academic Press, Burlington, MA, 2007, pp. 445–486.
38 International Programme on Chemical Safety. Cadmium. Environmental Health Criteria 134, 1992. Available at http://www.inchem.org/documents/ehc/ehc/ehc134.htm
39 European Community. Community Action Programme, Directive No. 89/677/EEC, 1992.
40 Sivaperumal P, Sankar T, Viswanathan V, Nair PG. Heavy metal concentrations in fish, shellfish and fish products from internal markets of India vis-à-vis international standards. *Food Chem* 2007;**102**:612–620.
41 Wang WX. Comparision of metal uptake and absorption efficiency in marine bivalves. *Environ Toxicol Chem* 2001;**20**: 1367–1373.
42 Chary NS, Kamala CT, Raj DS. Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. *Ecotoxicol Environ Saf* 2008;**69** :513–524.
43 Agency for Toxic Substances and Disease Registry. *Toxicological Profile for Cadmium (Final Report)*. NTIS Accession No. PB99-166621, 1999.
44 Drivsholm T, Hanse E, Maag J, Havelund S. Massestrømsanalyse for cadmium [Substance flow analysis for cadmium]. Environmental Project No. 557. Danish EPA, Copenhagen, 2000.
45 Organization for Economic Cooperation and Development. Risk Reduction Monograph No. 5: Cadmium. Background and national experience with reducing risk. OECD Environment Monograph Series No. 104. OECD Environment Directorate, Paris, 1994.
46 Arctic Monitoring and Assessment Programme. Forurensning i Arktis: Tilstandsrapport om det arktiske miljø[Pollution in the Arctic: Report on the present state of the Arctic environment]. AMAP, Oslo, 1997.
47 Arctic Monitoring and Assessment Programme. *Arctic Pollution 2002*. AMAP, Oslo, 2002.
48 He QB, Singh BR. Effect of organic matter on the distribution, extractability and uptake of cadmium in soils. *Eur J Soil Sci* 1994;**44**:641–650.
49 Thornton I. Sources and pathways of cadmium in the environment. In: Nordberg GF, Herber RFM, Alessio L (eds) *Cadmium in the Human Environment: Toxicity and Carcinogenicity*. IARC Scientific Publications, Lyon, 1992, pp. 149–162.
50 Clemens S. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie 2006;**88**: 1707–1719.
51 Franz E, Römkens P, van Raamsdonk L, van der Fels-Klerx I. A chain modeling approach to estimate the impact of soil cadmium pollution on human dietary exposure. J Food Prot 2008;**71**:2504–2513.
52 United Nations Environment Programme. Interim review of scientific information on cadmium. Available at http://www.chem.unep.ch/Pb_and_Cd/SR/Files/Interim_reviews/UNEP_Cadmium_review_Interim_Oct2006.doc (Version of October 2006).
53 United Nations Environment Programme. Draft final review of scientific information on cadmium. http://www.chem.unep.ch/Pb_and_Cd/SR/Draft_final_reviews/Cd_Review/Final_UNEP_Cadmium_review_Nov_2008.pdf (Version of November 2008).
54 OSPAR Commission. Cadmium (2002). Hazardous Substances Series. Available at http://www.ospar.org/documents/dbase/publications/p00151/p00151_background%20document%20on%20cadmium.pdf
55 Whyte AL, Raumati HG, Greening GE, Gibbs-Smith E, Gardner JP. Human dietary exposure to heavy metals via the consumption of greenshell mussels (Perna canaliculus Gmelin 1791) from the Bay of Islands, northern New Zealand. Sci Total Environ 2009;**407**:4348–4355.
56 Copes R, Clark NA, Rideout K, Palaty J, Teschke K. Uptake of cadmium from Pacific oysters (Crassostrea gigas) in British Columbia oyster growers. *Environ Res* 2008;**107**:160–169.
57 Warnau M, Ledent G, Temara A, Bouquegneau JM, Jangoux M, Dubois P. Heavy metals in Posidonia oceanica and Paracentrotus zividus from seagrass beds of the north-western Mediterranean. *Sci Total Environ* 1995;**171**: 95–99.
58 Warnau M, Biondo R, Temara A, Bouquegneau J-M, Jangoux M, Dubois P. Distribution of heavy metal in the echinoid Paracentrotus lividus (Lmk) from the Mediterranean Posidonia oceanica ecosystem: seasonal and geographical variations. *J Sea Res* 1998;**39**:267–280.

59 International Register of Potentially Toxic Chemicals. IRPTC legal file 1986, Vol. 1. United Nations Environment Programme, Geneva, 1987.
60 International Agency for Research on Cancer. *Beryllium, Cadmium, Mercury and Exposures in the Glass Manufacturing Industry*. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 58. IARC, Lyon, 1993. Available at http://monographs.iarc.fr/ENG/Monographs/vol58/volume58.pdf
61 Marzec Z, Schlegel-Zawadzka M. Exposure to cadmium, lead and mercury in the adult population from Eastern Poland, 1990–2002. *Food Addit Contam* 2004;**21**:963–970.
62 Nawrot T, Plusquin M, Hogervost J *et al*. Environmental exposure to cadmium and risk of cancer: a prospective population-based study. *Lancet Oncol* 2006 ;**7**:119–126.
63 Horiguchi H, Oguma E, Sasaki S *et al*. Comprehensive study of the effects of age, iron deficiency, diabetes mellitus and cadmium burden on dietary cadmium absorption in cadmium-exposed female Japanese farmers. *Toxicol Appl Pharmacol* 2004;**196**:114–123.
64 Agency for Toxic Substances and Disease Registry. Toxicological profile for cadmium, September 2012. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=48&tid=15.
65 Kobayashi E, Suwazono Y, Doch M, Honda R, Kido T. Influence of consumption of cadmium-polluted rice or Jinzu river water on occurrence of renal tubular dysfunction and/or Itai Itai disease. *Biol Trace Elem Res* 2009;**127**:257–268.
66 European Commission. European risk assessment report. Cadmium metal. Part II. Human health. EUR 22767 EN. Office for Official Publications of the European Communities, Luxembourg, 2007. Available at http://echa.europa.eu/documents/10162/4ea8883d-bd43-45fb-86a3-14fa6fa9e6f3
67 Chen L, Jin T, Huang B, Nordberg G, Nordberg M. Critical exposure level of cadmium for elevated urinary metallothionein: an occupational population study in China. *Toxicol Appl Pharmacol* 2006;**215**:93–99.
68 European Food Safety Authority. Statement on tolerable weekly intake for cadmium. *EFSA J* 2011;**9**(2):1975.
69 European Food Safety Authority. Cadmium dietary exposure in the European population. *EFSA J* 2012;**10**:2551.
70 European Commission. Commission Regulation No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF.
71 Directorate-General Health and Consumer Protection. Assessment of the dietary exposure to arsenic, cadmium, lead and mercury of the population of the EU Member States. SCOOP 3.2.11, March 2004. Available at http://ec.europa.eu/food/food/chemicalsafety/contaminants/scoop_3-2-11_heavy_metals_report_en.pdf.
72 Food and Agriculture Organization/World Health Organization. *Evaluation of Certain Food Additives and Contaminants (61st report of the Joint FAO/WHO ExpertCommittee on Food Additives)*. WHO Technical Report Series No. 922. WHO, Geneva, 2004.
73 L'Agence Federale pour la Securite de la Chaine Alimentaire. AVIS 35-2009. Estimation de l'exposition alimentaire au cadmium par la population Belge (dossier Sci Com No. 2009/13). Available at http://www.afsca.be/comitescientifique/avis/_documents/AVIS35-2009_FR_DOSSIER2009-13_000.pdf2009
74 Vroman V, Waegeneers N, Cornelis C *et al*. Dietary cadmium intake by the Belgian adult population. *Food Addit Contam A* 2010;**27**:1665–1673.
75 Hutchinson TC, Meema KM (eds) *Lead, Mercury, Cadmium and Arsenic in the Environment*. John Wiley & Sons, Chichester, UK, 1987.
76 World Health Organization. *Air Quality Guidelines*, 2nd edn. WHO Regional Office for Europe, Copenhagen, 2001, chapter 6.7, pp. 1–17.
77 Levin R, Brown MJ, Kashtock ME *et al*. Lead exposures in U.S. children, 2008: implications for prevention. *Environ Health Perspect* 2008;**116**:1285–1293.
78 Järup L. Hazards of heavy metal contamination. *Br Med Bull* 2003;**68**:167–182.
79 Agency for Toxic Substances and Disease Registry. Toxicological profile for lead, August 2007. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=96&tid=22
80 EFSA Panel on Contaminants in the Food Chain. Scientific Opinion on lead in food. *EFSA J* 2010;**8**:1–151.
81 Hazardous Waste Management Program Regulatory and Program Development Division, 2004, Department of Toxic Substances Control, pp. 1–119.
82 International Programme on Chemical Safety. Lead. WHO Food Additives Series 21. Available at http://www.inchem.org/documents/jecfa/jecmono/v21je16.htm.
83 Giblin A. Food, water and lead contamination. *LEAD Action News* 2010;**10**:1–18.
84 Koirala P, Khadka DBdr. Lead contamination on food and food products and its exposure to human. *Food Wave Magazine* 2007;**4**:28–31.
85 Food Safety Authority of Ireland. Mercury, lead, cadmium, tin and arsenic in food. Toxicology Factsheet Series Issue No. 1, May 2009. Available at www.fsai.ie/workarea/downloadasset.aspx?id=8412.
86 International Programme on Chemical Safety. Lead. WHO Food Additives Series 44. http://www.inchem.org/documents/jecfa/jecmono/v44jec12.htm.
87 Codex Alimentarius. Code of practice for the prevention and reduction of lead contamination in foods. CAC/RCP 56-2004. http://www.codexalimentarius.org/download/standards/10099/CXP_056e.pdf.
88 US Food and Drug Administration. Supporting document for recommended maximum level for lead in candy likely to be consumed frequently by small children. Available at http://www.fda.gov/Food/FoodborneIllnessContaminants/Metals/ucm172050.htm.

89 Directorate-General Health and Consumer Protection. Assessment of the dietary exposure to arsenic, cadmium, lead and mercury of the population of the EU Member States. SCOOP 3.2.11, March 2004. Available at http://ec.europa.eu/food/food/chemicalsafety/contaminants/scoop_3-2-11_heavy_metals_report_en.pdf.
90 European Food Safety Authority. Lead dietary exposure in the European population. *EFSA J* 2012;**10**(7):2831.
91 Mason RP, Sheu GR. Role of the ocean in the global mercury cycle. *Global Biogeochem Cycles* 2002;**16**:40.1–40.14.
92 Reichlmayer-Lais AM, Kirchgessner M. Lead: an essential trace element. In: Momçiloviç B (ed.) *Trace Elements in Man and Animals 7*. Institute of Medical Research and Occupational Health, University of Zagreb, Croatia, 1991.
93 Mergler D, Anderson HA, Chan LHM *et al*. Methylmercury exposure and health effects in humans: a worldwide concern. *Ambio* 2007;**36**:3–11.
94 Selin NE. Global biogeochemical cycling of mercury: a review. *Annu Rev Environ Resour* 2009;**34**:43–63.
95 Selin NE, Jacob DJ, Yantosca RM, Strode S, Jaeglé L, Sunderland EM. Global 3-D land–ocean–atmosphere model for mercury: present-day versus preindustrial cycles and anthropogenic enrichment factors for deposition. *Global Biogeochem Cycles* 2008;**22**:GB2011. doi: 10.1029/2007GB003040.
96 Fitzgerald WF, Lamborg CH. Geochemistry of mercury in the environment. In: Lollar BS (ed.) *Treatise on Geochemistry*. Elsevier, New York, 2005.
97 World Health Organization. *Environmental Health Criteria: Mercury*. WHO, Geneva, 1976.
98 Morel FMM, Kraepiel AML, Amyot M. The chemical cycle and bioaccumulation of mercury. *Annu Rev Ecol Syst* 2008;**29**:543–566.
99 Douglas TA, Loseto LL, Macdonald RW *et al*. The fate of mercury in Artic terrestrial and aquatic ecosystems: a review. *Environ Chem* 2012;**9**:321–355.
100 Watras CJ, Bloom NS. Mercury and methylmercury individual zooplankton: implications for bioaccumulation. *Limnol Oceanogr* 1992;**37**:1313–1318.
101 Baeyens W, Leermakers M, Papina T *et al*. Bioconcentration and biomagnification of mercury and methylmercury in North Sea and Scheldt Estuary fish. *Arch Environ Contam Toxicol* 2003;**45**:498–508.
102 Loseto LL, Stern GA, Deibel D *et al*. Linking mercury exposure to habitat and feeding behavior in Beaufort Sea beluga whales. *J Mar Syst* 2008;**74**:1012–1024.
103 Campbell LM, Norstrom RJ, Hobson KA, Muir DCG, Backus S, Fisk A. Mercury and other trace elements in a pelagic Arctic marine food web (Northwater Polynya, Baffin Bay). *Sci Total Environ* 2005;**351–352**:247–263.
104 Selin NE. Mercury rising: is global action needed to protect human health and the environment? *Environment* 2005;**47**:22–35.
105 Selin NE, Selin H. Global politics of mercury pollution: the need for multi-scale governance. *Rev Eur Commun Int Environ Law* 2006;**15**:258–269.
106 United Nations. Report of the United Nations Conference on the Human Environment, Stockholm, 5–16 June 1972. Available at http://www.un-documents.net/aconf48-14r1.pdf
107 Harada M. Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. *Crit Rev Toxicol* 1995;**25**:1–24.
108 Futatsuka M, Kitano T, Shono M *et al*. Health surveillance in the population living in a methyl mercury-polluted area over a long period. *Environ Res* 2000;**83**:83–92.
109 Skerfving S. Methylmercury exposure, mercury levels in blood and hair, and health status of Swedes consuming contaminated fish. *Toxicology* 1974;**2**:3–23.
110 Bakir F. Methyl mercury poisoning in Iraq. *Science* 1973;**181**:230–241.
111 Marsh DO, Clarkson TW, Cox C, Myers GJ, Amin-Zaki L, Al-Tiriki SAA. Feral methylmercury poisoning. Relationship between concentration in single strands of maternal hair and child effects. *Arch Neurol* 1987;**44**:1017–1022.
112 Magos L. Methylmercury poisoning. *Br Med Bull* 1975;**31**:241–242.
113 Eriksen JA, Gustin MS, Schorran DE, Johnson DW, Lindberg SE, Coleman JS. Accumulation of atmospheric mercury in forest foliage. *Atmos Environ* 2003;**37**:1613–1622.
114 López-Alonso M. Animal feed contamination by toxic metals. In: Fink-Gremmels J (ed.) *Animal Feed Contamination: Effects on Livestock and Food Safety*. Woodhead Publishing Series in Food Science, Technology and Nutrition No. 215. Woodhead Publishing, Cambridge, 2012, pp. 183–204.
115 Vos G, Hovens JPC, Delft WV. Arsenic, cadmium, lead and mercury in meat, livers and kidneys of cattle slaughtered in the Netherlands during 1980–1985. *Food Addit Contam* 1987;**4**:73–88.
116 Grandjean P, Weihe P, White RF *et al*. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicol Teratol* 1997;**19**:417–428.
117 Crump KS, Kjellström T, Shipp AM, Silvers A, Stewart A. Influence of prenatal mercury exposure upon scholastic and psychological test performance: benchmark analysis of a New Zealand cohort. *Risk Anal* 1998;**18**:701–713.
118 Davidson PW, Myers GJ, Cox C *et al*. Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles Child Development Study. *JAMA* 1998;**280**:701–707.
119 Chan HM, Egeland GM. Fish consumption, mercury exposure, and heart diseases. *Nutr Rev* 2004;**62**:68–72.
120 Stern AH. A review of the studies of the cardiovascular health effects of methylmercury with consideration of their suitability for risk assessment. *Environ Res* 2005;**98**:133–142.
121 Australia New Zealand Food Authority. Draft food standards code. Standard 1.4.1. Contaminants and restricted

substances. Available at http://www.comlaw.gov.au/Series/F2008B00618.

122 Food and Drug Administration. Mercury in fish: cause for concern? Available at http://www.fda.gov/ohrms/dockets/ac/02/briefing/3872_advisory%207.pdf.

123 Kaiser J. Toxicology: mercury report backs strict rules. *Science* 2000;**289**:371–372.

124 EFSA Panel on Contaminants in the Food Chain. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA J* 2012;**10**:2985. doi: 10.2903/j.efsa.2012.2985.

125 World Health Organization. Guidance for identifying populations at risk from mercury exposure. Issued by UNEP DTIE Chemicals Branch and WHO Department of Food Safety, Zoonoses and Foodborne Diseases, August 2008. Available at http://www.who.int/foodsafety/publications/chem/mercuryexposure.pdf.

CHAPTER 7

Geographical variation of land mineral composition

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Abstract: This chapter presents some basic but relevant information about the chemical composition of the earth's crust, emphasizing the classification of chemical elements in the crust, as well as their abundances. The main soil-forming factors and processes are described and their individual importance to soil formation is discussed, highlighting the contribution of humans as being of concern. Some considerations about soil geographical variations and mapping are also related due to the fact that soils can present high spatial variability on multiple scales, which are very relevant for agricultural purposes. Soil mapping activities and existing maps by several governmental institutions can help obtain a better understanding of agrosystems. Concepts such as bioavailability and speciation can also be found in this chapter, since they are very important in plant nutrition. Several methods for estimating bioavailability are reported including the traditional passive and sequential ones and modern passive sampler approaches, as well as their usefulness and drawbacks.

Keywords: crust chemical composition, total element abundances, soil-forming factors, soil geographic variation, soil mapping, bioavailability, extraction techniques, passive samplers

7.1 Chemical composition of the earth's crust

The earth is divided into three distinct units: the core, the mantle–crust and the atmosphere–hydrosphere system. The core, with a mass approximately one-third of the mass of the earth, is composed of an iron–nickel alloy. The mantle–crust, which contains two-thirds of the mass of the earth, is composed of primary silicates of magnesium, iron, aluminium and calcium. The atmosphere–hydrosphere system is dominated by oceans, a unique feature within the solar system [1].

Chemical elements in the earth can be classified according to their chemical behaviour, forming groups as such as lithophile, siderophile, chalcophile and atmophile. Lithophile elements are present in the silicate shell and are chemical elements that bind readily with oxygen. Elements that readily react with iron are called siderophile elements and are concentrated in the core. Chalcophile elements are able to bind with sulfur and are concentrated in the core but also appear in the mantle. The gaseous elements are concentrated in the atmosphere and are called atmophile elements [2].

The continental crust of the earth is composed of large continents and small islands. The total area of the earth's crust is approximately $8.1 \times 10^8\,km^2$ with a thickness ranging between 14 and 80 km (average 41 km), and covers 41% of the solid surface of the earth [3, 4]. The continental crust has a different and more complex chemical composition than the oceanic crust. As the continental crust consists of several types of rocks of different mineralogical and chemical compositions, accurately estimating the chemical composition is not easy. According to Wedepohl [5], the continental crust is composed mainly of 61.5% SiO_2, 15.1% Al_2O_3, 6.28% FeO (as total iron), 5.5% CaO, 3.7% MgO, 3.2% Na_2O, 2.4% K_2O and 0.68% TiO_2. This composition was estimated considering the average chemical composition of samples of mixed rocks obtained from several regions of Canada. Several other researchers conducting analogous

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studies with rock composite samples and sedimentary rocks have found similar results. In the chemical composition shown above, several trace and ultra-trace elements must be included. Some of these trace elements are of concern to the lives of animals and plants.

7.2 Natural abundance of chemical elements

The total element abundances of the earth have been estimated by several researchers and compiled by McDonough [1]. A number of famous geochemists such as Clarke and Washington (1924), Goldschmidt (1954), Poldervaart (1955) and Vinogradov (1962) made efforts to establish the abundance of chemical elements in the continental crust by analysing different rock samples collected from around the world [6]. Oxygen, magnesium, silicon and iron are the most abundant elements in the earth. The higher iron content is due to the presence of iron in the nucleus of the earth. The composition of the earth's crust is slightly different because the crust is composed mainly of silicates with other minerals present in minor amounts. Values for the abundances of several chemical elements in the continental crust of the earth can be found in classical geochemistry books [7, 8]. Major elements, called rock-forming elements, include oxygen, silicon, calcium, sodium and potassium as well as trace elements including micronutrients of concern in food science. The crust of the earth comprises only 0.29% of the earth's total mass.

7.3 Soil-forming factors and processes

Soil can be defined as the solid material present on the surface of the earth resulting from the interaction of chemical and physical weathering and biological activity on the parent and/or rock material. Soil appears as a natural phenomenon, and the factors and processes of soil formation and the characteristics and distribution of the different types of soil are studied in a science called pedology [9].

A soil system is normally described as a soil profile (variation of properties along the depth of the soil) characterized by distinct layers known as horizons. The horizons are normally designated by symbols and letters. In this way, soils are classified by the presence (or absence) of a particular horizon. In the case of agricultural soils, the presence of an organic (O horizon) is of concern because the O horizon results from the decomposition of organic materials such as plants and animals and acts as a nutrient source. The upper soil profile horizon (A horizon) is the most important for agriculture [9].

The main factors that are important for soil formation are parent material, climate, organisms, relief and time. The parent material is the material from which the soil has been developed and can vary from solid rock (mainly igneous rocks such as granite and basalt, as well as metamorphic and sedimentary rocks) to deposits like alluvium and boulder clay [10]. The parent material can influence the soil properties in several ways, but the mineral composition of the soil is the most important variable. Other variables are colour (depending on the amount of organic matter and the oxidation state of iron compounds), texture (distribution of different sizes of mineral particles), structure (particle morphology) and water permeability/drainage. Climate is a major factor that can produce different soils from the same parent material. Climate governs the rate and type of soil formation and also determines the distribution of vegetation. The main factors of concern are rain (precipitation influences leaching of soluble components from soils) and temperature (which is important in evaporation). Temperature also determines the rate of reaction (chemical reactions are influenced by temperature) and chemical and biological transformations, thus influencing weathering and humus formation. Organisms ranging from bacteria and fungi to macroscopic animals have important functions in soil formation. Microorganisms mainly support the decomposition of litter (mixture of dead plant materials) that can be mixed by macroorganisms, rendering the soil horizons less distinct when there is a lot of organic activity in the soil. In this context, humans have made an increasing contribution to soil formation. Although the time required for soil formation is very long, humans have increased deforestation and cultivated areas; used fertilizers and chemical products for insect, disease and weed control; constructed levees and dams for flood control; and improved drainage of the soil surface, which can affect chemical and physical transformations in soils [11, 12]. Vegetation ranging from small plants to large trees has an influence on humus quality and quantity. Relief can control soil formation according to slope (determinant in soil thickness) and gradient that

affect runoff and could lead to creation of microclimatic conditions. Time is also very important because soils develop very slowly. Young soils retain many of the original characteristics of the parent material, and over time these young soils will acquire many features due to the addition of organic matter and the activity of microorganisms. Over time, soil development will occur in deep profile and will be structured in well-defined horizons.

7.4 Soil geographic variation and mapping

Soils are characterized by the high spatial variation of properties on multiple scales, ranging from point measurements to global measurements [13]. According to Lin *et al*. [14], the quantification of soil spatial variability across multiple scales is significant in ecological modelling, environmental prediction, precision agriculture, and natural resource management. Using case studies of soils from the USA, these authors demonstrate that soil variability is a function of map scale, special location and specific soil properties. Even at scales of centimetres or less, considerable variability in soil measurements, such as fungal and bacterial biomass [15] and microbial communities, can be found [16].

Similarities in parent material, vegetation and climate would indicate that soil samples collected in close proximity are more similar in their soil properties and processes than soil samples separated by long distances [13]. These authors investigated how the variability of some soil properties (such as C and N concentration, particulate organic matter, and other parameters) increases from small (1 m) to large (1 km) scale within the same ecosystem (mixed hardwood forest in the USA). The results obtained show that increasing variability with increasing spatial scale was not valid for five of the eleven factors studied [13].

At present, the quality of soil resources is increasingly important for the sustainable productive capacity of an agrosystem, making soil a strategic developmental resource in many countries, especially countries where soil productivity is now declining as the demand for food grows [17].

Worldwide compatible soil databases (which include information harmonized in time and space) are crucial for establishing the current status of soils and guiding the shift of technologies from one area to another with similar characteristics and climate [18]. Institutions in a number of countries have made efforts to establish soil maps. The primary information source is a soil survey in which soil properties are collected and made available for land use decision-making purposes [19]. Recently, soil maps have been employed to provide chemical and physical data inputs within ecological and hydrological process models [20], which has been possible due to technological advances such as remote sensing and geographical information systems [19].

Predictive soil mapping, based on numerical and/or statistical models, relates environmental variables and soil properties coupled to geographic databases and is very important in establishing the relationship between these parameters to collect the best soil data, to produce and present the soil landscape, and to incorporate expert knowledge in model design [19]. The review paper published by Scull *et al*. [19] is an excellent source of information on predictive models and soil geographic variation.

However, the main problem with soil (also for vegetation, climate and other natural phenomena) is that soil varies spatially on any scale from millimeters to a higher scale. Every soil scientist understands clearly that the soil can change abruptly, but soil models are of concern, and the accuracy of these models is improving as modern technologies are developed. Such efforts to map soils have been made for several countries and economic blocs. As only one example, the Joint Research Centre from the European Commission maintains the European Soil Portal [21] as a part of the European Soil Data Centre, one of 10 environmental data centres in Europe. This Soil Portal presents data and information regarding soils at the European Community level. The Portal contains information such as soil datasets that include data and maps where some atlases dealing with European and African and Latin American (launched in 2013) soils, among others, can be found. A very interesting portion of the European Soil Portal is the 'Soil Threats Maps', where it is possible to navigate around the maps and obtain information about soil properties such as organic carbon content, soil erosion and soil pH. Many research reports and a list of recent publications of interest in soil science (with the access to the journal of publication) can also be found.

7.5 Bioavailability of chemical elements in soil

In soil science, bioavailability can be defined as the physical, chemical and biological interactions (processes) that determine the exposure of organisms (animals and plants) to chemicals associated with soils. Bioavailability is a concept that describes the ability of chemical species to interact with living organisms. In soils, bioavailability is a way that chemical substances can access the interior of a plant cell and subsequently take part in metabolic processes or exert a toxic effect within a particular tissue. The magnitude and nature of these effects will be determined by the chemical form and concentration of the compound at its active site [22]. At present, the determination of the composition of specific chemical species (speciation analysis) is of concern for establishing the speciation of an ecosystem to estimate the nature and toxicity of each chemical species (nutrients and toxicants) in soils [23].

In agriculture, the total soil concentration of a compound is not equivalent to its bioavailable or effective concentration. The concentration of plant nutrients is controlled by the soil matrix and also by fertilization. Physical and chemical processes in the aqueous phase are phytoavailable for only a small fraction of the total concentration. Considering the many factors that affect nutrient availability and the methods developed to predict the effective nutrient concentrations, these factors and methods can potentially be employed as a model for the development of suitable protocols to assess bioavailability processes for contaminants in soils. Although the majority of these protocols have been developed to predict phytoavailability of nutrients in potentially deficient conditions, a direct correlation with the bioavailable fraction of contaminants may be observed. Several soil characteristics such as heterogeneity, presence of colloids, superficial area of particles (as regulators of adsorption processes), differences in pH, differences in organic matter content, and pore spaces hamper the ability to ultimately predict the fate of nutrients in a soil system. Differences in uptake efficiencies across plant species also make the bioavailability estimation more difficult [22].

The key soil properties that play a role in determining chemical bioavailability are soil pH, cationic and anionic exchange capacity, texture (clay content), soil type and soil organic matter content [24]. To assess soil bioavailability, numerous assays have been developed, including chemical extraction techniques and tests employing microorganisms, invertebrates, plants and higher organisms. Chemical extractions are commonly used to estimate the fraction of the constituent in soil that may be taken up by plants, is mobile or impacts soil biota [24]. Bioavailability in soils may also be estimated by using toxicity tests (bioassays) to assess toxic responses through the inhibition of a metabolic function, by changes in microbial population structure, by mortality or malformations or by the accumulation of chemical species. Bioassays would normally be conducted to offer supplementary information and can be used to make decisions [24].

7.6 Extraction techniques for estimating bioavailability of nutrients and metals in soils

A suite of tools can be implemented to describe bioavailability processes in soils. However, no single tool has been developed that can universally describe or measure bioavailability, and approaches that have attempted this universal application have failed. Several complementary tools that characterize different bioavailability processes constitute a better choice for matching bioavailability. Risk managers should ideally consider processes that influence contaminant concentration, chemical form, or transformation such as biological processes that affect uptake and linkages between internal concentrations and possible adverse effects in receptors.

Different laboratory tests for a single bioavailability process may therefore yield different types of information, and these laboratory tests must be considered carefully in evaluating the relevance and meaning of the various types of data obtained. Additionally, tests that combine the effects of multiple processes may have limited relevance to understanding the mechanisms (if that is the primary objective), while tests that constrain certain variables for direct elucidation of mechanisms may have less relevance for assessment of the overall species toxicity [22].

The majority of extraction procedures were proposed with the goal of releasing toxic and nutrient species from the solid surface to the pore water. Thus, these extraction procedures are most successful (i.e. predictive) when biological uptake is dominated by a pore-water pathway (e.g. plant uptake of metals). Extraction

methods are operationally defined procedures for estimating bioavailability. Several drawbacks can arise from these methods, including the impossibility of identification of chemical forms of nutrients and contaminants [22].

The main extraction methods currently in use for estimating bioavailability in soils are described briefly in the following sections. Numerous varieties of extraction methods have been proposed for estimating the bioavailability of organic and inorganic species, involving chemical extraction for (non)-metal nutrients and/or contaminants and extraction using organic solvents or solid-phase sorbents for organic contaminants. These techniques attempt to provide site-specific measurement of the bioavailable fraction of a contaminant as opposed to the total extractable contaminant based on rigorous extraction procedures, and these extraction methods are more simple and reliable [22].

All of these extractions (single or multiple reagents, single or sequential extractions) depend on ion activity, pH, concentration of the reagents, soil/reagent ratio, order of application of the reagents, time, kinetics of extraction, stirring and temperature [22, 25].

7.6.1 Exchangeable cations

These extraction methods use neutral salt solutions to replace the cations present in the soil as an exchangeable complex, and cation concentrations determined by these procedures are therefore referred to as 'exchangeable'. The analysis of exchangeable cations retained by the negative charges of clays is important in evaluating the transfer rate of the ionic compounds through soil profiles (leaching) and allows the kinetics of exchange versus time and space to be identified. The displacement of the exchangeable cations depends on the nature of the cations used to move them. The reagent should move the exchangeable cations as selectively as possible, without being too aggressive and without dissolving products that are not involved in adsorption–desorption. In practice, a range of neutral salt extractions have been used, including $Ca(NO_3)_2$, $CaCl_2$, $Sr(NO_3)_2$, $NaNO_3$, NH_4NO_3, NH_4Cl and $MgCl_2$, but extraction using neutral normal ammonium acetate at pH 7.0 is still the most commonly used method. In soils able to fix potassium, NH_4^+ can partially replace fixed K^+, yielding results for exchangeable K^+ that are too high, although their ionic radii are almost the same [22, 25–27].

7.6.2 Extractions with Mehlich-I, Mehlich-II and Mehlich-III solutions

Mehlich-1 extraction or the double-acid extraction method (0.05 mol/L HCl + 0.0125 mol/L H_2SO_4) was developed primarily for determining labile P in acidic soils but is now also applied for extraction of K, Ca, Mg, Mn, Cu and Zn. Soils should be relatively low in organic matter content (<5%) and acid (pH <6.5). This extraction method is not suitable for alkaline soils. The great advantage is that the Mehlich-1 extraction is low cost and easy to use. The preferential extraction of calcium compounds is a disadvantage.

This extraction solution was changed by its author, and extraction solution Mehlich-II was created. Mehlich-II solution consists of a solution that contains 0.2 mol/L NH_4Cl + 0.2 mol/L CH_3COOH + 0.015 mol/L HF + 0.012 mol/L HCl, with a pH of approximately 2.5, but the excessive corrosion of laboratory apparatus caused another modification in solution composition, giving rise to the Mehlich-III solution. This new extraction solution is composed of CH_3COOH (0.02 mol/L) + NH_4NO_3 (0.25 mol/L) + NH_4F (0.015 mol/L) + HNO_3 (0.013 mol/L) + EDTA (0.001 mol/L) and was proposed in order to provide the capability of determining several nutrients in the same extraction. The presence of ammonium fluoride and EDTA provides a better estimative of phosphorus concentration in neutral and basic soils and allows Cu, Mn and Zn extraction, and this solution can be employed to extract P, Na, K, Ca, Mg, Mn, Cu and Zn [22, 25, 28–33].

7.6.3 Oxalic acid/ammonium oxalate (under darkness)

This extraction solution is also called Tamm reagent and uses oxalic acid/ammonium oxalate buffer at a suggested pH of 3.0 with stirring for 4 hours in darkness. The method allows the dissolution of allophane, gels, iron, aluminium organic complexes, aluminium and iron hydrated oxides (ferrihydrite, feroxyhyte), providing an estimate of total non-crystalline iron. If several treatments (two to three) are performed, some Al and Fe compounds can be solubilized to a considerable extent [25, 27].

7.6.4 Dithionite–citrate–bicarbonate (DCB) method

This method is based on a reduction process, especially because of the presence of dithionite below pH 9–10. Biologically reducible elements such as iron

or manganese are reduced and maintained in solution by complexation with citric acid in the system buffered at pH 7.3 with sodium bicarbonate.

This method makes the solubilization of pedogenic oxides and hydroxides possible: crystalline iron oxides (hematite, goethite), non-crystalline iron oxides and iron and aluminium organic complexes as well as exchangeable iron and manganese oxides, and some non-crystalline compounds with a ratio of SiO_2 to Al_2O_3 of less than 0.5.

The optimum pH for reduction is between 7 and 8, and as the dithionite solution rapidly loses its reduction properties, complexation avoids reoxidation as well as the precipitation of iron sulfide, allowing solubilization of the elements. The temperature should be set at 75 °C to accelerate the reactions, to limit the appearance of colloidal sulfur and iron sulfide, and to minimize dithionite decomposition. This temperature should not be exceeded, and localized overheating should be avoided by using a water bath [25].

7.6.5 EDTA and DTPA methods

EDTA (ethylenediaminetetraacetic acid) is an aminopolycarboxylic acid with six coordination bonds suitable for the formation of chelates. In alkaline solution (pH 10), 1 : 1 complexes are formed with pedogenic elements. An excess of complexing reagent is needed for satisfactory control of the dissolution rate. In this extraction, the forms of iron extractable with EDTA appear to be linked with the most active forms in pedogenesis, which is the 'free' least crystalline compounds with extensive surface contact and thus with great reactivity [22, 25].

In agricultural studies, the availability of metals for plants (for establishing nutritional factors or possible chlorosis) is often checked using other complexing reagents such as diethylenetriaminepentaacetic acid (DTPA) or ethylenediamine di(*o*-hydroxyphenylacetic acid) (EDDHA) [22, 25].

DTPA soil extraction could potentially predict micronutrient deficiencies in both neutral and calcareous soils according to Lindsay and Norvell [34]. When correctly employed, DTPA soil extraction is effective, reproducible and accurate. For calcareous soils, micronutrients such as Zn, Fe, Mn and Cu are usually scarcely soluble, and nutrient deficiencies are very common. As a consequence, plants may manipulate the rhizosphere to access the required (insoluble) micronutrients. As an example, grass plants produce phytosiderophores in the soil solution, and these compounds have the ability to chelate metals, reducing the concentration of free metal ions. To overcome this equilibrium change, a portion of the labile cations in the solid phase can solubilize and be available for complexation and absorption by plants [35]. The DTPA extraction was developed to mimic the behaviour of plant roots under these circumstances. Since its inception, the DTPA extraction has become one of the most widely used tests to predict micronutrient availability in soil systems [36].

7.6.6 Pyrophosphate extraction

This method is used to analyse the forms complexed with organic matter (especially iron) and to differentiate the spodic and podzolic horizons where displacement of these complexes can be observed. A good correlation can generally be obtained between extracted Al and Fe and organic carbon. Pyrophosphate has a peptizing action and is effective only if the soil is under the influence of organic matter. The time factor of 16 hours is regarded as critical, particularly when the results of this method are compared with the results obtained by EDTA extraction after a contact time of 90 days [25, 27].

7.6.7 Exchange resins

Exchange resins have been widely employed to quantify free ions, soluble fractions and labile concentrations of metals in soils. These different fractions can be assessed by varying the resin, the ratio between resin and soil solution, and the equilibration time. The common procedure involves circulating soil solution or soil solution extracted with water or with a dilute salt through an acceptor solution that contains a resin impregnated with a specific cation. The amount of metal exchangeable onto the resin is then operationally defined as corresponding to a portion of the total present in the soil solution [22].

7.6.8 Sequential extractions

Passive extracts measure only the instantaneous bioavailable fraction of the nutrient/contaminant and offer no information on the solid-bound nutrient/contaminant. Sequential extractions have therefore been developed to quantify the distribution of metals in different solid phases. Each successive treatment is more drastic in chemical reaction or of a different concentration and nature than the previous treatment, so each solid phase

is defined as having different potential bioavailability [22, 25]. Metals in soil solution or exchangeable metals are considered the most labile and bioavailable. Organically bound metals and inorganically bound metals are progressively less labile. The residual fraction extracted using strong acid is seen as unavailable [22]. In general, the procedures using sequential extraction function in the following manner: (i) exchangeable fraction (containing metals weakly adsorbed or exchangeable in soil surface interactions); (ii) acid-extracted fraction (containing metals that are extracted at a pH of approximately 5.0; (iii) manganese and iron oxide-associated fraction (solubilized metals that are unstable under reduction conditions); (iv) oxidized fraction (metal fraction that is free because of organic matter and sulfide oxidation; and (v) residual fraction (including metal fraction that is associated with mineral crystalline structures, not bioavailable to plants) [22, 37–39].

Although sequential extractions can reveal changes in how metals are bound to the solid phases, these procedures may be more suitable for qualitative than quantitative determination of metal partitioning. As soils consist of heterogeneous layered aggregates, even with a specific extractant for a specific mineral phase, some phases may be leached progressively instead of completely by their selective extractant, but conditions used during the extraction procedures are difficult to extrapolate to naturally occurring processes. Despite these concerns, efforts to standardize sequential extractions are occurring, and although changes in the operationally defined fractions of metals in soil may provide some indication of changes in bioavailability, at present sequential extractions are best used in comparative experiments [22].

Two sequential extraction procedures are currently employed: the Tessier and the BCR methods. The Tessier method proposed in 1979 is based on five steps: (i) exchangeable fraction (1 mol/L $MgCl_2$ at pH 7 or 1 mol/L NaAc at pH 8.2); (ii) carbonate fraction (1 mol/L NaAc at pH 5.0, adjusted by HAc); (iii) oxides and hydroxides of iron and manganese fraction (0.04 mol/L $NH_2OH \cdot HCl$ in 25% (v/v) HAc at 96 °C); (iv) organic matter and sulfide fraction [0.02 mol/L HNO_3 + 30% (v/v) H_2O_2 (pH 2 with HNO_3) at 85 °C, cooling and NH_4Ac in 20% (v/v) HNO_3]; and (v) residual fraction (digestion using HNO_3, HCl and HF) [37–40]. The BCR method proposed by the Standards, Measurements and Testing Programme (formerly BCR) to harmonize procedures for the sequential extraction among the members of the European Community is based on four steps: (i) exchangeable/acid-extracted fraction (0.11 mol/L HAc at 22 ± 5 °C for 16 hours, under mechanical stirring); (ii) reductive fraction (0.5 mol/L $NH_2OH \cdot HCl$ in HNO_3 at 22 ± 5 °C for 16 hours under mechanical stirring); (iii) oxidizing fraction [8.8 mol/L H_2O_2 (pH 2 with HNO_3) at 22 ± 5 °C for 1 hour + 1 hour (twice), and 1 mol/L NH_4Ac at 22 ± 5 °C for 16 hours under mechanical stirring]; and (iv) residual fraction (digestion by using aqua regia) [39–44].

7.6.9 Passive samplers

Passive samples can also be used for bioavailability measurements in soils. These samplers have the advantage of providing integrative temporal measurements for single species or species groups (fractionation of species), which is of concern for nutritional and environmental purposes [22]. Diffusive gradients in thin films (DGT) were first described by Davison and Zhang in 1994 [45] and have since been used for evaluating the bioavailability of metal species in aquatic ecosystems and are now employed for soil systems. Briefly, DGT devices are introduced into the soil (the soil must be wetted to provide chemical species diffusion from soil to the gel binding agent), and the mobile species can accumulate in a thin hydrogel containing a chelating resin (or other adequate sorbent). After a deployment time, the species retained by the resin are eluted, and the concentration of these species is measured by a proper analytical technique. Based on Fick's laws, the bulk concentration in soils can easily be determined [45].

To date, there are a number of papers in the literature dealing with DGT in a soil environment. In a paper published by Glaesner *et al* [46]., the physical properties, chemical reactivity and potential bioavailability of mobilized phosphorus in soil columns during leaching events, with and without manure application, were studied, and manure application was observed to have an influence on P leachability. Plant-essential elements such as manganese can be determined in soils using the DGT technique. In this case, DGT devices were deployed in hydrated soils for times ranging between 12 hours and 6 days, and the concentration of manganese was observed to be dependent on redox conditions. The concentration of manganese could be estimated although the availability process of manganese is not fully understood [47]. A DGT system was also used to evaluate

cadmium concentrations in soils from rice field cultivation in China. The DGT assay was observed to be the best predictor of grain cadmium concentration from soil measurements [48].

Recent developments in DGT-based techniques employing mixed binding gel layers have been proposed by Huynh *et al*. [49] to measure arsenic and trace metals (Cd, Cu, Pb and Zn) simultaneously in soils from an impacted area. In this case, a double layer containing Chelex-100 (chelating resin) and ferrihydrite (iron oxide) was developed and used to study possible competitive retention behaviour among the analytes and concomitant soil components.

A model for dynamic uptake of micronutrients by the rhizosphere environment using DGT devices was proposed by Lehto *et al*. [50]. Application of the model in soils was discussed in mathematical simulations of real situations. As a model element, zinc in hyperaccumulator and non-accumulator plants was studied because the DGT technique can behave as a reliable surrogate for transport and release of metal species that affect plant uptake in soils. Metal distribution (Mn, Cd, Cu, Co and Ni) in the soil profile can also be investigated by deployment of DGT devices. The results from a depth of 15 cm show localized reductive dissolution of manganese oxides in soils treated with sewage sludge [51].

A very interesting way to study processes in soil is by mapping the concentration differences by time and depth. Santner *et al*. [52] proposed ferrihydrite as a binding agent to monitor labile phosphate in soils. The authors show the possibility of chemically mapping phosphorus due to microbial activity in soils. To accomplish this chemical mapping, laser ablation was used to scan the thin gel-binding surface. High-resolution mapping was performed for labile phosphorus using a Zr-oxide binding gel with conventional detection by the molybdenum blue method. The authors demonstrated that the coloration technique can be used for submillimetre scale imaging that can allow observation of microenvironments in soils, aiding studies of mobility processes [53].

7.6.10 Biological assays

The use of biological indicators together with chemical methods to evaluate bioavailability is currently very important. Plant bioassays can be employed for a range of organic and inorganic substances. For this task, two strategies can be used. Plant tissue can be analysed to determine the concentrations of contaminants, and the results of this analysis can determine whether the contaminants are toxic or non-toxic. Otherwise, the growth and vigour of the plant when cultivated in a medium containing the contaminant can be measured. These tests have been used throughout agriculture for decades [22]. For metals, the utility of plant bioassays depends on the particular metal, its chemical form and its route of uptake. Different plants pick up the same element in different ways, and variations in metal concentration in soils may not reflect tissue concentration of the metal. For some elements, the concentration in plants may not vary significantly and could be independent of soil concentration [22]. No single metal concentration is associated with growth suppression and phytotoxicity across all plant species. Plant growth suppression and phytotoxicity depend on the speciation of the metal.

Invertebrate bioassays comprise a selection of tests used to estimate the uptake of contaminants and nutrients (such as metals) and their toxicity to soil invertebrates. These assays can be better performed in the field. Information about tissue accumulation, growth and reproduction and mortality can be gathered from these test results. Several invertebrate species can be employed including protozoa, nematodes, earthworms, crustaceans and insects [22].

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Abbreviations

BCR: Bureau Communautaire de Références (succeeded by Standards, Measurements and Testing Programme) from European Union
DCB: dithionite–citrate–bicarbonate
DGT: diffusive gradients in thin films
DTPA: diethylenetriaminepentaacetic acid
EDDHA: ethylenediamine di(*o*-hydroxyphenylacetic acid)
EDTA: ethylenediaminetetraacetic acid

References

1 McDonough WF. The composition of the earth. In: Tersseyre R, Majewsky E (eds) *Earthquake Thermodynamics and Phase Transformations in the Earth's Interior*. Academic Press, San Diego, CA, 2001.

2 Larimer JW. The cosmochemical classification of the elements. In: Kerridge JF, Matthews MS (eds) *Meteorites and the Early Solar System*. University of Arizona Press, AZ, 1988.

3 Cogley JG. Continental margins and the extent and number of the continents. *Rev Geophys Space* 1984;**22**:101–122.

4 Christensen NI, Mooney WD. Seismic velocity structure and composition on the continental crust: a global view. *J Geophys Res* 1995;**100**(B7):9761–9788.

5 Wedepohl KI. The composition of the continental crust. *Geochim Cosmochim Acta* 1995;**59**:1217–1232.

6 Taylor SR. Abundance of chemical elements in the continental crust: a new table. *Geochim Cosmochim Acta* 1964;**28**:1273–1285.

7 Krauskopf KB, Bird DK. *Introduction to Geochemistry*. McGraw Hill, New York, 1995.

8 Wedepohl KH. *Geochemistry*. Holt, Rinehart and Winston, New York, 1971.

9 Birkeland PW. *Pedology, Weathering, and Geomorphological Research*. Oxford University Press, London, 1974.

10 Jenny H. *Factors of Soil Formation: A System of Quantitative Pedology*. McGraw-Hill, New York, 1941.

11 Dudal R, Nachtergaele FO, Purnell MF. The human factor of soil formation. In: *Transactions 17th WCSS, 14–21 August 2002, Bangkok, Thailand*. Symposium 18, Vol. II, Paper 93.

12 Dudal R. The sixth factor of soil formation. *Eurasian Soil Sci* 2005;**38**:S60–S65.

13 Garten CT Jr, Kanga S, Brice DJ, Schadt CW, Zhou J. Variability in soil properties at different spatial scales (1 m – 1 km) in a deciduous forest ecosystem. *Soil Biol Biochem* 2007;**39**:2621–2627.

14 Lin H, Wheeler D, Bell J, Wilding L. Assessment of soil spatial variability at multiple scales. *Ecol Model* 2005;**182**:271–290.

15 Morris SJ. Spatial distribution of fungal and bacterial biomass in southern Ohio hardwood forest soils: fine scale variability and microscale pattern. *Soil Biol Biochem* 1999;**31**: 1375–1386.

16 Grundmann GL, Debouzie D. Geostatistical analysis of the distribution of NH4+ and NO2-oxidizing bacteria and serotypes at the millimeter scale along soil transect. *FEMS Microbiol Ecol* 2000;**34**:57–62.

17 Batjes NH. Soil parameter estimates for the soils types of the world for use in global and regional modeling. ISRIC Report 2002/02c. Available at http://www.isric.eu/isric/webdocs/docs/ISRIC_Report_2002_02c.pdf (accessed 20 July 2013).

18 Baumgardner MF. Soil databases. In: Sumner ME (ed.) *Handbook of Soil Science*. CRC Press, Boca Raton, FL, 1999.

19 Scull P, Franklin J, Chadwick OA, McArthur D. Predictive soil mapping: a review. *Prog Phys Geogr* 2003;**27**:171–197.

20 Burrough PA, McDonnell RA. *Principles of Geographic Information Systems*. Clarendon Press, Oxford, 1998.

21 European Soil Portal. Available at http://eusoils.jrc.ec.europa.eu/ (accessed 7 July 2013).

22 Committee on Bioavailability of Contaminants in Soils and Sediments, National Research Council. *Bioavailability of Contaminants in Soils and Sediments: Processes, Tools, and Applications*. National Academies Press, Washington, DC, 2003.

23 Cornelis R, Caruso J, Crews H, Heumann K. *Handbook of Chemical Speciation: Techniques and Methodology*. John Wiley & Sons, Chichester, 2003.

24 Naidu R. *Chemical Bioavailability in Terrestrial Environments*. Elsevier, Amsterdam, 2008.

25 Pansu M, Gautheyrou J. *Handbook of Soil Analysis: Mineralogical, Organic and Inorganic Methods*. Springer-Verlag, Berlin, 2006.

26 Voguel A. *Vogel's Qualitative Inorganic Analysis*, 7th edn. Longman, Essex, 1996.

27 Otero XL Álvarez E, Fernández-Sanjurjo MJ, Macías F. Micronutrients and toxic trace metals in the bulk and rhizospheric soil of the spontaneous vegetation at an abandoned copper mine in Galicia (NW Spain). *J Geochem Explor* 2012;**112**:84–92.

28 Bortolon L. *Métodos de avaliação da disponibilidade de nutrientes para as plantas em solos do Rio Grande do Sul*. Masters thesis, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil, 2005.

29 Mehlich A. Determination of P, Ca, Mg, K, Na, and NH4 by North Carolina soil testing laboratories. North Carolina Soil Test Division Mimeo, North Carolina Department of Agriculture, Raleigh, 1953.

30 Mehlich A. New extractant for soil test evaluation of phosphorus, potassium, magnesium, calcium, sodium, manganese and zinc. *Commun Soil Sci Plant Anal* 1978;**9**:477–492.

31 Mehlich A. Mehlich 3 soil test extractant: a modification of Mehlich 2 extractant. *Commun Soil Sci Plant Anal* 1984;**15**: 1409–1416.

32 North Carolina State University. Methods of phosphorus analysis for soils, sediments, residuals, and waters. Southern Cooperative Series Bulletin No. 396, June 2000. Available at http://www.sera17.ext.vt.edu/Documents/Methods_of_P_Analysis_2000.pdf (accessed 30 August 2013).

33 Hardy DH, Tucker MR, Stokes CE. Crop fertilization based on North Carolina soil tests. North Carolina Department of Agriculture and Consumer Services, Agronomic Division. Available at www.ncagr.gov/agronomi/pdffiles/obook.pdf (accessed 1 October 2013).

34 Lindsay WL, Norvell WA. Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Sci Soc Am J* 1978;**42**:421–428.

35 Marschner H. *Mineral Nutrition of Higher Plants*, 2nd edn. Academic Press, New York, 1995.

36 Loeppert RH, Inskeep WP. Iron. In: Sparks D (ed.) *Methods of Soil Analysis. Part 3: Chemical Methods*. Soil Science Society of America Book Series 5. American Society of Agronomy and the Soil Science Society of America, Madison, WI, 1996.

37 Tessier A, Campbell PGC, Bisson M. Sequential extraction procedure for the speciation of particulate trace metals. *Anal Chem* 1979;**51**:844–851.

38 Gleyzes C, Tellier S, Astruc M. Fractionation studies of trace elements in contaminated soils and sediments: a review of sequential extraction procedures. *Trends Anal Chem* 2002;**21**: 451–467.

39 Marin B, Valladon M, Polve M, Monaco A. Reproducibility testing of a sequential extraction scheme for the determination of trace metal speciation in a marine reference sediment by inductively coupled plasma-mass spectrometry. *Anal Chim Acta* 1997;**342**:91–112.

40 Abollino O, Malandrino M, Giacomino A, Mentast E. The role of chemometrics in single and sequential extraction assays: a review. Part I. Extraction procedures, uni- and bivariate techniques and multivariate variable reduction techniques for pattern recognition. *Anal Chim Acta* 2011;**688**:104–121.

41 Rauret GJ. Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. *J Environ Monit* 1999;**1**:57–61.

42 Rauret G, López-Sánchez JF, Lück D, Yli-Halla M, Muntau H, Quevauviller Ph. The certification of the extractable contents (mass fractions) of Cd, Cr, Cu, Ni, Pb and Zn in freshwater sediment following sequential extraction: BCR-701. BCR Information Reference Materials Report EUR 19775EN. European Communities, Belgium, 2001.

43 Quevauviller P. Operationally-defined extraction procedures for soil and sediment analysis. Part 3: new CRMs for trace-element extractable contents. *Trends Anal Chem* 2002;**21**:774–785.

44 Sutherland RA. BCR®-701: a review of 10 years of sequential extraction analyses. *Anal Chim Acta* 2010;**680**:10–20.

45 Davison W, Zhang H. In situ speciation of trace components in natural waters using thin-film gel. *Nature* 1994;**367**:546–548.

46 Glaesner N, Donner E, Magid J, Rubæk GH, Zhang H, Lombi E. Characterization of leached phosphorus from soil, manure, and manure-amended soil by physical and chemical fractionation and diffusive gradients in thin films (DGT). *Environ Sci Technol* 2012;**46**:10564–10571.

47 Mundus S, Tandy S, Cheng H *et al*. Applicability of diffusive gradients in thin films for measuring Mn in soils and freshwater sediments. *Anal Chem* 2011;**83**:8984–8991.

48 Williams PN, Zhang H, Davison W *et al*. Evaluation of in situ DGT measurements for predicting the concentration of Cd in Chinese field-cultivated rice: impact of soil Cd:Zn ratios. *Environ Sci Technol* 2012;**46**:8009–8016.

49 Huynh T, Zhang H, Noller H. Evaluation and application of the diffusive gradients in thin films technique using a mixed-binding gel layer for measuring inorganic arsenic and metals in mining impacted water and soil. *Anal Chem* 2012;**84**:9988–9995.

50 Lehto NJ, Davison W, Zhang H, Tych W. Analysis of micronutrient behaviour in the rhizosphere using a DGT parameterised dynamic plant uptake model. *Plant Soil* 2006;**282**:227–238.

51 Luo J, Zhang H, Davison W *et al*. Localised mobilisation of metals, as measured by diffusive gradients in thin-films, in soil historically treated with sewage sludge. *Chemosphere* 2013;**90**:464–470.

52 Santner J, Prohaska T, Luo J, Zhang H. Ferrihydrite containing gel for chemical imaging of labile phosphate species in sediments and soils using diffusive gradients in thin films. *Anal Chem* 2010;**82**:7668–7674.

53 Ding S, Wang Y, Xu D, Zhu C, Zhang C. Gel-based coloration technique for the submillimeter-scale imaging of labile phosphorus in sediments and soils with diffusive gradients in thin films. *Environ Sci Technol* 2013;**47**: 7821–7829.

CHAPTER 8

Variation of food mineral content during industrial and culinary processing

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Abstract: Food is usually cooked in order to improve the palatability of the product. Different cooking processes such as steaming, boiling, frying, stewing, roasting, baking or grilling can be used. Minerals can be lost during cooking procedures due to their solubility in water or due to heating. Food processing and cooking procedures can also vary food composition (vitamins, proteins, fatty acids and anti-nutrients) causing changes in mineral species and their bioavailability. The influence of industrial processes (including food fortification) and cooking procedures on the composition of minerals in food as well as mineral speciation and bioavailability is summarized in this chapter.

Keywords: food, mineral, cooking, industrial process, bioavailability, fortification and speciation

8.1 Introduction

Food is the primary source of minerals for humans. Ca, Cl, Mg, Na, K and P are considered major essential elements; Co, Cu, Cr, F, Fe, Mn, Mo, Ni, Se, I and Zn minor essential elements, while Li, B, Si, V, As, Sn and Pb are classified as possible essential elements in very small quantities [1–3]. The content of these minerals in food depends on many factors, such as type of food (animal or vegetable), genetic origin, agricultural procedures and geographical location. Other important factors in food mineral composition are industrial processing and cooking procedures. Food is usually cooked in order to improve the palatability of the product. Different cooking processes such as frying, boiling, baking or grilling can be used. In some cases, additives are added during food processing, thus increasing the mineral concentration. In other cases, minerals are lost during cooking procedures due to their solubility in water or due to heating. Moreover, food processing and cooking procedures can also vary food composition (vitamins, proteins or fatty acids) causing changes in mineral species and in their bioavailability. The influence of industrial processes and cooking procedures on the composition of minerals in food as well as mineral speciation and bioavailability will be summarized in this chapter.

8.2 Effects of refrigeration on mineral composition

Refrigeration and freezing are common practices in food storage to increase food lifetime. The effects of these processes on the mineral composition of different types of food products have been studied by some authors. Mohamed *et al*. [4] studied the effect of the refrigeration process on Ca, P and Fe concentration during storage of two millet cultivars. The samples were stored for 30 and 60 days at room temperature (25°C) and at 4°C, and the authors concluded that the refrigeration process had no effect on the contents of the elements studied.

The effect of freezing on the arsenic concentration in an algal sample (*Cystoseira mediterranea*) was tested by Pell *et al*. [5]. The total arsenic concentration in samples stored at temperatures of –18°C and –80°C for different periods of time, as well as at room temperature, was

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determined. The authors found that the level of arsenic in all frozen samples was approximately 60% lower than that in the non-frozen samples. Moreover, the arsenic species present in frozen and non-frozen samples were also studied and no changes in arsenic species occurred during the freezing process. It was suggested that the losses of total arsenic are related directly to the freezing/defrosting process; in this process arsenic compounds were probably removed when broken cells were rinsed away after defrosting.

8.3 Effects of cooking procedures on the mineral content of different types of foods

Different cooking processes such as steaming, boiling, frying, stewing, roasting, baking or grilling can be used before food consumption. The cooking process applied depends on the type of food. During heating, considerable changes in the composition (vitamins, fatty acids, minerals) or transformations can occur. Several studies have been performed to evaluate the changes in mineral composition during cooking procedures of foodstuffs such as vegetables, fish and meat. In general, cooking procedures produce mineral losses, which are particularly high in vegetables [6]. Mineral losses during cooking were reported by Kimura *et al*. [6] for spinach and pork after different cooking processes (boiling, steaming, frying and stewing); losses of 30–94% and 1–76% were obtained for spinach and pork, respectively. The highest losses in spinach were obtained for Fe and Mg followed by K, Na, Zn and P. The highest losses in pork were obtained for Zn, followed by Na, K, Ca, Fe, Mg and P. In general, boiling produced the highest losses of minerals, followed by heating with steam, stewing and frying. Recently, Gonçalves *et al*. [7] studied the effect of cooking (roasting and boiling) on mineral profiles of sweet chestnuts. Cooking significantly affected the mineral composition of the chestnuts, except for P, Fe, Cu and Mn contents, and boiling led to a severe leaching of K, Ca, Mg and B (mineral loss percentage 12–14%).

Minerals (Fe, Ca and Mg) leached from the chickpeas into boiling water during cooking was studied by Avola *et al*. [8]. The leached percentages depended on cultivar. Cooking treatment reduced the levels of calcium in by 55–80%, of Mg by 68–78%, and Fe by 23–31%.

Seaweeds bioaccumulate essential elements such as Co, Cr, Mo, Ni, Se, V, Mg, Ca, Fe and I, among others, at higher rates than land vegetation [9]. However, as a result of environmental pollution, seaweeds can also concentrate non-essential elements such as As, Pb or Cd [10, 11]. Several studies have been performed to quantify the concentration of these elements in different types of seaweed samples [12–17]. In general, the results obtained in these studies indicate that the element concentration in seaweeds depends on the seaweed family and show a mineral content much higher than those reported for edible land plants. Large differences could be observed in elemental composition among the samples, especially for some elements such as As and I, which were present in high concentrations in brown seaweed, mainly in Kombu samples. These studies were performed in raw seaweed; however, seaweeds are subjected to different cooking procedures before consumption. Ichikawa *et al*. [18] studied the effect of cooking procedures on the arsenic concentration in edible brown algae *Hijikia fusiforme*. This seaweed was selected because it has a high content of inorganic arsenic (~50%). The seaweed sample was soaked and then boiled in pure water for 20 min at 90°C. Total arsenic was then determined in soaking water, cooking water and in the cooked sample. It was observed that 28–58% of total arsenic leached into the soaking water, and 49–60% of total arsenic was leached during cooking. Therefore, the authors concluded that 88.7–91.5% of total arsenic can be removed by cooking. García-Sartal *et al*. [19] studied the effects of cooking procedures on the concentration of As, Co, Cr, Cu, Ni, Se and Zn from edible seaweed. Four edible seaweeds (Kombu, Wakame, sea lettuce and Nori) were cooked following the manufacturer's recommendations (boiling in water), and element concentrations were calculated in the raw and cooked samples. The authors concluded that the metals were released into the cooking water during the heating treatment, with the exception of Ni and Zn from Kombu, Cr, Fe and Zn from Wakame, and Zn from Nori, for which almost 100% was retained. On the other hand, the retention percentages for Ni and Cu in Wakame and As in Nori were lower than 25%.

The effect of cooking procedures on arsenic concentrations was also studied in rice samples. Sun *et al*. [20] determined the arsenic concentration in two different types of rice samples (A and B). The samples were washed three times with Millipore water and then cooked in a

Table 8.1 Mineral losses obtained after different cooking processes.

Food	Minerals	Losses (%)	Reference
Spinach (boiled)	Na, K, Ca, Mg, Zn	56.9, 72.4, 60.0, 87.1, 29.4, 47.8	[6]
Spinach (steam)	Na, K, Ca, Mg, Zn	60.6, 60.4, 48.0, 78.3, 93.5, 30.4	[6]
Chickpea (boiled)	Fe, Ca, Mg	23–31, 55–80, 68–82	[8]
Chestnuts (boiled)	K, Ca, Mg, B	13, 12, 12, 12	[7]
Sea bream (*Sparus aurata*) (steaming, boiling, oven cooking, grilling, frying)	Ca, Mg, K, Na, Fe, Zn, Cu, Mn	3–65, 6–30, 3–36, 43–72, 19–29, 15–41, 10–37, 50	[21]
Rainbow trout (*Oncorhynchus mykiss*) (boiled)	Na, K, Ca, Mg, P, Zn, Mn, Cu	26, 21, 4, 41, 27, 67, 47, 76	[22]
Colocasia esculenta (L) Schott	P, Ca, Mg, K, Zn	13–32, 14–42, 0–8, 31–67, 5–62	[25]
Rice (boiled)	P, K	51, 27	[26]
Rice (boiled)	Ca, Mg, Fe, P, Na, K	5.6–39.13, 5.6–17.94, 26.40–71.21, 4.44–12.0, 52.0–73.52, 4.47–20.90	[27]
Beans, chickpeas, lentils (traditional and microwave cooking)	Ca, Fe, Zn	9.7–36.4, 14.2–31, 11.1–28.9	[28]
Macaroni	K, Na	68–61	[29]
White bean (*Phaseolus vulgaris*) (soaking and cooked)	Na, K, Ca, Mg, P, Fe, Mn, Cu, Co, Zn	28–56, 32–39, 42–57, 22–33, 21–33, 29–41, 30–54, 27–66, 31–37, 13–24	[30]
Seaweed	As, Co, Cr, Cu, Ni, Se, Zn	49–79, 5–41, 12–55, 17–82, 0–76, 18–68, 0–36	[19]
Pork (boiled)	Na, K, P, Ca, Mg, Fe, Zn	48, 57, 58, 51, 48, 22, 60	[6]
Pork (stewing)	Na, K, P, Ca, Mg, Fe, Zn	58, 61, 32, 54, 66, 34, 77	[6]
Pork (fry)	Na, K, P, Ca, Mg, Fe, Zn	76, 55, 1, 53, 44, 61, 84	[6]
Pork (steam)	Na, K, P, Ca, Mg, Fe, Zn	13, 67, 4, 67, 52, 34, 72	[6]
Horse, lamb, ostrich	Fe (horse and Lamb), Zn (ostrich), Cu (ostrich),	26–20 (Fe), 13–24 (Zn), 21–29 (Cu)	[23]

beaker for 30 min with a rice to water ratio of 1 : 3. Arsenic was then determined in raw rice (0.490 and 0.338 mg/kg in samples A and B, respectively) and cooked rice (0.389 and 0.314 mg/k[1] in cooked samples A and B, respectively). Therefore, 20.6% and 7.1% of arsenic in samples A and B, respectively, was removed during the washing and cooking procedure. The authors concluded that the arsenic is desorbed only during the washing procedure because rice is cooked until no water is left to discard.

The effect of different cooking methods on the mineral content in fish samples was also studied. Amira Mnari *et al*. [21] evaluated the effects of different cooking methods (steaming, boiling, grilling, and frying using different kinds of oils) on the mineral contents of wild and farmed sea bream (*Sparus aurata*). These authors found that frying sea bream in olive, soybean, corn and sunflower oils produced a significant decrease in the mineral content. This may be due to the leaching of minerals from the fish into the cooking medium during the heating treatment. Boiling in water involves an exchange of minerals between the fish and cooking medium which caused significant changes in the mineral content.

Gokoglu *et al*. [22] studied the effects of cooking methods (frying, boiling, baking, grilling and microwaving) on the mineral contents of rainbow trout. The authors observed that boiling caused losses in mineral content, with high reductions in Na, K, P, Mn, Zn and Mg (Table 8.1). Baked samples showed insignificant reductions with the exception of decreases in Mg (34%), P (20%), Zn (60%) and Mn (59%). Na and K contents of microwave-cooked fish increased (33 and 43%, respectively), while Zn and Mn contents decreased (61 and 13%, respectively). Meanwhile, Mg, Zn and Mn contents of grilled fish decreased (21, 64 and 47%, respectively), and K content increased (24%) using this cooking procedure. Taking into account the results

obtained, the authors concluded that baking and grilling are the best cooking methods for rainbow trout as far as healthy eating is concerned.

Regarding meats, the influence of cooking processes on the retention of micronutrients in some meat cuts, such as beef (sirloin, fillet, roast beef, topside, thick flank), veal (fillet), lamb (chop), horse (fillet), ostrich (fillet, sirloin, leg), pork (saddle, loin, chump chop), chicken (breast, leg-lower part, leg-thigh, wing), turkey (breast, leg-lower part, leg-thigh) and rabbit (whole) was evaluated by Lombardi-Boccia *et al*. [23]. The meat cuts were cooked using different methods that avoided the use of any other ingredients. Poultry legs and wings were oven cooked at 180°C for 50 min; breast and the meat cuts were hand-trimmed of all visible fat and cooked in an iron-free pan at medium heat until the red colour disappeared. Beef fillet and roast beef were underdone. The results obtained indicated that there is great variability in micronutrient content in meats among those studied. Cooked meats showed higher trace element (Fe, Zn and Cu) concentrations compared with the raw samples. This can be due to moisture losses occurring during cooking and the fact that the cooking methods used in this study did not require water, which probably allowed for a higher retention of the trace elements. Iron losses were detected in horse, lamb and in two beef cuts (topside and fillet, with losses of 24 and 12%, respectively). Zn losses were observed in two ostrich cuts (fillet and leg, 24% losses for both) and in three beef cuts (13–17% losses). Cu losses were only detected in ostrich leg (21%), beef sirloin (29%) and fillet (25%). Perelló *et al*. [24] studied the changes in mineral composition (As, Cd, Hg and Pb) in some foods including meat (veal steak, loin of pork, breast and thigh of chicken, and steak and rib of lamb) using different cooking procedures (frying, grilling, roasting and boiling), and concluded that metal concentrations in meat decreased and the metal losses depended on the cooking procedure.

The mineral losses obtained in studies using different cooking processes and various types of foods are summarized in Table 8.1 [6–8, 21–23, 25–30].

8.4 Bioaccessibility and bioavailability of minerals after cooking

The total amount of a pollutant, or nutrient, does not reflect the amount that is bioavailable for humans, since only a portion of the total content is absorbed and participates in body functions [31]. Food components are released during digestion, and only a portion of these compounds will be available for body physiological functions and storage [32]. Different terms have been used to describe the bioavailable fraction of a food. It is important to differentiate between two biological terms: bioaccessibility and bioavailability. *Bioaccessibility* is defined as the fraction of an element that is released from the food matrix after ingestion and solubilized in the intestinal lumen. However, *bioavailability* describes the fraction of the solubilized element which is absorbed in the intestinal tract and reaches the circulatory system [33]. Consequently, element bioaccessibility itself does not provide a direct measure of element bioavailability, since not all soluble element species are absorbable [34]. The bioavailability of a compound depends on the physical properties of the food matrix.

In vivo or *in vitro* methods can be used to estimate the bioavailability of a nutrient; *in vitro* methods are preferred over *in vivo* methods since the latter are expensive, difficult to reproduce and ethically controversial [35]. Most of the bioaccessibility and bioavailability studies of elements in food were performed in raw food. Since cooking processes can produce element transformations, these studies must be performed in the cooked sample in order to obtain an accurate measure of the potential risk for human health. Some procedures used for studying element bioaccessibility and bioavailability in different food samples are summarized in Table 8.2.

A study on the bioavailability of certain trace elements in cooked seaweed was performed by García-Sartal *et al*. [19]. The scheme of the *in vitro* digestion method used by these authors is shown in Figure 8.1. This method involves a simulated gastrointestinal digestion with pepsin during the gastric stage, followed by an intestinal stage with pancreatin and bile salts. The procedure was performed by weighing 0.5 g of powdered seaweed, raw or cooked, into a 100-mL Erlenmeyer flask. A volume of 20 mL of ultrapure water was then added and after 15 min the pH was adjusted to 2.0 with 6 M hydrochloric acid solution. Then, 0.15 g of a freshly prepared gastric solution (pepsin 6.0% m/v) dissolved in 0.1 M hydrochloric acid was added. The flasks were then covered and incubated at 37°C with orbital-horizontal shaking at 150 rpm for 120 min. The flasks were then placed in an ice-water bath to stop the enzymatic digestion. After this treatment, the supernatant was the *in vitro* gastric digest. The procedure continued by adding to the gastric digest 5 mL of intestinal solution

Table 8.2 Element bioaccessibility and bioavailability studies in different cooked samples.

Food sample	*In vitro* bioaccessibility or bioavailability process	Elements	Reference
Edible seaweed	*Bioavailability* 1 0.5 g of dry sample with 20 mL of ultrapure water and adjust pH 2.0 with 6.0 M HCl 2 Add 0.15 g of gastric solution (pepsin 6.0% m/v) dissolved in 0.1 M HCl and incubate at 37 °C and shaking at 150 rpm for 120 min 3 Add 5 mL of intestinal solution (pancreatin 0.4% m/v and bile salts 2.5% m/v dissolved in 0.1 M sodium hydrogen carbonate) 4 To Introduce dialysis membrane (10 kDa) filled with 20 mL of 0.15 N PIPES solution (pH 7.5 adjusted with HCl) and incubate at 37 °C shaking at 150 rpm for 120 min	As, Co, Cr, Cu, Fe, Ni, Se, Zn	[36]
Cereals and pulses	*Bioavailability* Gastric digestion by incubation with pepsin (pH 2.0) at 37 °C for 2 h. To simulate intestinal digestion, segments of dialysis tubing (molecular mass cut-off 10 kDa) containing 25 mL sodium bicarbonate solution were placed in Erlenmeyer flask containing the gastric digest and incubated at 37 °C with shaking for 30 min or longer until the pH of the digest reached 5.0. Then, 5 μL of the pacreatin–bile extract mixture was added and the incubation was continued for 2 h or longer until the pH of the digest reached 7.0	Fe and Zn	[38]
Farmed marine fish	*Bioaccessibility* First, 6 mL of artificial saliva was added to the fish sample (4.5 g) and incubated for 5 min. Then, 12 mL of artificial gastric juice was added and incubated for 2 h. Finally, a mixture of 12 mL of artificial duodenal juice, 6 mL of artificial bile, and 2 mL of HCO_3^- was added and incubated for further 2 h. All three simulating processes were incubated at 37 °C	As, Cd, Cu, Fe, Se and Zn	[39]
Black scabbard fish and edible crab	*Bioaccessibility* Digestion with four digestive juices (saliva, gastric, duodenal and bile). Digestion of 5 g of fish sample using 50 mL in a rotating water bath (60 rpm) at 37 °C throughout each digestive phase: saliva (5 mL, 5 min), stomach (gastric juice: 12 mL, 2 h) and small intestine (duodenal juice: 12 mL, 5 min; bile juice: 6 mL, 2 h). The reaction was stopped at the end of each digestion phase by lowering the temperature to 4 °C, and the tubes were centrifuged at 10,000 *g* for 10 min at 4 °C, in order to separate the digestion fluids (aqueous phase) from the particulate residue	Hg, Cd, As	[41]
Fish samples	*Bioaccessibility* Gastric digestion: 25 g sample + 75 mL gastric juices (pepsin, NaCl, pH 1.8) and shaking water bath (pH 3, 37 °C, 4 h) Intestinal digestion: adjust pH 6.8 ($NaHCO_3$); add 50 mL intestinal juice (pancreatin, NaCl, α-amylase and bile salts) and shaking in a water bath (37 °C, 4 h). Adjust pH7. Centrifugation at 3500 rpm. Filtration (0.45 μm)	Se, Hg	[42]
Pigeon pea cultivars	*HCl extractability* 1 g of sample + 50 mL 0.03 N HCl. Incubated at 37 °C in a shaker-cum-water bath for 3 h to simulate conditions that occur in human stomach	Zn, Cu, Ca, Fe	[43, 44]
Bathua (*Chenoodium album*) and fenugreek (*Trigonella foenum graecum*) leaves	*HCl extractability* 1 g of sample + 50 mL 0.03 N HCl. Incubated at 37 °C in a shaker-cum-water bath for 3 h to simulate conditions that occur in human stomach	Ca, Zn	[45]

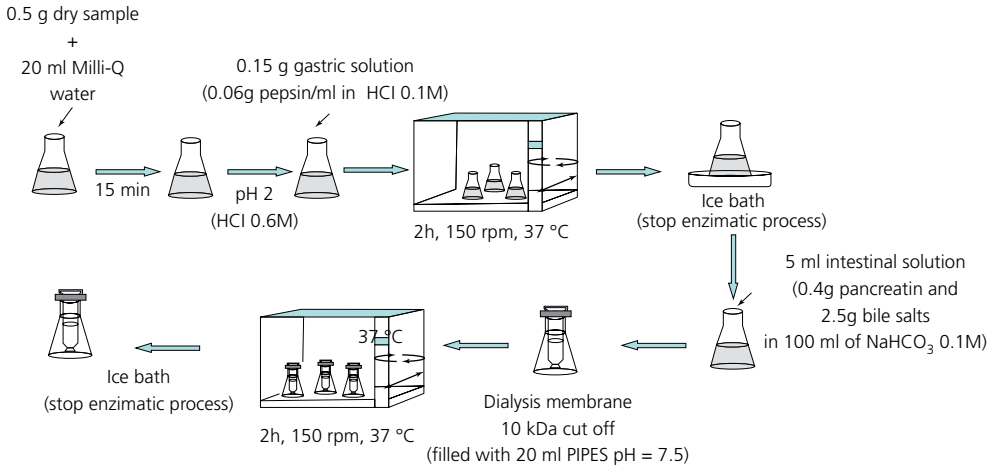


Figure 8.1 *In vitro* digestion procedure.

(pancreatin 0.4% m/v and bile salts 2.5% m/v dissolved in 0.1 M sodium hydrogen carbonate). At this point, dialysis membranes, filled with 20 mL of a 0.15 N piperazine-*N*,*N*′-bis(2-ethane-sulfonic acid) or PIPES solution (pH 7.5 adjusted with hydrochloric acid), were placed inside the flasks. Intestinal digestion took place in the incubation chamber at 37°C with orbital-horizontal shaking at 150 rpm for 120 min. The enzymatic reaction was stopped by immersing the flasks in an ice-water bath. The membranes were removed and their outer surface was rinsed with ultrapure water and the dialysis membrane containing solution (dialysate) and the residual or non-dialysable fraction (remaining slurries in the flasks) were transferred to polyethylene vials and weighed separately. The dialysability percentage was calculated using the dialysable element concentration and the total element concentration. Low dialysability percentages of 1.0–16.0%, 3.4–27%, 8.2–12.9%, 9.7–21.0% and 14.6–61.3% were found for Cr, Co, Ni, As and Se, respectively. The dialysability percentages depend on the type of seaweed studied, and this can be due to differences in seaweed composition (fat, protein, carbohydrates and fibre). In general, Wakame, followed by Kombu, is the seaweed that presents the highest bioavailability percentages for the elements under study. García-Sartal *et al*. [36] studied the changes in arsenic dialysability percentages in four seaweed samples before and after cooking. The results obtained (Table 8.3) shown that the dialysability percentages in cooked seaweed were close to those found in raw seaweed, except in the case of sea lettuce, for which dialysability percentages were lower (As species become less bioaccessible for this kind of sample). In another study performed by Moreda-Piñeiro *et al*. [37], a bioavailability study of various elements (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, V and Zn) in different types of raw seaweeds, including sea spaghetti, and a canned seaweed (mixture of sea spaghetti and *Saccorhiza polyschides*) was performed. The dialysability percentages obtained for canned seaweed (containing principally sea spaghetti) were lower than the dialysability percentages obtained for raw sea spaghetti. This suggests that the cooking procedure changes the major nutrient composition and consequently metal bioavailability.

Table 8.3 Arsenic dialysability (%) obtained in raw and cooked seaweed samples.

Seaweed	Raw seaweed	Cooked seaweed
Kombu	14.0 ± 0.6	13.7 ± 0.8
Wakame	15.0 ± 0.8	13.8 ± 1.0
Nori	17.0 ± 0.4	15.3 ± 1.0
Sea lettuce	17.0 ± 1.5	7.4 ± 0.2

The influence of heat processing (pressure cooking and microwave heating) on the bioavailability of Zn and Fe from cereals and pulses consumed in India was studied by Hemalatha *et al*. [38]. The authors found that both heating processes produced an important reduction in Zn bioaccessibility from cereals and pulses while, in

general, Fe bioaccessibility was significantly enhanced during the heating treatment for all the food grains studied. The changes in the bioaccessibility of both elements could be due to the nature of the interaction of the two metals with food proteins on heat processing, and also to the variations in other constituents of the food matrix.

He *et al*. [39] evaluated the effects of cooking processes, including boiling, steaming, frying and grilling, on the bioaccessibility of several trace elements (As, Cd, Cu, Fe, Se and Zn) from the muscle of two farmed marine fish species (seabass *Lateolabrax japonicus* and red seabream *Pagrosomus major*). Bioaccessibility measurements of trace elements were performed using an *in vitro* digestion method consisting of three steps simulating the digestive processes in the mouth, stomach and small intestine. The cooking process generally decreased the bioaccessibility of all the elements in the two fish muscles studied, but was especially significant for Cu and Zn where frying (Cu, 13.0–27.2%; Zn, 30.0–41.4%) and grilling (Cu, 17.7–26.0%; Zn, 30.1–38.5%) caused larger decreases than boiling (Cu, 11.7–18.9%; Zn, 20.1–26.3%) and steaming (Cu, 13.2–21.0%; Zn, 22.4–33.6%). Decreases in bioaccessibilities after cooking were previously found in shellfish [40]. Changes in the bioaccessibility could be due to protein degradation and losses of water and other soluble constituents during the cooking processes. The most significant influence on the bioaccessibility of Cu and Zn may be attributed to their storage patterns. Both elements are stored mainly in metallothioneins and insoluble ligands in the form of less easily degradable complexes and less digestible proteins.

Bioaccessibility of Hg, Cd and As in cooked black scabbard fish (Hg: fried, steamed, grilled) and edible crab (Cd and Hg: steamed and boiled) was assessed by Maulvault *et al*. [41] using an *in vitro* digestion method with four digestive juices (saliva, gastric, duodenal and bile). In general, Hg, Cd and As bioaccessibility increased throughout the digestion process. The bioaccessible fractions were lower in the mouth step and higher at the end of the digestion process because protein and lipid digestion occurs in the stomach and small intestine with the release of specific enzymes (pepsin and pancreatin) and pH alterations. Regarding Hg, no statistically significant differences in bioaccessibility was observed between uncooked (45%), grilled (42%) and steamed (33%) samples, whereas lower bioaccessibility was observed in fried samples (24%) compared with uncooked samples. This change could be due to structural changes during the cooking treatments that might affect the solubility and release of Hg, or induce Hg complexation with other components of the sample. Cd and As show high bioaccesibility in raw and cooked samples (up to 100%) and no statistically significant differences were detected between boiled and steamed samples. Selenium bioaccesibility in tuna using an *in vitro* method was evaluated by Cabañero *et al*. [42] before and after cooking (microwave oven at high power; 100%, 650 W for 4 min) of a tuna sample. The bioaccessibility percentages obtained were around 50% in both cases (cooked and uncooked samples) and the value in the cooked tuna was slightly higher than in uncooked tuna. The heating process applied to the sample produced losses of water and vitamins and protein degradation, and might consequently affect the efficiency of Se release by the digestive enzymes.

HCl extractability has been used by different authors as a useful indicator of mineral bioavailability. The HCl extractabilities of Ca, P and Fe [43] and Cu and Zn [44] from pigeon pea after various domestic processing (soaking and dehulling) and cooking methods (ordinary cooking and pressure cooking) were studied. Pigeon pea is a good source of dietary essential minerals and is also rich in anti-nutrients like polyphenols, phytic acid, saponins and tripsin inhibitor activity [44]. Ordinary cooking of pigeon peas was performed in water using a mass of water three times the weight of seeds. Pressure cooking was done at 121°C (15 psi) for 15 min. The minerals in the samples were extracted with 0.03 N HCl by shaking the contents at 37°C for 3 hours. The results obtained show the reduction in mineral concentration during the processing and cooking. Moreover, the HCl extractability of these dietary essential minerals was enhanced significantly (4–50%) when the pigeon pea seeds were processed and cooked, possibly due to reduction in phytate content during the cooking process (6–45% reduction) [43], which is known to chelate the minerals. This effect was also observed by Kala-Yadav *et al*. [45] when studying the effect of domestic processing (dried, blanched and cooked in an open pan and a pressure cooker) on total extractable Ca and Zn content of bathua (*Chenopodium album*) and fenugreek (*Trigonella foenum graecum*) leaves. Blanching and cooking processes produced a reduction in Ca and Zn content, increasing the HCl extractability of these minerals. This increment could be due to the removal of anti-nutrients like oxalic acid and phytic acid during the blanching and cooking of these leaves.

8.5 Changes in essential trace element content during industrial processing

Some industrial procedures involving food result in a decrease in mineral content. The addition of minerals to these products to the levels at which they occur naturally, or fortifying them above the levels expected naturally, is a frequent technological practice. On the other hand, many compounds used as food additives contain metallic cations in their structure, principally Ca, Na, K, Mg and Al [46].

The industrial process applied depends on the food sample, and different steps such as storage, cooking, canning, freezing and peeling can be applied. Usually, normal storage has minimal influence on mineral composition, and some changes are related to variations in humidity, microbiological transformation or chemical contamination [46].

Changes in mineral composition during industrial processing are important in vegetables and have been reported by several authors. Steps such as washing, blanching or peeling (in fruit) can remove an important source of the minerals present in the food sample. The effect of industrial processing on the mineral content of white asparagus was studied by Amaro *et al*. [47, 48]. In this case, fresh asparagus were washed (with a machine), peeled (manually by trained staff) and then blanched. Blanching was performed by immersion in hot water for 3 min at 90°C in a vertical autoclave with automatic regulation of pressure and temperature. Spears were then cut at 210 mm and divided into three portions of 70 mm each. The results obtained showed some significant changes during the processing in the essential trace elements. Overall, there was a decrease in Fe and Mn levels, while there were increases in Cu, K, Mg, P and Zn content. These modifications are mostly related to the peeling process. The Fe decrease after peeling can be associated with the higher concentration of this element in asparagus peel. The increment in the levels of the other elements during processing can be related to the smaller concentrations of these elements in the peel.

The influence of dehulling on nutritional composition of several varieties of lentil (*Lens culinaris*) was studied by Wang *et al*. [49]. These authors found that dehulling significantly decreased Ca, Cu, Fe, Mg and Mn levels in lentils. Khalil [50] studied the effect of processing on the chemical and biological value of guar and faba beans. In this case, the effect of soaking, germination, autoclaving (121°C for 45 min) and cooking on mineral composition of these legumes was studied. Results showed that there was a slight decrease in mineral contents due to the heating treatments, with the exception of Mg and Mn for guar, and Na and Mn for faba bean. Significant decreases in the contents of Na, K, Cu, Mn and Mg were observed in germinated guar and faba bean compared with the raw material, while a slight increase in Fe and Zn in germinated guar and faba beans was found.

The influence of industrial processing on the elemental composition (Mg, Al, Si, P, S, Cl, K, Ca, Ti, Mn, Fe, Ni, Cu, Zn and Rb) of mate tea leaves was studied by Giulian *et al*. [51]. The process consisted of three steps: (i) roasting (toasting in wood flames for about 3 min at 500°C); (ii) drying in a furnace by conventional gases originating from wood burning (30 min at 100°C); and (iii) grinding and storage. The results showed that the roasting and drying steps caused an increase in the concentration of the majority of the elements with the exception of Mg, Al, Cu and Zn (which did not show any significant change), and Cl (which decreased its concentration). The authors attributed this increment to a dynamic mechanism that correlates both the huge biomass burning at high temperatures and the contamination of the flames and fog due to the composition of the wood used in these processes. The storage step also produced changes in element composition. Decreases in K, Ca, Cl and Zn were observed after the storage step while an increase in the concentrations of Mn, Al and Si was found.

8.6 Fortification of foods with minerals

Although food is usually an important source of minerals, some foods are fortified with minerals such as Fe, Ca, Mg, Cu, Zn, Se and I in order to prevent micronutrient deficiencies in the population. Worldwide, the three most common forms of micronutrient malnutrition are iron, vitamin A and iodine deficiency. Some of the food fortification programmes are promoted by the governments of developing countries but they also exist in some industrialized countries as a means of restoring micronutrients lost by food processing [52]. These programmes are focused on certain groups, such as children and pregnant women, having the highest risk of micronutrient

Table 8.4 Mineral fortificants used for specific foods.

Food vehicle	Fortificant
Iron	
Low extraction (white) wheat flour or degermed cornflour	Dry ferrous sulfate, ferrous fumarate, electrolytic iron (×2 amount), encapsulated ferrous sulfate, encapsulated ferrous fumarate
High extraction wheat flour, cornflour, corn masa flour	Sodium iron EDTA, encapsulated ferrous sulfate (×2 amount), encapsulated ferrous fumarate (×2 amount)
Pasta	Dry ferrous sulfate
Dry milk	Ferrous sulfate plus ascorbic acid
Fluid milk	Ferric ammonium citrate, ferrous bisglycinate, micronized ferric pyrophosphate
Cereals-based	Ferrous sulfate
Cocoa products	Ferrous fumarate plus ascorbic acid; ferric pyrophosphate (×2 amount) plus ascorbic acid
Iodine	
Milk, bread and salt	Potassium iodate and potassium iodide
Zinc	
Breakfast cereals	Zinc oxide
Infant formulas	Zinc sulfate
Calcium	
Infant formulas	Carbonate; chloride; citrate and citrate malate; gluconate; glycerophosphate; lactate; monobasic, dibasic, tribasic phosphate; orthophosphate; hydroxide; oxide
Selenium	
Milk-based infant formulas	Sodium selenite and sodium selenate

deficiencies. Another example is the fortification of food with iodine in iodine-deficient areas.

Many compounds are used for food fortification, but the selected fortificant must meet a number of requisites, such as (i) safety; (ii) must not cause unacceptable sensory problems at the level of the intended fortification; (iii) low cost; (iv) bioavailability; and (v) the potential interactions between the fortificant and other components present in the food sample that might interfere with the metabolic utilization of the fortificant must be known [52]. For example, metallic ions present in some mineral additives may cause the instability of vitamins; in some cases, the interaction of vitamin and mineral additives may impact and affect other quality factors such as colour [53]. The World Health Organization (WHO) and the Food and Agricultural Organization of the United Nations has published guidelines on food fortification with micronutrients that include a list of the different compounds used for mineral food fortification [52]. Selection of a fortificant depends on the food matrix and its bioavailability. Some examples of fortificants are shown in Table 8.4. Regarding iron, the presence of inhibitors in the diet (phytates and certain phenolic compounds) can reduce iron bioavailability. To mitigate this effect, the addition of ascorbic acid or sodium ethylendiaminetetraacetic acid (EDTA) and the removal of phytates are recommended in iron-fortified foods. It is also important to emphasize that in some cases high concentrations of a mineral can inhibit the absorption of another. This is the case of Ca and Fe. High levels of calcium inhibit the absorption of Fe from foods. The co-addition of ascorbic acid (added in some iron-fortified foods to increase iron bioavailability) can help overcome the inhibitory effect of calcium on iron absorption [52].

Dominguez *et al.* [54] studied the effect of different iron salts used to fortify infant formulas (ferrous lactate, ferrous sulfate, EDTA–Fe(III) sodium salt hydrate and encapsulated ferrous sulfate) on the bioavailability of Fe, Cu and Zn. Results showed that Fe(III)–EDTA was the most useful salt for iron fortification because it gives the highest iron bioavailability after *in vitro* digestion. For Cu, both Fe(III)–EDTA and ferrous sulfate offered useful results, while Zn is more bioavailable when milk is fortified with Fe(III)–EDTA, ferric diphosphate and ferric lactate. Therefore, the authors concluded that Fe(III) was the best iron salt for fortifying infant formulas.

Several plants, such as garlic, onion, shallot, broccoli or pumpkin, have the ability to accumulate and transform inorganic forms of selenium into bioactive organic compounds [55, 56]. Therefore, the most effective way

to increase the amount of selenium in plants is to use Se-enriched fertilizers. Consumption of these vegetables could be an effective way of reducing dietary selenium deficiencies and increasing health benefits. The effect of different fertilizer treatments on the total selenium and selenium species has been studied by several authors. In general, increases in selenium content in the growth mixture as well as length of exposure resulted in proportional increases in the total selenium content in vegetables. This increment was higher when the soil was fertilized with Se(VI) than with Se(IV) and selenomethionine (SeMet) [56]. However, better transformation of selenium into organic species was observed for plant grown in the presence of selenite. Kápolna *et al.* [57] also studied the effect of Se concentration used for enrichment (10 and 100 μg/mL as Se(IV) or SeMet) of onion selenium concentration and also selenium speciation. The authors concluded that enrichments with either Se(IV) or SeMet at 100 μg/mL concentration resulted in 80% or more inorganic selenium in plants. This Se concentration is too high for the onion plants to metabolize either inorganic or organic selenium into anti-carcinogenic species like methylselenocysteine (MeSeCyst), and is not recommended for producing selenium supplements. The highest amount of these anti-carcinogenic species (85%) was observed in the case of onion bulbs enriched with 10 μg/mL Se(IV).

Selenium supplementation of animal feed is the most economic alternative for increasing the selenium content in meat, milk and eggs. The effect of the selenium species added to the diet was evaluated. In general, higher concentrations of selenium in meat were obtained in animals fed a diet rich in selenized yeast, which contains mainly selenomethionine [58].

Regarding milk, several studies have been developed to obtain whole milk naturally fortified with selenium [59]. The bioavailability of selenium in cows' milk after supplementation of cow feed with different forms of selenium was also studied by Muñiz-Naveiro *et al.* [60]. The authors studied the effect of selenium supplementation with inorganic selenium (sodium selenite) and organic selenium (selenized yeast containing 90% selenomethionine) at different concentrations on the bioavailability of selenium in cows' milk. The authors concluded that supplementation with organic selenium at levels higher than 0.4 μg/g forage was recommended for producing milk with higher selenium bioavailability without altering the original milk composition.

8.7 Changes in element speciation during the cooking procedure

The toxicity and bioavailability of the elements not only depends on their concentration but also on their chemical form. For example, inorganic arsenic species, i.e. As(III) and As(V), are well known for their toxicity. Other arsenic species such as arsenobetaine (AB) and arsenosugars, widely found in marine animals and seaweed, respectively, are considered negligibly toxic [61]. Chromium can be present in the environment as Cr(III) and Cr(VI). These species differ in their physicochemical properties and consequently in their biochemical reactivity. Cr(III) is considered a trace essential element and is involved in the metabolism of lipids, glucose and proteins in mammals, while Cr(VI) presents high toxicity because of its carcinogenic effects due to its high oxidation potential and because it easily permeates biological membranes [62, 63]. Therefore, the determination of elemental species in food is important for human nutrition studies and to evaluate risks for human health [63].

Food is usually cooked, and different cooking processes such as frying, boiling, baking or grilling can be used. As mentioned above, these thermal treatments can produce alterations in the total element concentration but also changes in the chemical species that could pose a risk to human health. Several studies have been performed in recent years to evaluate possible changes on elemental speciation and their bioavailability after cooking. There is a lack of information on element speciation of food after cooking, and there is a sparse literature on arsenic speciation.

Arsenic is an element present in high concentrations in some foods such as fish, molluscs, seaweed and rice. Because of the different toxicity of arsenic species, several studies have been performed to estimate changes in arsenic speciation after cooking. Laparra *et al.* [64] determined the concentration of total arsenic and inorganic arsenic in raw and cooked *Hizikia fusiforme* samples. The cooking procedure was performed by boiling the sample in water for 20 min at 100°C. The concentrations of As obtained in raw samples ranged from 125.8 to 131.7 μg/g (dry weight) for total As, and from 79.7 to 87.7 μg/g for inorganic As. The cooking procedure caused an important reduction in the contents, 30–40% for total As and 46–50% for inorganic arsenic. Total arsenic and inorganic arsenic were also determined in the soluble fractions obtained after applying a gastric

and gastrointestinal stage to raw and cooked samples. The results showed that bioaccessibility varied in the raw samples in the range 55.6–73.9% for total As and 54.0–66.5% for inorganic As. After cooking, bioaccessibility remained practically constant for total As (61.9–74.6%), and increased for inorganic As (78.3–84.4%). This could be due to the fact that the boiling procedure might decrease the fibre content and produce denaturation of protein, permitting greater accessibility of enzymes during proteolysis; therefore, boiling might facilitate solubilization of As(III) in the cooked seaweed. García-Sartal *et al*. [65] studied the effect of the cooking procedure on arsenic speciation in the bioavailable fraction from edible seaweed samples (Kombu, Wakame, Nori and sea lettuce). The samples were cooked following the manufacturer's instructions (cooking in boiling water at 100°C), and the arsenic species were determined in the raw and the cooked samples. The results obtained showed that 69% of arsenic in Kombu, 50% in Wakame, 71% in Nori and 34% in sea lettuce was removed from the seaweed matrix and released into the cooking water during the cooking procedure. The arsenic bioavailability varied from 11 to 29% in cooked samples. Arsenosugars were the main arsenic species found in raw and cooked seaweed samples as well as in the bioavailable fractions. These results suggest that the heat treatment and the acid environment and the enzymes used in the *in vitro* gastrointestinal digestion were not sufficient to produce changes in arsenic species in the four seaweeds studied. These results agree with the results obtained in previous studies by Almela *et al*. [66] examining arsenic species content in raw and cooked edible seaweed and bioaccessibility of arsenosugars. The study was performed on three types of seaweed samples (*Hizikia fusiforme*, *Undaria pinnatifida* and *Porphira* sp.); *H. fusiforme* and *U. pinnatifida* were boiled (100°C) or soaked, and *Porphira* sp. was baked (200°C, 5 min) or toasted over a flame.

The effect of cooking temperature on chemical changes in species of organic arsenic in seafood was studied by Devesa *et al*. [67]. The changes in arsenic speciation in some seafood samples (dory, hake, sole and sardine) using different cooking treatments (frying, baking and grilling) were studied. Experiments using different cooking temperatures (from 90 to 160°C) were also performed. The total arsenic contents in raw seaweed varied with the type of seaweed. AB is practically the total quantity of arsenic. Losses of total arsenic were not observed before these cooking treatments at the different temperatures; nevertheless, transformations of AB into tetramethylarsonium ion (TMA^+) were observed after the cooking process. The concentration of TMA^+ found in the cooked samples depended on cooking process, temperature and cooking time. Increments in cooking times and temperatures produce increments in TMA^+ concentration. The effect of grilling and frying were similar, whereas baking produced low levels of TMA^+.

Taking into account the results obtained in these studies on arsenic speciation, further studies will be necessary in other type of samples and with other elements.

8.8 Conclusion

Changes in mineral composition as well as mineral bioavailability are produced during industrial and culinary processing. In general, heating at high temperatures causes mineral losses. Changes in element bioavailability are also produced during the cooking process. These can be due to changes in food composition during the cooking procedure, such as protein degradation, water losses or other soluble constituents, and the removal of anti-nutrients like oxalic acid and phytic acid.

Regarding chemical speciation, although in recent years there has been an increase in element speciation studies in food samples, few papers have been published on changes in elemental species and their bioavailability during food processing (including food fortification). Because the toxicity and the bioavailability of an element depend on the chemical species, further studies will be necessary in this field to assure food safety.

Abbreviations

AB: Arsenobetaine
MeSeCyst: Methylselenocysteine
PIPES: Piperazine-*N*,*N*′-bis(2-ethane-sulfonic acid)
SeMet: Selenomethionine
TMA^+: Tetramethylarsonium ion
WHO: World Health Organization

References

1 Fairweather-Tait SJ, Hurrell RF. Bioavailability of minerals and trace elements. *Nutr Res Rev* 1996;**9**:295–324.

2 O'Dell BL, Sunde RA. *Handbook of Nutritionally Essential Mineral Elements*. Marcel Dekker, New York, 1997.

3 Nielsen FH. Ultratrace elements in nutrition: current knowledge and speculation. *J Trace Elem Exp Med* 1998;**11**: 251–274.

4 Mohamed EA, Ali NA, Ahmed SH, Mohamed-Ahmed IA, Babiker EE. Effect of refrigeration process on antinutrients and HCl extractability of calcium, phosphorus and iron during processing and storage of two millet cultivars. *Innovative Romanian Food Biotechnology* 2010;**8**:13–21.

5 Pell A, Márquez A, Rubio R, López-Sánchez JF. Effects of sample processing on arsenic speciation in marine macroalgae. *Anal Method* 2013;**5**:2543–2550.

6 Kimura M, Itokawa Y. Cooking losses of minerals in foods and its nutritional significance. *J Nutr Sci Vitaminol* 1990;**36**: S25–S33.

7 Gonçalves B, Borges O, Rosa E, Coutinho J, Silva AP. Effect of cooking on free amino acid and mineral profiles of sweet chestnut (Castanea sativa Mill.). *Fruits* 2012;**67**:207–214.

8 Avola G, Patanè C, Barbagallo RN. Effect of water cooking on proximate composition of grain in three Sicilian chickpeas (Cicer arietinum L.). *LWT Food Sci Technol* 2012;**49**: 217–220.

9 Rupérez P. Mineral content of edible marine seaweeds. *Food Chem* 2002;**79**:23–26.

10 MacArtain P, Gill CIR, Brooks M, Campbell R, Rowland IR. Nutrional value of edible seaweeds. *Nutr Rev* 2007;**65**: 535–543.

11 Ródenas de la Rocha S, Sánchez-Muñiz FJ, Gámez-Juaristi M, Marín MTL. Trace element determination in edible seaweeds by an optimized and validated ICP-MS method. *J Food Compos Anal* 2009;**22**:330–336.

12 Besada V, Andrade JM, Schultze F, González JJ. Heavy metals in edible seaweeds commercialised for human comsumption. *J Mar Syst* 2009;**75**:305–313.

13 Almela C, Clemente MJ, Vélez D, Montoro R. Total arsenic, inorganic arsenic, lead and cadmium contents in edible seaweed sold in Spain. *Food Chem Toxicol* 2006;**44**:1901–1908.

14 Moreda-Piñeiro A, Peña-Vázquez E, Bermejo Barrera P. Significance of the presence of trace and ultratrace elements in seaweed. In: Se-Kwon Kim (ed.) *Handbook of Marine Macroalgae: Biotechnology and Applied Phycology*. John Wiley & Sons, Chichester, 2012, pp. 116–170.

15 Rao P, Mantri VA, Ganesan K. Mineral composition of edible seaweed Porphyra vietnamensis. *Food Chem* 2007;**102**: 215–218.

16 Hwang Y, Park S, Park G, Ghoi S, Kim M. Total arsenic, mercury, lead and cadmium contents in edible dried seaweed in Korea. *Food Addit Contam B* 2010;**3**:7–13.

17 Romarís-Hortas V, García-Sartal C, Barciela-Alonso MC, Moreda-Piñeiro A, Bermejo-Barrera P. Characterization of edible seaweeds harvasted on Galician coast (northwestern Spain) using recognition techniques and major and trace element data. *J Agric Food Chem* 2010;**58**:1986–1992.

18 Ichikawa S, Kamoshida M, Hanaoka K, Hamano M, Maitani T, Kaise T. Decrease of arsenic in edible brown algae Hijikia fusiforme by the cooking process. *Appl Organometal Chem* 2006;**20**:585–590.

19 García-Sartal C, Barciela-Alonso MC, Moreda-Piñeiro A, Bermejo-Barrera P. Study of cooking procedure on the bioavailability of As, Co, Cr, Cu, Fe, Ni, Se and Zn from edible seaweed. *Microchem J* 2012;**108**:92–99.

20 Sun GX, Wiele TV, Alava P, Tack F, Laing GD. Arsenic in cooked rice: effect of chemical, enzimatic and microbial processes on the bioaccessibility and speciation in the human gastrointestinal tract. *Environ Pollut* 2012;**162**:241–246.

21 Amira Mnari B, Sonia FZ, Nadiha D *et al*. Effects of different cooking methods on the mineral contents of wild and farmed sea bream (Sparus aurata). *Int J Food Sci Technol* 2012;**47**:1964–1969.

22 Gokoglu N, Yerlikaya P, Cengiz E. Effects of cooking methods on the proximate composition and mineral contents of rainbow trout (Oncorhynchus mykiss). *Food Chem* 2004;**84**:19–22.

23 Lombardi-Boccia G, Lanzi S, Aguzzi A. Aspects of meat quality: trace elements and B vitamins in raw and cooked meats. *J Food Compos Anal* 2005;**18**:39–46.

24 Perelló G, Martí-Cid R, Llobet JM, Domingo JL. Effect of various cooking processes on the concentration of arsenic, cadmium, mercury and lead in foods. *J Agric Food Chem* 2008;**56**:11262–11269.

25 Lewu MN, Adebola PO, Afolayan AJ. Effect of cooking on the mineral contents and anti-nutrial factors in seven accessions of *Colocasia esculenta* (L.) Schott growing in South Africa. *J Food Compos Anal* 2010;**23**:389–393.

26 Noreen N, Shah H, Anjum F, Masood T, Faisal S. Variation in mineral composition and phytic acid content in different rice varieties during home traditional cooking processes. *Pak J Life Soc Sci* 2009;**7**:11–15.

27 Abbas A, Farooq M, Nisar MF, Rafique S, Komal T, Naeem S. Effect of boiling on mineral contents in rice. *World Appl Sci J* 2010;**10**:1038–1041.

28 Sebastia V, Barbera R, Farre R, Lagarda MJ. Effects of legume processing on calcium, iron, and zinc contents and dialysabilities. *J Sci Food Agric* 2001;**81**:1180–1185.

29 Yaseen AAE. Effect of processing conditions and cooking on retention of minerals in macaroni. *Nahrung* 1993;**37**: 449–455.

30 El Maki HB, Abdel Rahaman SM, Idris WH, Hassan AB, Babiker EE, El Tinay AH. Content of antinutritional factors and HCl-extractability of minerals from white bean (Phaseolus vulgaris) cultivars: influence of soaking and/or cooking. *Food Chem* 2007;**100**:362–368.

31 Intawongse M, Dean JR. *In-vitro* testing for assessing oral bioaccessibility of trace metals in soil and food samples. *Trends Anal Chem* 2006;**25**:876–886.

32 Moreda-Piñeiro J, Moscoso-Pérez C, López-Mahía P, Muniategui-Lorenzo S, Prada-Domíngez D, Moreda-Piñeiro A. *In-vivo* and *in-vitro* testing assess the bioaccessibility and the bioavailability of arsenic, selenium and mercury species in food samples. *Trends Anal Chem* 2011;**30**:324–345.

33 Caussy D. Case studies of the impact of understanding bioavailability: arsenic. *Ecotoxicol Environ Saf* 2003;**56**:164–173.

34 Laparra JM, Velez D, Barbera R, Montoro R, Farre R. Bioaccessibility and transport by Caco-2 cells of organo-arsenical species present in seafood. *J Agric Food Chem* 2007;**55**:5892–5897.

35 Parada J, Aguilera JM. Food microstructures affects the bioavailability of several nutrients. *J Food Sci* 2007;**72**:R21–R23.

36 García-Sartal C, Romarís-Hortas V, Barciela-Alonso MC, Moreda-Piñeiro A, Domínguez-González R, Bermejo-Barrera P. Use of an in vitro digestion method to evaluate the bioaccessibility of arsenic in edible seaweed by inductively coupled plasma-mass spectrometry. *Microchem J* 2011;**98**:91–95.

37 Moreda-Piñeiro J, Moreda-Piñeiro A, Romarís-Hortas V *et al*. Trace metals in marine foodstuff: bioavailability estimation and effect of major food constituents. *Food Chem* 2012;**134**:339–345.

38 Hemalatha S, Platel K, Srinivasan K. Influence of heat processing on the bioaccessibility of Zn and iron from cereals and pulses consumed in India. *J Trace Elem Med Biol* 2007;**21**:1–7.

39 He M, Ke CH, Wang WX. Effects of cooking and subcellular distribution on the bioaccessibility of trace elements in two marine fish species. *J Agric Food Chem* 2010;**58**:3517–3523.

40 Amiard JC, Amiard-Triqued C, Charbonnier L, Mesnil A, Rainbow PS, Wang WX. Bioaccessibility of essential and non-essential metals in commercial shellfish from western Europe and Asia. *Food Chem Toxicol* 2008;**46**:2010–2022.

41 Maulvault AL, Machado R, Afonso C *et al*. Bioaccessibility of Hg, Cd and As in cooked black scabbard fish and edible crab. *Food Chem Toxicol* 2011;**49**:2808–2815.

42 Cabañero AI, Madrid Y, Cámara C. Selenium and mercury bioaccessibility in fish samples: an in vitro digestion method. *Anal Chim Acta* 2004;**526**:51–61.

43 Duhan A, Khetarpaul N, Bishnoi S. Content of phytic acid and HCl-extractability of calcium, phosphorus and iron as affected by various domestic proccessing and cooking methods. *Food Chem* 2002;**78**:9–14.

44 Duhan A, Khetarpaul N, Bishnoi S. HCl-extractability of zinc and cooper as affected by soaking, dehulling, cooking and germination of high yielding pigeon pea cultivars. *J Food Compos Anal* 2004;**17**:597–604.

45 Kala-Yadav S, Sehgal S. Effect of domestic proccessing on total and extractable calcium and zinc content of Bathua (*Chenopodium album*) and Fenugreek (*Trigonella foenum graecum*) leaves. *Plant Food Hum Nutr* 1999;**53**:255–263.

46 Nabrzyski M. Functional role of some minerals in food. In: Szefer P, Nriagu JO (eds) *Mineral Components in Foods*. Chemical and Functional Properties of Food Components Series. CRC Press, Boca Raton, FL, 2007, pp. 123–161.

47 Amaro MA, Zurera G, Moreno R, Cañal C. Effect of industrial proccesing on the mineral content of white asparagus. *Int J Food Sci Technol* 1997;**32**:401–406.

48 Amaro-López MA, Moreno-Rojas R, Zurera-Cosano G, Sánchez-Segarra PJ. Nutritional changes in the essential trace elements content of asparagus during industrial processing. *Food Res Int* 1999;**32**:479–486.

49 Wang N, Hatcher DW, Toews R, Gawalko EJ. Influence of cooking and dehulling on nutrional compostion of several varieties of lentils (Lens culinaris). *LWT Food Sci Technol* 2009;**42**:842–848.

50 Khalil MM. Effect of soaking, germination, autoclaving and cooking on chemicals and biological value of guar compared with faba bean. *Nahrung/Food* 2001;**45**:246–250.

51 Giulian R, dos Santos CEI, Shubeita SM, da Silva LM, Yoneama ML, Dias JF. The study of the influence of industrial processing on the elemental composition of mate tealeaves (Ilex paraguarienses) using PIXE technique. *LWT Food Sci Technol* 2009;**42**:74–80.

52 Allen A, de Benoist B, Dary O, Hurrell R. *Guidelines on Food Fortification with Micronutrients*. World Health Organization/Food and Agricultural Organization of the United Nations, Rome, 2006.

53 Chaudhari R, Fanion M. Technical aspects of micronutrient addition to foods. In: Ottaway PB (ed.) *Food Fortification and Supplementation: Technological, Safety and Regulatory Aspects*. CRC Press, Boca Raton, FL, 2008.

54 Domínguez R, Barreiro T, Sousa E *et al*. Study of the effect of different iron salts used to fortify infant formulas on the bioavailability of trace elements using ICP-OES. *Int Dairy J* 2004;**14**:1081–1087.

55 Ellis RD, Salt ED. Plants selenium and human health. *Curr Opin Plant Biol* 2003;**6**:273–279.

56 Pyrzynska K. Selenium speciation in enriched vegetables. *Food Chem* 2009;**114**:1183–1191.

57 Kápolna E, Fodor P. Speciation analysis of selenium enriched green onions (Allium fistulosum) by HPLC-ICP-MS. *Microchem J* 2006;**84**:56–62.

58 Davis PA, McDowell LR, Wikinson NS *et al*. Comparative effects of various dietary levels of Se as selenium selenite or Se yeast on blood, wool, and tissue Se concentrations of wether sheep. *Small Ruminant Res* 2008;**74**:149–158.

59 Muñiz-Naveiro O, Domínguez-González R, Bermejo-Barrera A *et al*. Selenium content in cows' milk supplemented with two dietary selenium sources. *J Agric Food Chem* 2005;**53**:9817–9822.

60 Muñiz-Naveiro O, Domínguez-González R, Bermejo-Barrera A, Bermejo-Barrera P, Cocho JA, Fraga JM. Study of the bioavailability of selenium in cows' milk after a supplementation of cow feed with different forms of selenium. *Anal Bioanal Chem* 2006;**385**:189–196.

61 Sharma VK, Shon M. Aquatic arsenic: toxicity, speciation, transformations and remediation. *Environ Int* 2009;**35**:743–759.

62 Cornelis R, Caruso J, Crews H, Heumann K (eds) *Handbook of Elemental Speciation II. Species in the Environment, Food,*

Medicine and Occupational Health. John Wiley & Sons, Chichester, 2005.

63 Ebdon LP, Cornelis R, Crews H, Donard OFX, Quevauviller P. *Trace Element Speciation for Environment, Food and Health*. Royal Society of Chemistry, London, 2001.

64 Laparra JM, Vélez D, Montoro R, Barberá R, Farré R. Bioaccessibility of inorganic arsenic species in raw and cooked Hijikia fusiforme seaweed. *Appl Organometal Chem* 2004;**18**:662–669.

65 García-Sartal C, Barciela-Alonso MC, Bermejo-Barrera P. Effect of cooking procedure on the arsenic speciation in the bioavailable (dialyzable) fraction from seaweed. *Microchem J* 2012;**105**:65–71.

66 Almela C, Laparra JM, Vélez D, Barberá R, Farré R, Montoro R. Arsenosugars in raw and cooked edible seaweed: characterization and bioacessibility. *J Agric Food Chem* 2005;**53**: 7344–7351.

67 Devesa V, Martínez A, Súñez MA, Vélez D, Almela A, Montoro R. Effect of cooking temperatures on chemical changes in species of organic arsenic in seaweed. *J Agric Food Chem* 2001;**49**:2272–2276.

CHAPTER 9
Speciation analysis of food

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Abstract: The main problems related to the presence of the specific chemical forms of mineral elements in foods are discussed in a general framework of their bioavailability and bioaccessibility with regard to both the essential and toxic elements. The oxidation state and the chemical inorganic and organic forms are related to the evolution of minerals from the earth's crust to the waters and biota and their amplification and/or detoxification through the food chain. Special cases concerning the most studied elements are related to their natural cycles and recent changes in the legal framework.

Keywords: speciation, bioavailability, bioaccessibility, oxidation state, chemical forms

9.1 Speciation, bioaccessibility and bioavailability

Trace elements play an important role in the life of our planet. Some elements can be highly toxic to various life forms; others are considered essential. Moreover, there are some elements that can be beneficial at low concentrations, whereas at high concentrations they become toxic, depending on the separation between deficiency, essentiality and toxicity of the element in question. Many of these effects depend strongly on the particular chemical form in which the element is present in the system and it seems clear that the total content of an element does not reflect or provide the desired information about its toxicity and/or uptake.

As Templeton *et al.* [1] stated in the International Union of Pure and Applied Chemistry (IUPAC) guidelines for terms related to chemical speciation, often these different chemical forms of a particular element or its compounds are referred to as 'species'. In that paper, the authors presented an attempt to provide a clear definition of chemical speciation. They proposed that the chemical species of an element are its specific forms such as its isotopic composition, electronic or oxidation state, and/or complex or molecular structure. On the other hand, the speciation of an element is the distribution of this element among the defined chemical species in a system.

Other important terms that should be defined together with speciation in the fields of human nutrition and food quality and safety are bioaccessibility and bioavailability. Bioaccessibility has been defined as the fraction of a compound which is released from the food matrix in the gastrointestinal lumen and thereby made available for intestinal absorption [2]. Mastication in the mouth initiates the process and several digestive fluids, containing different enzymes, continue to break down the food matrix in the stomach and throughout the remainder of the gastrointestinal lumen [3]. This process is influenced by the composition of the digested food matrix, the synergisms and antagonisms of the different components, but also by physicochemical properties such as pH, temperature and texture of the matrix [4].

Bioavailability is defined as the rate and extent to which the compound present in food is absorbed, retained or metabolized through normal pathways. For this purpose the concept of 'total utilization' defines the fraction of a nutrient that is ultimately used in metabolism after its digestion, absorption and distribution [5].

All the aforementioned aspects must be taken into account to provide a complete picture of the mineral profile of foods and its importance in human nutrition and the main objective of this chapter is to focus on the speciation problem without considering the analytical

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methodologies suitable for demonstrating speciation in foods which will be considered in detail in Chapter 18.

9.2 From the isotopic composition to the molecular structure

The periodic table is composed by 118 elements. The first 98 elements exist naturally though some are found only in trace amounts and were initially discovered by synthesis in laboratories. Elements with atomic numbers 99 to 118 have only been synthesized, or claimed to be so, in laboratories, with elements 113, 115, 117 and 118 still awaiting confirmation. Figure 9.1 shows the periodic table with the 118 elements, their atomic number and electronic configuration. The insets of Figure 9.1 highlight the most stable isotopes and the possible oxidation states of Cr and As, as examples of the complexity of mineral element behaviour with regard to their presence in nature and impact on human health.

9.2.1 Isotopic composition

When an element has atoms that differ in the number of neutrons in the nuclei, these atoms are called isotopes of the element. All isotopes of one element have identical chemical properties, and thus it is difficult to separate isotopes by chemical processes. However, the physical properties of the isotopes, such as their masses, boiling points and freezing points, are different.

Stable isotopes do not spontaneously disintegrate by any known mode of decay. To date, 270 stable nuclides and over 1700 radionuclides have been identified. Commonly analysed stable isotope systems include those of oxygen, carbon, nitrogen, hydrogen and sulfur. These elements are commonly called environmental isotopes: they are the naturally occurring isotopes of elements found in abundance in the environment. The stable isotopes of these elements serve as tracers of water, carbon, nutrient and solute cycling. The main applications of stable isotope analysis include hydrogeology cycle measurements [6], palaeotemperature measurement in palaeoclimatology [7], analysis of the origin of drugs in forensic science [8], in doping control to distinguish between endogenous and exogenous sources of hormones [9], and in food analysis where the content of hydrogen and oxygen are reliable indicators of climatic characteristics, as are the $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios, which depend on local agricultural practices and animal diets [10]. Thus, stable isotope ratio has been demonstrated to be a good descriptor for different food products, providing unique and representative fingerprints that make it possible to differentiate between food samples of different Protected Designation of Origin [11]. Additionally, nowadays modern inductively

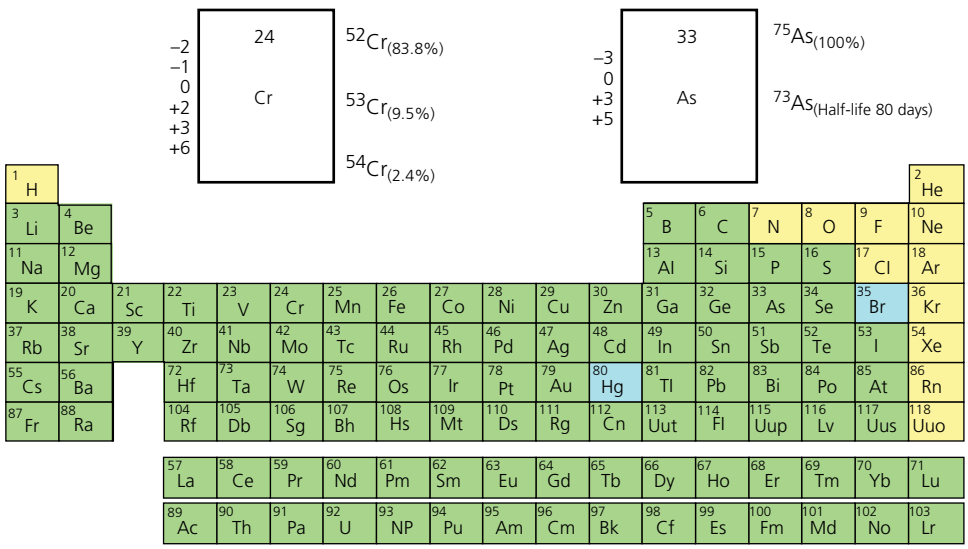


Figure 9.1 Periodic table of the elements. *Inset*: stable isotopes and possible oxidation states of Cr and As as examples of the complexity of mineral element behaviour. (See insert for colour representation of the figure.)

coupled plasma (ICP)–mass sprectrometry (MS) techniques are suitable for evaluating the presence of different isotopes in foods.

9.2.2 Electronic or oxidation state

Oxidation state is defined by IUPAC as the charge an atom might have when electrons are counted according to an agreed-upon set of rules.

1 The oxidation state of a free element (uncombined element) is zero.
2 For a simple (monatomic) ion, the oxidation state is equal to the net charge on the ion.
3 In a particular molecule or ion, hydrogen has an oxidation state of 1 and oxygen has an oxidation state of −2 when they are present in most compounds. Exceptions to this are that hydrogen has an oxidation state of −1 in hydrides of active metals, e.g. LiH, and oxygen has an oxidation state of −1 in peroxides, e.g. H_2O_2.
4 The algebraic sum of oxidation states of all atoms in a neutral molecule must be zero, while in ions the algebraic sum of the oxidation states of the constituent atoms must be equal to the charge on the ion [12].

As an example of the importance of the oxidation state, the toxic effect of the aqueous species of arsenic varies from As(III), which is 10 times more toxic than As(V), to the As organic species which are 70 times less toxic than As(III) [13]. Another classical example of the different levels of toxicity due to different oxidation states of the same element is the case of chromium. The relevant oxidation states of chromium are Cr(0), Cr(III) and Cr(VI) and there are marked differences of these oxidation states regarding their presence in foods. Cr(III) is considered to be essential while Cr(VI) is carcinogenic [14].

Moreover, oxidation state can also affect the absorption and elimination of an element. The Fe(II) ion is soluble under physiological conditions and diffuses freely across membranes, whereas Fe(III) does not enter cells readily and is prone to hydrolysis in aquatic and biological systems.

9.2.3 Complex or molecular structure

The highest structural level at which speciation analysis will be considered in this chapter is the molecular level, including macromolecules. The distribution of an element among different compounds, molecules and/or complexes profoundly affects its transport and bioavailability by determining its charge, solubility and diffusion coefficient.

The practical importance of inorganic speciation analysis is well illustrated by occupational exposure to nickel and its compounds. Nickel chloride and sulfate are water soluble and present low toxicity. On the other hand, nickel oxides and sulfides are highly insoluble in water, but their bioavailability may be influenced by biological ligands. Trinickel disulfide (subsulfide) (Ni_3S_2) is a potent carcinogen in animals [15].

Complexation with inorganic and organic ligands produces coordination complexes with differing degrees of thermodynamic stability and lability. The distribution of species in a metal–ligand complex also depends on concentration, stoichiometry, pH and ionic strength. This means that such species cannot normally be separated from each other without distribution changes. However, the lability of inorganic and organic complexes varies within very wide limits, from complexes that form and dissociate rapidly, to systems in slow exchange, to complexes that, for most practical analytical purposes, can be considered inert. For many metals, formation of hydroxides in aerobic aqueous environments at neutral pH is a key determinant of their solubility and bioavailability. Most metals, excluding alkali and alkaline earth elements, form one or more hydroxocomplexes under natural conditions [16]. A large variety of inorganic complexes can be found in the environment. Anions such as SO_4^{2-} and Br^- generally form weak complexes which are not very important in open fresh waters, but could be important in certain waters such as marine, well water or soil solutions. Other anions such as F^- and CO_3^{2-} are important for specific elements. However, it is clear that changes in pH can dramatically modify the equilibrium between species, being of special concern for aquatic environments and having a tremendous effect on the presence of minerals in foods.

Other type of compounds considered in this chapter are the organometallic compounds. They are defined as compounds containing a metal–carbon bond with a largely covalent character. In general, in the environment, metals undergo only biomethylation (Hg, Ge and Sn) (Figure 9.2). However, in some cases, alkylated species were introduced into the environment for specific purposes; for example, lead alkyl derivatives were used as antiknock agents in car motors, manganese alkyl-derivatives and manganese carboxylic species were

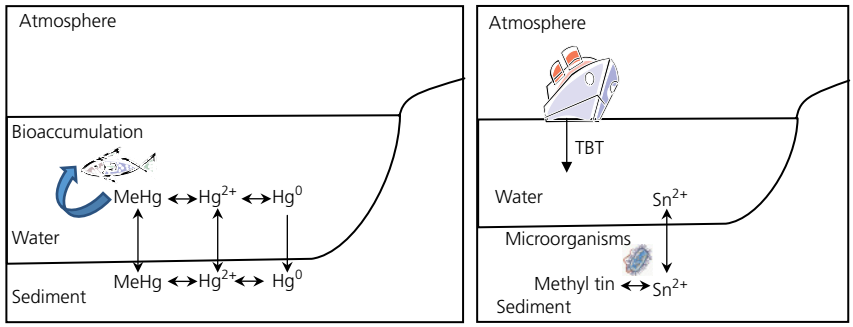


Figure 9.2 Natural biomethylation processes that increase the toxicty of Hg and Sn.

employed as additives in aircraft fuels, and tributyltin (TBT) was used as an antifouling agent in boat paints that ultimately are liberated to the environment and can enter the food chain. Biomethylation is generally restricted to microorganisms in sediments and soils and generally increases metal toxicity [17]. On the other hand, there are specific cases, like that of As, where the natural methylation process involves a strong reduction in the toxicity of this element [13]. In addition to the products of natural bioalkylation, manufactured organometallics can be released into the environment and can follow different detoxification or amplification routes. Those of major interest in speciation analysis include compounds of As, Sn, Hg and Pb [18].

Going a step further, we can consider macromolecular structures. Natural organic matter is a heterogeneous mixture of organic compounds derived from decomposition of biotic matter. In the environment, binding to dissolved natural organic matter, such as the polyanions humic and fulvic acids, contributes to the pattern of transport and availability of elements such as Cd, Cu and Pb, thereby enhancing solubility and mobility in the environment [19]. This phenomenon continues inside organisms because of the link between minerals and transportation proteins, and the generation of mineral complexes with sugars or lipid compounds.

9.3 Challenging elements in food for speciation analysis

Food products typically contain more than 60 elements. Usually, they are classified into two groups: the major components, including K, Na, Ca, Mg, Cl, S, P and C, and the trace elements. Among the trace elements, which are usually present in amounts of less than 50 ppm, are the essential nutritive elements (Fe, Cu, I, Co, Mn, Zn, Cr, Ni, Si, F, Mo and Se), the non-nutritive but non-toxic elements (Al, B and Sn), and the non-nutritive toxic elements (Hg, Pb, As, Cd and Sb) [20]. Additionally, it must be noted that the nutritional value of a food containing a given mineral depends not only on its content but also on its bioavailability for humans.

The examination of trace element speciation in food is extremely important for understanding the biological activity. Some trace elements attract the interest of researchers and have been and are extensively studied. This is case for Se, As and Hg. On the other hand, other elements such as Co, I, Mn, Fe, Zn, Cu and Mo have received little attention thus far [21].

Arsenic represents a relevant example of the importance of speciation in relation to food safety. The chemical forms of arsenic dominating in most seafoods, arsenobetaine, is considered non-toxic, whereas inorganic arsenic is highly toxic and should be monitored and controlled in relevant foods and in drinking water (Figure 9.3). Additionally, the numerous studies made on As in foods have evidenced the presence of chemical forms of this element like monomethylarsine and dimethylarsine and the presence of arsenosugars and arsenolipids not well known until now. Another classical example is mercury. Organic mercury compounds, of which methylmercury is the most common, are of special concern because of their enhanced toxicity. The route of human exposure to methylmercury is mainly through the diet especially via fish and shellfish that bioaccumulate this compound [22].

Two stable chromium species are known in the environment: Cr(III) and Cr(VI). Cr(III) appears to be essential for mammalian species and, together with insulin, is

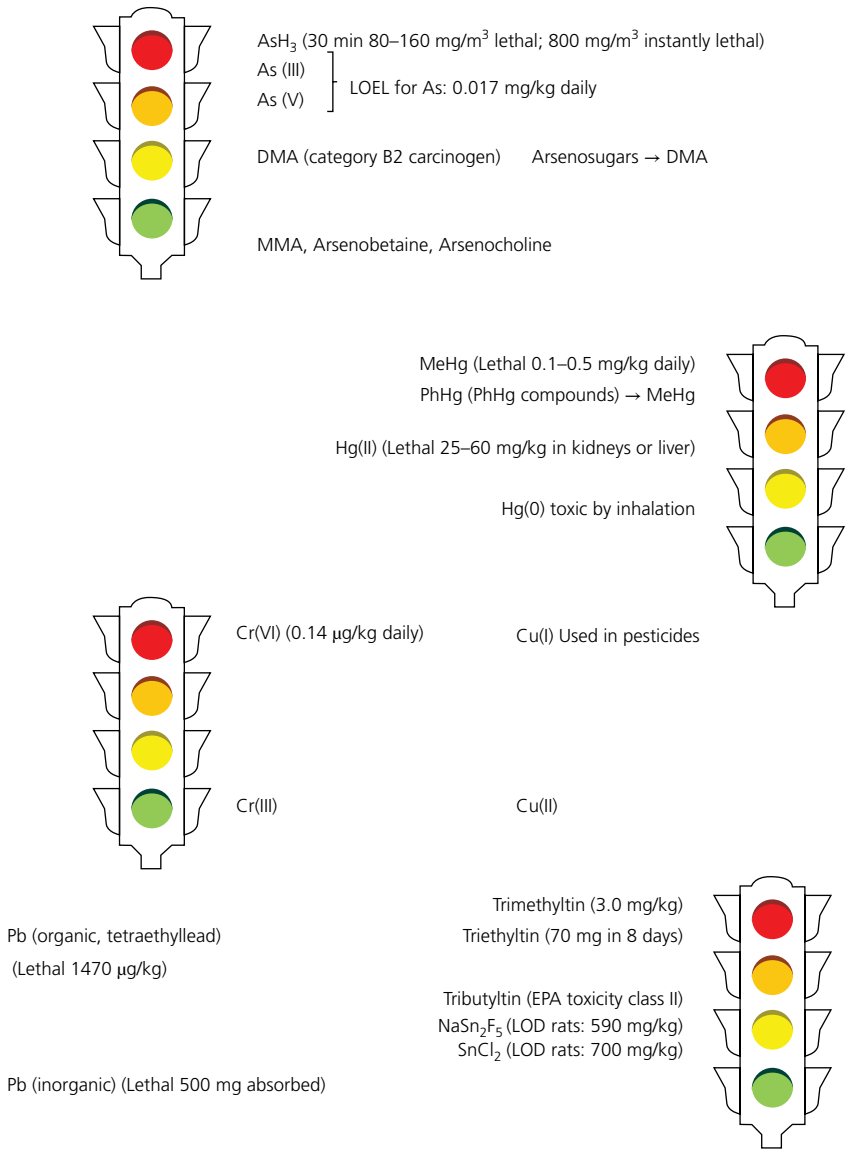


Figure 9.3 Toxicity of different species of mineral elements with regard to food safety. (See insert for colour representation of the figure.)

responsible for removing glucose from the bloodstream. Conversely, hexavalent chromium has toxic and carcinogenic effects. The toxic nature of the Cr(VI) ions is attributed to their high oxidation potential and their relatively small size, which enables them to penetrate biological cell membranes. Similar behaviour could be considered for copper: Cu(II) is an essential element with key importance in redox enzymes, while Cu(I) is a very toxic compound.

In contrast to the inorganic forms of selenium, the organoselenium compounds are of considerable interest and several of them play essential roles in cell biochemistry and nutritional science. In feed ingredients, such as forage, grains, and oilseed meals, selenium is incorporated into the amino acids and proteins as selenomethionine and selenocysteine.

A special case of the importance of the chemical forms of a specific element on its toxicity is shown by tin and

lead, elements 50 and 82 in the periodic table, both corresponding to the 4A series. Pb has a clearly toxic nature, while Sn manifests a practically innocuous character, so that it was traditionally used in canned foods as a sacrifice anode in order to avoid the dissolution of Pb from the welding [23].

With regard to the lethal dose of the inorganic chemical forms of Pb and Sn, it can be seen that 500 ng absorbed inorganic lead is lethal, as compared with 700 ng/kg body weight $SnCl_2$ or 590 ng/kg body weight $NaSn_2F_5$. However, with regard to the organic forms of these elements, the lethal dose of Pb as tetraethyl lead is as low as 1470 μg/kg body weight, whereas the lethal dose of the organic Sn species trimethyltin is 3 mg/kg body weight and that of triethyltin 80 mg in 8 days. Another organic Sn species, TBT, is classified as a toxicity class II compound by the United States Environmental Protection Agency (US EPA). TBT has created havoc in the Arcachon Bay area of France [24] because of its impact on molluscs, specifically oysters, thus moving many authorities to strictly limit the use of TBT as a marine antifouling agent [25].

9.4 The cycle of the elements from nature to food sources and metabolites

It is well known that certain substances move endlessly through the earth's biosphere, hydrosphere, atmosphere and lithosphere, existing in different forms and being used by different organisms at different times, in a similar way to the water cycle. Water evaporates into the atmosphere from the land and the sea. Plants and animals use and reuse water and release water vapour into the air. Once in the air, water vapour circulates and can condense to form clouds and precipitation, which fall back to earth.

The main sources and some pathways of element movements in the environment are shown in Figure 9.4. Ecological systems are characterized by a high degree of complexity, where the interpretation of ecological patterns is hindered by the multiple processes co-occurring at different spatial and temporal scales. Each of the elements has its own biogeochemical cycle and each cycle has a unique pathway characterized by reservoirs,

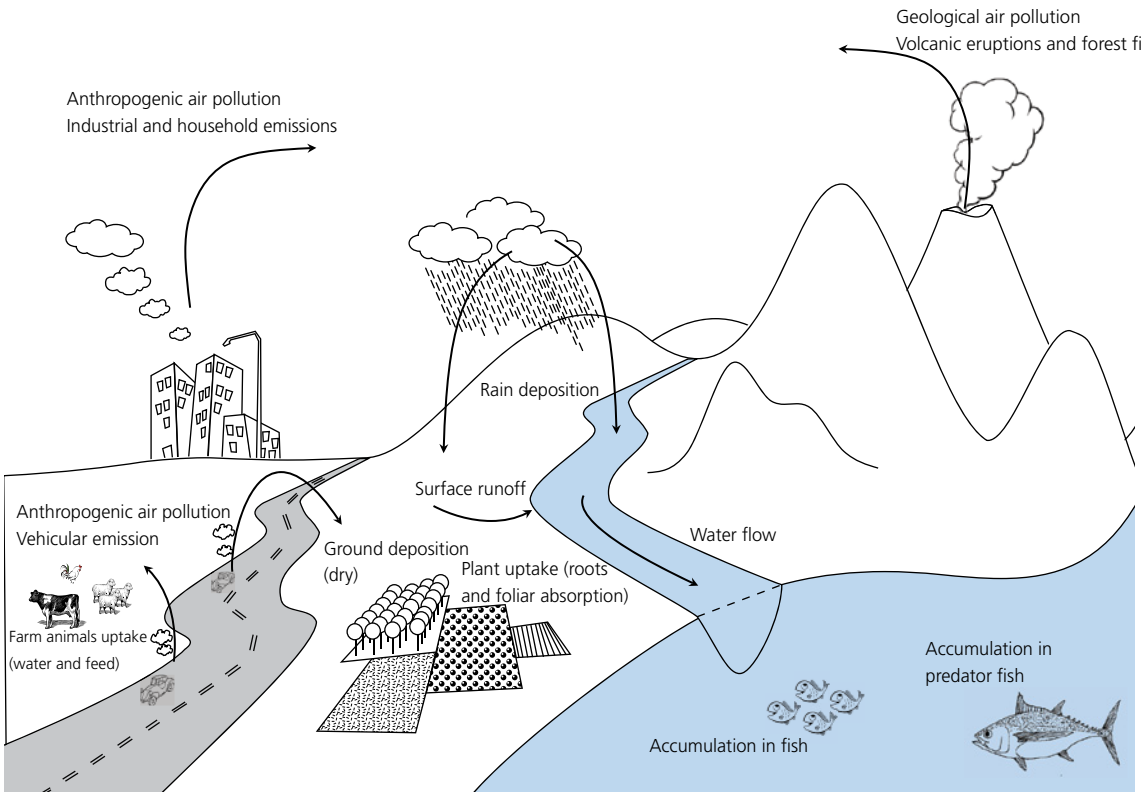


Figure 9.4 Main sources and some pathways of the movement of elements in the environment.

exchange pools and residence times. Moreover, human activities can influence those biogeochemical cycles.

We focus on the nitrogen cycle as an example. Nitrogen is required for the manufacture of all amino acids and nucleic acids. However, atmospheric nitrogen cannot be used for these tasks and most organisms are dependent on the nitrogen cycle as a source of nitrogen. Nitrogen exists in the atmosphere as N_2 or in the soil as ammonium (NH_4^+), ammonia (NH_3), nitrite (NO_2^-) or nitrate (NO_3^-). Nitrogen is assimilated into living organisms through three stages: nitrogen fixation, nitrification, and plant metabolism. Nitrogen fixation is a process by which atmospheric N_2 is converted to NH_4^+ or NO_3^- after fixation by microorganisms (fixation also occurs via the action of lightning). During nitrification, ammonium is converted by bacteria into nitrite, and finally into nitrate. In the final stage, plants absorb ammonia and nitrate, which are incorporated into metabolic pathways. Once the nitrogen has entered the plant metabolic pathway, it may be transferred to animals. Nitrogen is released back to the cycle by denitrifying bacteria which convert NO_3^- into N_2, by detrivorous bacteria that convert organic compounds into ammonia, or by animal excretion of ammonia, urea or uric acid. It can be concluded that chemical forms and not just total nitrogen are important to assure the metabolic pathway and the same occurs for all the elements which migrate from the soil to water and foods.

9.4.1 Uptake and accumulation

Plants play a major role in the entry of metals and metalloids into the food chain [26]. Wastewater irrigation, solid waste disposal, sludge applications, vehicular exhaust and industrial activities are the major sources of soil contamination with heavy metals. Metals from both natural and pollutant sources have the potential to be assimilated by the plant through foliar or root absorption processes. On the other hand, the absorption of chemical species by plant roots is dependent on a number of plant factors, including (i) the pH and redox potential of the medium; (ii) the chemical form of the minerals; (iii) physical processes such as root intrusion, water and ion fluxes and their relationship to the kinetics of metal solubilization in soils; (iv) biological parameters, including kinetics of membrane transport, ion interactions, and metabolic fate of absorbed ions; and (v) the ability of plants to adapt metabolically to changing metal stresses in the environment.

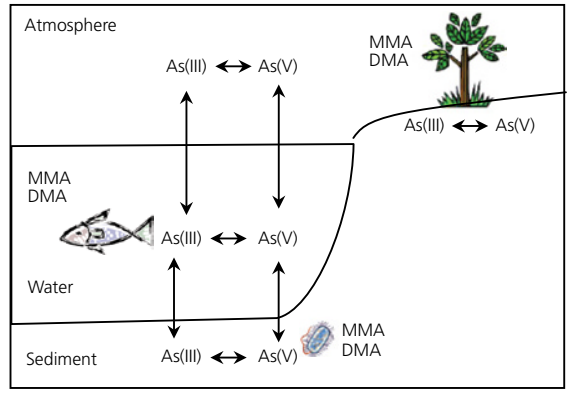


Figure 9.5 Schematic representation of the arsenic cycle in nature. MMA, monomethylarsine; DMA, dimethylarsine.

Figure 9.5 shows a schematic representation of the arsenic cycle in the environment and plants. Arsenic sources in the environment are natural (geological) or the result of human activities (anthropogenic) based on its use in pharmaceuticals and pesticides, and the equilibrium between arsenate, As(V), and arsenite, As(III), in soil solutions is mainly dependent on the redox conditions of soils. Microbial transformation of inorganic to organic arsenic compounds generally occurs through methylation, as one or more OH groups are replaced by CH_3 groups, the products of this reaction being monomethyl-, dimethyl- and trimethyl-arsines [27]. Arsenate is taken up in roots by phosphate transporters and arsenite is taken up by a subclass of aquaporins, some of them also transporting silicon. Methylated forms of As are also taken up by aquaporins and Si transporters. Inside plants, these types of transporters are also involved in the distribution of As between organs and tissues. As(V) is enzymatically reduced to As(III) in plant cells by arsenate reductase, leading to the conversion of glutathione to its oxidized form. Arsenite can be effluxed to the environment by a root Si transporter or methylated. A cascade of methylation can then transform As into a gaseous form that is volatilized. Another pathway of detoxification occurs by the synthesis of phytochelatins corresponding to a three-step enzymatic condensation of three amino acids: cysteine, glutamate and glycine. The synthesis of phytochelatins and their complexation to As(III) is coordinated with transport of the phytochelatin–As(III) complex to the vacuolar subcellular compartment through the activity of two members of a subclass of ATP-binding cassette

(ABC) transporters, ABCC1 and ABCC2 [28]. These mechanisms explain the decontamination of As through the food chain in the case of fish, but recently it has been observed that in rice the main arsenic species are the inorganic ones, so that strict control of As in rice produced in polluted areas is necessary [29].

Among the common pollutants that affect plants, lead is one of the most toxic and frequently encountered [30]. Lead continues to be used widely in many industrial processes and occurs as a contaminant in all environmental compartments (soils, water, atmosphere, and living organisms). Despite a long history of its beneficial use by humankind, lead is now recognized as a chemical of great concern in the new European Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulations [31]. In soils, lead may occur as a free metal ion, complexed with inorganic constituents or as organic ligands; alternatively lead may be adsorbed onto particle surfaces [32]. Because of its strong binding with organic and/or colloidal materials, it is believed that only small amounts of the lead in soil are soluble and thereby available for plant uptake [33]. The main pathway by which plants accumulate metals is via root uptake from soil solution. At the molecular level, the mechanism by which lead enters roots is still unknown but may involve ionic channels, cyclic nucleotide-gated ion channels and low-affinity cation transporters [34]. Additionally, it must be taken into consideration that pH changes, due for example to acid rain, dramatically modifies the availability of lead for plants and fish and thus enters the food chain.

The case of mercury is the opposite of that for As. The species of mercury discharged to the environment is unimportant because any specie of mercury may be converted by microorganisms to methylmercury, a highly toxic compound. Over 90% of the total Hg in fish tissue is methylmercury [35]. It can be obtained by fish from food and from water passing over the gills during respiration. In addition, methylmercury can be produced within the fish gastrointestinal tract [36] and on the external slime layer [37], but the amount of methylmercury contributing to tissue concentrations via these processes has not been quantified and is assumed to be insignificant. This mercury is accumulated in fish, shellfish and fish-eating birds and mammals, resulting in biomagnification of the mercury from the water that enters the food chain (see Figure 9.2).

It is generally known that plants grown in acid soils due to aluminium solubility at low pH have reduced root systems and exhibit a variety of nutrient-deficiency symptoms, with a consequent decrease in yield. Different forms of aluminium occur in soil solution: $Al(OH)^{2+}$ and $Al(OH)_2^+$ at pH 4–5, Al^{3+} at pH 5.5–7, and $Al(OH)_4$ at pH 7–8. Other complex ions suchas $AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+}$ (Al_{13}) and Al^{3+} are almost certainly toxic. The following Al species are toxic for wheat roots in the following increasing order: $AlF_2^+ < AlF^{2+} < Al^{3+} < Al_{13}$. Al ions translocate very slowly to the upper parts of plants [38]. Most plants contain no more than 0.2 mg Al per gram dry mass. However, some plants, known as Al accumulators, may contain over 10 times more Al without any injury. Tea plants are typical Al accumulators: the Al content in these plants can reach as high as 30 mg/g dry mass in old leaves [39]. Approximately 400 species of terrestrial plants, belonging to 45 families, have so far been identfied as hyperaccumulators of various toxic metals [40]. It is clear that tolerant food crops or animals, if exposed to heavy metals in their growth medium, may be dangerous as carriers of toxic metals in the food chain, leading to food toxicity.

On the other hand, some elements are intentionally added to farm animal alimentation by addition to feedstuffs to prevent deficiency-related diseases. Selenium-related diseases occur among farm animals in many parts of the world and are due to the low selenium concentrations in forage crops; these may be due to either low levels of selenium in the soil or the soil selenium being unavailable to the plants. In these cases, selenite and selenium yeast are used as feed supplements for dairy cows. Phosphorus in fish feed is added in the form of phosphates. However, the availability of phosphate in fish is quite low and should be increased by feed supplements such as phytase.

9.4.2 Intake, bioavailability, bioaccessibility and excretion

As discussed, metals are ubiquitous in the environment as a result of both natural and anthropogenic activities, and humans are exposed to them through various pathways, especially the food chain. For humans, food is the major non-occupational source of exposure to heavy metals [41]. As essential part of food, vegetables are important source of these metals. Vegetables cultivated in contaminated soils take up heavy metals in sufficient quantities to cause potential health risks to consumers [42]. This process of bioaccumulation of metals occurs in all living organisms as a result of exposure to metals

in food and the environment, including food animals such as fish and cattle.

The main concern in relation to the toxicity of mercury in the general population is the potential effect of organic forms of mercury on the brain and intellectual development in young children. Mercury is relatively widely distributed in food at very low levels, primarily in the less toxic inorganic form; the most toxic form of mercury, methylmercury, is found in significant levels only in fish and seafood, in particular top predatory fish. Lead also has an effect on brain and intellectual development in young children, while long-term exposure in both children and adults can cause damage to the kidneys and reproductive and immune systems in addition to effects on the nervous system. Lead contamination of food arises as a result of environmental emissions such as mining and the nowadays diminished use of leaded petrol. However, like mercury, lead can accumulate in fish and shellfish and in addition can be found at high levels in the offal (liver and kidney) of food animals. Cadmium is toxic to the kidney, while exposure to high levels of tin from food in incorrectly manufactured tins can cause gastrointestinal irritation and upsets. Cadmium is present at low levels in most foods, with commodities such as cereals, fruit, vegetables, meat and fish making the largest contribution to dietary exposure. The highest levels of cadmium are found in the offal (kidney and liver) of mammalians, in mussels, oysters and scallops, and in certain wild mushrooms. Exposure to inorganic arsenic is of concern because of its cancer-causing properties. Data on arsenic occurrence show that fish and seafood account for over 90% of total exposure to arsenic in food [43] but with increasing levels of As being reported in farm chicken [44] and rice [45]. Moreover, organotins occur mainly in aquatic organisms and intake of seafood may be an important source of human exposure. Other sources of exposure to tin include canned foods, but in this case the chemical forms of Sn seem to be safer than TBT.

In addition to knowing the total amount of mineral contaminant and the involved species, it is also important to know the bioavailability of such chemical forms. It is often not known how much of the ingested contaminant is actually taken up in the human body. It must be considered that only the fraction of the contaminant that is released from the food (bioaccessible) and which is bioavailable (concentration in blood, organ and tissues) can exert toxic effects. Studies in experimental animals and humans indicate oral bioavailability of compounds to be dependent on the food product, food processing or food preparation. This hampers an accurate risk assessment of ingested toxic compounds in humans [46]. Moreover, *in vitro* models in which the digestive processes are simulated in a simplified manner, allowing for measurement of the bioaccessibility of ingested contaminants from food as an indicator of oral bioavailability, have also been successfully developed and provide valuable information regarding food safety.

It is clear that the danger following exposure to heavy metals comes from the fact that they are accumulated in renal and liver tissue and poorly excreted in the urine. Plasma heavy metals (toxic or not) exist as non-diffusible (protein-bound) and diffusible (complexed and ionized) species. Acute intoxications of divalent heavy metals by absorption of a salt initially lead to an increase in the inorganic form in the plasma. Heavy metal ions bind rapidly to albumin, the most abundant protein in plasma, with affinity to albumin probably not the same for all heavy metals. They readily bind to the free sulfhydryl group of terminal cysteine residues and to histidine residues [47]. However, a small fraction escapes binding and the plasma also contains the free form of the heavy metal. The ionized form of heavy metals is the toxic form. It appears that conjugates and bound forms of filtered heavy metals are not toxic by themselves. Finally, renal uptake following acute intoxication is probably due to the membrane transport of both albumin-bound and free forms of heavy metals. During chronic intoxication, the concentration of heavy metal-binding proteins increases in the blood. Heavy metals are known to be potent inducers of metallothioneins and glutathione in renal and liver tissues [48]. Their synthesis is induced by the free form of the heavy metal [49]. Metallothioneins are low molecular weight cysteine-rich proteins and glutathione is a tripeptide containing one cysteine. Both peptides, synthesized in the liver and the kidney, protect against heavy metal toxicity by trapping the metal inside the cells by conjugate formation [47]. The renewal of liver cells during intoxication leads to the release of these conjugates into the systemic circulation where they are subsequently delivered to the kidneys [50]. Because of this, speciation studies regarding the molecular weight of associated chemical forms of toxic elements are of great importance in evaluating their toxicity.

9.5 The role of elemental speciation in legislation

As discussed in the previous sections, the biological activities and toxicity of mineral and trace elements are highly dependent on the involved species. In turn, advances in toxicological knowledge of species must move forward hand in hand with more specific legislation of hazardous substances. Although legislators are becoming increasingly aware of the importance of elemental speciation, there is a slow process of implementation of speciation information into legislation, probably due to the complexity of analytical instrumentation, the high sensitivity required in most cases, together with the lack of reliable and detailed toxicological data.

Some examples of the legislation related to elemental species include European Community (EC) regulation of marine pollution. List II of substances includes organic compounds of phosphorus, silicon and tin and substances which may form such compounds in the marine environment, excluding those which are biologically harmless or which are rapidly converted in the sea into substances which are biologically harmless, and the following elements and their compounds: arsenic, chromium, copper, lead, nickel and zinc [51]. List I of the EC directive on groundwater pollution includes organohalogen, organophosphorus and organotin compounds and mercury and cadmium compounds [52]. The Regulation for the Restriction of the Use of Certain Hazardous Substances in Electrical and Electronic Equipment 2005 [53] includes hexavalent chromium. Concentration of Cr(VI) is also regulated in cement [54]. Organotins have been banned in antifouling paints [25, 55], especially TBT and triphenyltin.

9.6 Conclusions and future trends

The field of speciation is changing and much research has moved from the bench to real life, thus providing appropriate methodologies and data about the species present in the environment, water and biota and their transformation and transport through the food chain. It is true that most speciation studies are focused on a reduced list of elements and chemical species, and additional efforts must be made to characterize the chemical forms of essential and toxic elements in foods in order to provide data to law and policy-makers. Interdisciplinary studies covering basic and applied analytical chemistry and environmental and nutritional aspects must be taken into consideration in a complementary perspective. Special developments will be produced in the next few years due to improvements in high-performance instruments like ICP-MS using high-resolution MS and through the coupling between liquid chromatography and MS detectors but additional efforts must also be made to provide cheap vanguard screening alternatives suitable to be used in control laboratories around the world.

Abbreviations

ABC: ATP binding cassette
EC: European Community
ICP: Inductively coupled plasma
IUPAC: International Union of Pure and Applied Chemistry
LC: Liquid chromatography
MS: Mass spectrometry
REACH: Registration, Evaluation, Authorisation and Restriction of Chemicals
TBT: Tributyltin
US EPA: United States Environmental Protection Agency

References

1 Templeton DM, Ariese F, Cornelis R *et al*. Guidelines for terms related to chemical speciation and fractionation of elements. Definitions, structural aspects, and methodological approaches (IUPAC Recommendations 2000). *Pure Appl Chem* 2000;**72**:1453–1470.
2 Saura-Calixto F, Serrano J, Goñi I. Intake and bioaccessibility of total polyphenols in a whole diet. *Food Chem* 2007;**101**:492–501.
3 Gropper SS, Smith JL. *Advanced Nutrition and Human Metabolism*, 6th edn. Wadsworth, Belmont, CA, 2013, pp. 33–62.
4 Neilson AP, Ferruzzi MG. Influence of formulation and processing on absorption and metabolism of flavan-3-ols from tea and cocoa. *Annu Rev Food Sci Technol* 2011;**2**:125–151.
5 Schümann K, Elsenhans B. The impact of food contaminants on the bioavailability of trace metals. *J Trace Elem Biol* 2002;**16**:139–144.
6 Clark I, Fritz P. *Environmental Isotopes in Hydrogeology*. CRC Press, Boca Raton, FL, 1997.
7 McDermott F. Palaeo-climate reconstruction from stable isotope variations in speleothems: a review. *Quaternary Sci Rev* 2004;**23**:901–918.

8 Ehleringer JR, Cooper DA, Lott MJ, Cook CS. Geo-location of heroin and cocaine by stable isotope ratios. *Forensic Sci Int* 1999;**106**:27–35.

9 Cawley AT, Flenker U. The application of carbon isotope ratio mass spectrometry to doping control. *J Mass Spectrom* 2008;**43**:854–864.

10 Kelly S, Heaton K, Hoogewerff J. Tracing the geographical origin of food: the application of multi-element and multi-isotope analysis. *Trends Food Sci Technol* 2005;**16**:555–567.

11 Gonzalvez A, Armenta S, de la Guardia M. Trace-element composition and stable-isotope ratio for discrimination of foods with Protected Designation of Origin. *Trends Anal Chem* 2009;**28**:1295–1311.

12 McNaught AD, Wilkinson A. *IUPAC Compendium of Chemical Terminology*, 2nd edn. Blackwell Scientific Publications, Oxford, 1997. XML online corrected version: http://goldbook.iupac.org (2006) created by M Nic, J Jirat and B Kosata.

13 Pontius FW, Brown KG, Chen CJ. Health implications of arsenic in drinking water. *J Am Water Works Assoc* 1994;**86**:52–63.

14 Katz SA, Salem H. The toxicology of chromium with respect to its chemical speciation: a review. *J Appl Toxicol* 1993;**13**:217–224.

15 International Agency for Research on Cancer. *Chromium, Nickel and Welding*. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. **49**. IARC, Lyon, 1990.

16 Perera N. *Hydrolysis and cyanide speciation of some heavy metals relevant to the fate of cyanide in the environment*. PhD thesis, Murdoch University, Perth, 2001.

17 Craig PJ. *Organometallic Compounds in the Environment*, 2nd edn. John Wiley & Sons Ltd, Chichester, 2003.

18 Wasik A, Namieśnik J. Speciation of organometallic compounds of tin, lead, and mercury. *Pol J Environ Stud* 2001;**10**:405–413.

19 van Schaik JWJ. *Binding of metals to macromolecular organic acids in natural waters. Does organic matter?* PhD thesis, Swedish University of Agricultural Sciences, Uppsala, 2008.

20 deMan JM. *Principles of Food Chemistry*. Aspen Publishers, Gaithersburg, MD, 1999.

21 Ruzik L. Speciation of challenging elements in food by atomic spectrometry. *Talanta* 2012;**93**:18–31.

22 Jokai Szatura Z. *Challenges in mercury speciation*. PhD thesis, Corvinus University of Budapest, Budapest, 2007.

23 Capar SG. Changes in lead concentration of foods stored in their opened cans. *J Food Safety* 1978;**1**:241–245.

24 Ruiz JM, Bachelet G, Caumette P, Donard OF. Three decades of tributyltin in the coastal environment with emphasis on Arcachon Bay, France. *Environ Pollut* 1996;**93**:195–203.

25 Organotin Antifouling Paint Control (33 U.S.C. 2401) (1988).

26 Colangelo EP, Guerinot ML. Put the metal to the petal: metal uptake and transport throughout plants. *Curr Opin Plant Biol* 2006;**9**:322–330.

27 O'Neill P. Arsenic. In: Alloway BJ (ed.) *Heavy Metals in Soils*, 2nd edn. Blackie Academic & Professional, Glasgow, 1995, pp. 105–121.

28 Briat JF. Arsenic tolerance in plants: "Pas de deux" between phytochelatin synthesis and ABCC vacuolar transporters. *Proc Natl Acad Sci USA* 2010;**107**:20853–20854.

29 Meharg AA. Arsenic in rice: understanding a new disaster for South-East Asia. *Trends Plant Sci* 2004;**9**:415–417.

30 Grover P, Rekhadevi P, Danadevi K, Vuyyuri S, Mahboob M, Rahman M. Genotoxicity evaluation in workers occupationally exposed to lead. *Int J Hyg Environ Health* 2010;**213**:99–106.

31 European Commission. Regulation (EC) No. 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Available at http://ec.europa.eu/enterprise/sectors/chemicals/reach/index_en.htm

32 Uzu G, Sobanska S, Aliouane Y, Pradere P, Dumat C. Study of lead phytoavailability for atmospheric industrial micronic and sub-micronic particles in relation with lead speciation. *Environ Pollut* 2009;**157**:1178–1185.

33 Punamiya PM, Datta R, Sarkar D, Barber S, Patel M, Das P. Symbiotic role of *Glomus mosseae* in phytoextraction of lead in vetiver grass [*Chrysopogon zizanioides* (L.)]. *J Hazard Mater* 2010;**177**:465–474.

34 Pourrut B, Perchet G, Silvestre J, Cecchi M, Guiresse M, Pinelli E. Potential role of NADPH-oxidase in early steps of lead-induced oxidative burst in *Vicia faba* roots. *J Plant Physiol* 2008;**165**:571–579.

35 Spry DJ, Wiener JG. Metal bioavailability and toxicity to fish in low-alkalinity lakes: a critical review. *Environ Pollut* 1991;**71**:243–304.

36 Rudd JWM, Furutani A, Turner MA. Mercury methylation by fish intestinal contents. *Appl Environ Microbiol* 1980;**40**:777–782.

37 Jensen S, Jernelov A. Biological methylation of mercury in aquatic organisms. *Nature* 1969;**223**:753–754.

38 Ma JF, Zheng SJ, Matsumoto H, Hiradate S. Detoxifying aluminum with buck-wheat. *Nature* 1997;**390**:569–570.

39 Matsumoto H, Hiraseva E, Morimura S, Takahashi E. Localization of aluminum in tea leaves. *Plant Cell Physiol* 1976;**17**:627–631.

40 Baker AJM, McGrath SP, Reeves RD, Smith JAC. Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In: Terry N, Banuelos G (eds) *Phytoremediation of Contaminated Soil and Water*. Lewis Publishers, Boca Raton, FL, 2000, pp. 85–107.

41 World Health Organization. Assessment of Chemical Contaminants in Foods. Report on the result of the UNEP/FAO/WHO programme on health related environmental monitoring. United Nations Environment Programme, Nairobi, 1988. Available at http://www.who.int/iris/handle/10665/62913

42 Alam MGM, Snow ET, Tanaka A. Arsenic and heavy metal contamination of vegetables grown in Samta village, Bangladesh. *Sci Total Environ* 2003;**308**:83–96.

43 Food Safety Authority of Ireland. Mercury, lead, cadmium, tin and arsenic in food. Toxicology Fact Sheet Series, Issue No. 1, May 2009. Available at www.fsai.ie/workarea/downloadasset.aspx?id=8412

44 Rutherford DW, Bednar AJ, Garbarino JR, Needham R, Staver KW, Wershaw RL. Environmental fate of roxarsone in poultry litter. Part II. Mobility of arsenic in soils amended with poultry litter. *Environ Sci Technol* 2003;**37**: 1515–1520.

45 Abedin MJ, Cresser MS, Meharg AA, Feldmann J, Cotter-Howells J. Arsenic accumulation and metabolism in rice (*Oryza sativa* L.). *Environ Sci Technol* 2002;**36**:962–968.

46 Versantvoort CHM, van de Kamp E, Rompelberg CJM. Development and applicability of an in vitro digestion model in assessing the bioaccessibility of contaminants from food. RIVM report 320102002/2004. National Institute for Public Health and Environment, Ministry of Health, Welfare and Sports, The Netherlands. 2004. Available at http://hdl.handle.net/10029/8885

47 Ferguson CJ, Wareing M, Ward DT, Green R, Smith CP, Riccardi D. Cellular localization of divalent metal transporter DMT-1 in rat kidney. *Am J Physiol* 2001;**280**:F803–F814.

48 Zalups RK. Molecular interactions with mercury in the kidney. *Pharmacol Rev* 2000;**52**:113–143.

49 Klaassen CD, Liu J, Choudhuri S. Metallothionein: an intracellular protein to protect against cadmium toxicity. *Annu Rev Pharmacol Toxicol* 1999;**39**:267–294.

50 Thevenod F. Nephrotoxicity and the proximal tubule. Insights from cadmium. *Nephron Physiol* 2003;**93**:87–93.

51 European Union. Council Decision 75/437/EEC of 3 March 1975 concluding the Convention for the prevention of marine pollution from land-based sources. Available at http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:31975D0437

52 European Union. Council Directive 80/68/EEC of 17 December 1979 on the protection of groundwater against pollution caused by certain dangerous substances. Available at http://europa.eu/legislation_summaries/environment/water_protection_management/l28017b_en.htm

53 European Union. Directive 2011/65/EU of the European Parliament and of the Council of 8 June 2011 on the restriction of the use of certain hazardous substances in electrical and electronic equipment. Available at http://www.linak.co.uk/corporate/pdf/english/misc/rohs%20directive.pdf

54 European Union. Directive 2003/53/EC of the European Parliament and of the Council, amending for the 26th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (nonylphenol, nonylphenol ethoxylate and cement). Available at http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32003L0053

55 European Union. Regulation (EC) No. 782/2003 of the European Parliament and of the Council of 14 April 2003 on the prohibition of organotin compounds on ships. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:115:0001:0011:EN:PDF

CHAPTER 10
Atomic absorption spectrometry

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Abstract: The chapter first describes the basis of atomic absorption spectrometry (AAS) and then discusses the main characteristics of flame atomization (FAAS), electrothermal atomization (ETAAS) and the techniques that involve release of a vapour (hydride generation, HG-AAS, and cold vapour, CV-AAS). A critical overview of the advantages and limitations of AAS, when dealing with food analysis, including sample treatment and possible use as a tool for speciation purposes, is presented. In addition to the possibility of directly using solid or undigested samples in ETAAS with a saving in time and reagents and minimizing the risk of contamination or analyte loss, recent advances in instrumentation, as well as the advantages of the combination with a solid or liquid microextraction stage, are discussed. Recent literature is provided to show that these advances considerably increase the performance of AAS and counteract the drawbacks inherent to the sequential operation mode. The relative low cost of acquisition and maintenance, moderate consumption of gas, versatile use and extreme sensitivity in some atomization modes make AAS a useful technique for the routine determination of a large number of elements in food analysis.

Keywords: flame atomic absorption spectrometry (FAAS), electrothermal atomization-atomic absorption spectrometry (ETAAS), cold vapour-atomic absorption spectrometry (CV-AAS), hydride generation-atomic absorption spectrometry (HG-AAS), speciation

10.1 Atomic spectrometry: history

Spectroscopy is based on the fact that when energy is supplied to matter, part of the energy can be absorbed or emitted as electromagnetic radiation at frequencies which are characteristics of the radiant species (and of the mechanism responsible for the radiation). Spectrometry refers to the quantitative measurement of the intensity of such electromagnetic radiation. The frequencies measured allow the radiant species to be identified, while the intensity of the radiation is related to the amount of matter which is responsible for the absorption or emission process [1].

Although the ability of a prism to produce a rainbow of colours has been known for many centuries, the first documented reference to this phenomenon is probably due to Georgius Agricola [2], who in 1556 gave details concerning the identification of chemical substances based on the colour observed when they are introduced in a flame. However, Newton [3] was the first to decompose white light into the colours of the spectrum using a prism, and, almost a century later, the existence of infrared and ultraviolet (UV) radiation was pointed out. The scientific work of Kirchhoff and Bunsen [4] is considered the starting point for our present knowledge of the subject, and played a relevant role in the development of many aspects of theoretical and practical chemistry at the end of the nineteenth and the beginning of the twentieth centuries [5].

The atomic absorption process was known and understood by chemists for decades but its use in the analytical laboratory was almost null until the publication by Walsh of the relevant article 'The application of atomic absorption spectra to chemical analysis' [6]. A few years later in 1963 the first instrument for flame atomic absorption spectrometry (FAAS) was marketed. The need to increase sensitivity soon resulted in new atomization modes based on the ideas of L'vov, giving rise to

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electrothermal atomization atomic absorption spectrometry (ETAAS) [7]. The first ETAAS instrument was launched in 1970. Significant advances involving, for example, platform atomization, the use of pyrolytic coatings, chemical modifiers and background correction devices were rapidly introduced, turning the technique into a robust, sensitive and reliable analytical tool as indeed had been foreseen by Walsh. Other improvements made during the last two decades of the twentieth century were the transversal mode of heating the electrothermal atomizer, the use of suspensions and the direct introduction of solid samples in the atomizer.

The fate of atomic absorption spectrometry (AAS) as an analytical technique was apparently darkened at the beginning of the 1990s due to the spectacular development (and reduction in price) of plasma-based instruments involving optical emission or mass measurements [8]. However, the present reality is much more positive and, as pointed out by Welz [9], important advances have been made in the use of continuum sources [10] and solid sampling. Recently, AAS in general and ETAAS in particular have experienced renewed drive as easily available techniques connected with liquid-phase microextraction processes [11]. Since ETAAS only requires a low volume of sample solution, an extremely high sensitivity, similar or even better than that reached by inductively coupled plasma mass spectrometry (ICP-MS), is achieved. It is interesting that during the period 2008–2012, 955 analytical papers dealt with FAAS and 64 of them involved a microextraction stage. During the same period, 603 articles dealt with ETAAS and more than 10% used microextraction.

This chapter deals with the main atomization techniques used in AAS, namely FAAS and ETAAS in addition to those involving vapour generation. Other techniques that cover similar application fields in analytical chemistry, such as plasma-based techniques with optical or mass measurements, are described in Chapters 11 and 12, respectively.

10.2 Introduction

Interaction of electromagnetic radiation with atoms involves electronic transitions. For the purposes discussed here, the UV-visible zone (180–780 nm) is used, the energy of the photons corresponding to the energy differences between upper excited levels and lower and ground levels. For the atomic spectra to be obtained, the sample must be in the form of atoms in a gas phase. The atoms behave independently, and no interactions distort the energy of the transitions. In suitable experimental conditions, because the energy electronic levels of the atoms are quantized, the transitions result in a series of very narrow spectral lines rather than relatively wide bands. This is the essential difference that provides atomic techniques with their most relevant analytical advantage: selectivity.

The samples to be analysed are not usually in a gas phase but they are present at room temperature as liquids or solids. This means that a mechanism is required to obtain a cloud of atoms in gas phase in a way that is reproducible. Once this atomic vapour is obtained, the absorption, emission or fluorescence phenomena can be studied. For our purposes, there are three basic atomization modes, the most simple being a flame (FAAS). Other ways are based on the use of electrothermal energy (ETAAS) or on the prior generation of a vapour involving volatile molecules of the analyte that are then easily atomized.

10.3 Electromagnetic radiation: interaction with atoms

Electromagnetic radiation is a form of energy that for most of the discussion involving analytical spectrometry may be considered as a wave, although some of its properties are better described by considering it as consisting of discrete particles of energy (photons). It is useful to keep both aspects in mind, and think of photons as particles of energy radiating from a source and characterized by an electromagnetic wave.

As occurs with every periodical wave, the electromagnetic radiation may be characterized by the distance covered by the wave to pass the same position twice (wavelength, λ) and the time elapsed for this repetition (period, τ). Associated with wavelength is the frequency (υ), the number of waves that pass a fixed point in the unit of time.

The velocity of the electromagnetic radiation in a vacuum (c) is 2.998×10^8 m/s. The velocity in other media (v_i) is the product of the frequency and the wavelength ($v_i = \upsilon \cdot \lambda$), and changes with the refraction index (n_i) of the medium through which it is travelling, a parameter that is always higher than unity. Consequently, λ also changes, in such a way that the energy of the radiation

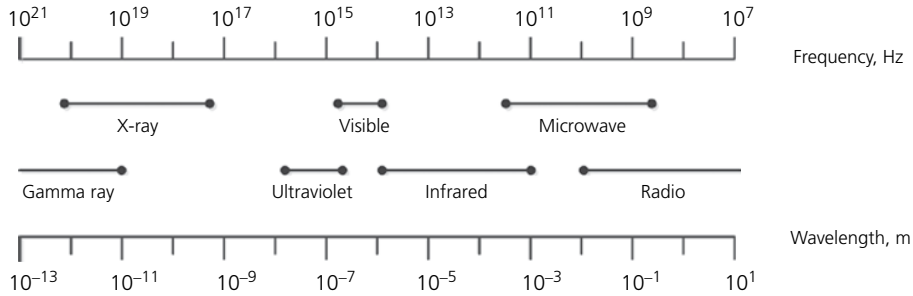


Figure 10.1 The electromagnetic spectrum.

(E) remains constant. This energy is directly proportional to the frequency of the electromagnetic radiation,

$$E = h \cdot \upsilon = h\frac{c}{\lambda} = h \cdot c \cdot \bar{\upsilon} \qquad (10.1)$$

where h is the Planck constant (6.62×10^{-34} J·s) and $\bar{\upsilon}$ is called the wavenumber (number of waves that occur per centimetre) and is sometimes used instead of frequency. From the above, it is important to note that only frequency is truly characteristic of a particular radiation. As schematized in Figure 10.1, the electromagnetic spectrum covers a wide range of energy, although for AAS purposes only the narrow UV-visible region corresponding to 180–780 nm (1 nm = 10^{-9} m) is used.

When a beam of photons with energy matching the difference in energy between some of the allowed levels of the outer electrons irradiates the atomic cloud that contains atoms of the analyte, the energy may be transferred, promoting electrons to a higher level (Figure 10.2). Assuming that the number of target atoms in the cloud is high enough, the decrease in the intensity of the beam after passing through the atomic cloud may be measured, thus providing the analyst with a specific tool (AAS) that allows the concentration of atoms to be calculated.

It should be noted that other processes involving electromagnetic radiation occur simultaneously, and the real situation is rather complicated. Irradiation of the atomic cloud by suitable photons may result in the release of electromagnetic radiation of the same or slightly higher wavelength than used for the irradiation. This is the basis of atomic fluorescence, which needs a source of high energy to be useful for analytical purposes. Even in the absence of exciting radiation, the atomic cloud releases electromagnetic radiation because, simply to obtain the atomic cloud, energy must be supplied to the

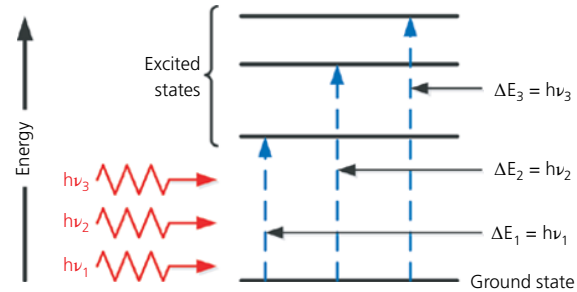


Figure 10.2 The absorption process.

sample. The high temperature reached by the cloud means that part of the atoms must be in an excited state, whose relaxation is achieved by emitting radiation corresponding to the energy differences of the outer electrons. That is, UV-visible light is released even though no prior absorption has occurred. This is the atomic emission process which is treated in detail in Chapter 12. For atoms of the same element, several transitions are possible, in accordance with the different levels of energy available. The most probable transition gives rise to the most intense absorption or emission signal. This line is called the resonance line and is usually selected to carry out the measurements with maximum sensitivity.

Leaving aside atomic fluorescence spectroscopy, which is useful only for a reduced number of elements, the atomic absorption and atomic emission techniques have the same purpose, i.e. the direct determination of a large number of elements in very low concentrations. Both analytical techniques have their own advantages and drawbacks. A significant fact that deserves to be emphasized is that the sensitivity of atomic emission spectroscopy (AES) increases with temperature, a parameter which is not so relevant in AAS. This represents an advantage for AES since lower analyte concentrations can be

measured by using a higher temperature in the atomizer. However, this is also inconvenient because of the difficulty involved in controlling temperatures if they are high.

10.4 Relationship between concentration and analytical signal

Due to its own nature, the atomic absorption process obeys the general law of absorption which, in this case, is written as

$$\log \frac{P_0}{P} = A = k \cdot C \qquad (10.2)$$

where P_0 and P are the intensities of the monochromatic beam of radiation entering and emerging from the atomic cloud, respectively, A is the absorbance, and C is the concentration of the solution used to obtain the atomic cloud. The proportionality constant, k, depends on a large number of factors (analyte, atomization conditions, particular instrument used, etc.) but remains invariable for a given experimental set. This simple equation is the basis for the analytical use of AAS, provided that the condition imposed by the general law of absorption (monochromatic radiation) is fulfilled.

The similarity with classical UV-visible molecular spectrophotometry is evident, although one essential difference is that in such a case the absorption is due to molecules or ions that remain static in a cuvette. In contrast, in AAS the particles responsible for absorption are atoms that have to be measured in a dynamic system maintained at a high temperature inside the atomizer.

Taking into account all the above, the basic components of a simplified AAS instrument as schematized in Figure 10.3 are as follows.

- A source capable of providing monochromatic radiation of sufficient intensity (source).
- A device (atomizer) to transform the sample (usually a liquid) into a gas phase containing the atoms. The analyte concentration inside the atomizer has to be representative of that of the original liquid sample.
- Devices (wavelength selector and detector) allowing the radiation absorbed by the analyte in the atomizer to be measured without distortion caused by the emission process, the presence of stray light or other phenomena.

10.5 The source of electromagnetic radiation

For the law of absorption to be obeyed, the measurements must be carried out using monochromatic radiation. This posed a practical problem during the earlier stages of AAS development because the width of the atomic absorption lines may often be less than 0.002 nm. From a theoretical point of view, they should be even narrower but the Doppler effect caused by the relative motion of photons and atoms inside the atomizer, the collisions with other atoms and the existence of magnetic and electric fields result in their widening [12]. The instrumental problem that hindered the development of the technique is that a conventional monochromator cannot isolate wavelength bands narrower than 0.1 nm, so that the amount of radiation impinging the atomic cloud is not significantly reduced by the extremely narrow atomic absorption signal.

The problem was elegantly solved by Walsh. Since the Doppler effect is a factor that affects the peak width, Walsh suggested that the atomic vapour inside the atomizer could be irradiated with the light emitted by another atomic vapour of the same analyte, which would overcome the need for a high-resolution monochromator. This idea resulted in what is now the most commonly used source for AAS purposes, the hollow cathode lamp (HCL). Figure 10.4 shows a scheme of this device. The lamp with an inert gas (low-pressure Ar or Ne) inside, has two electrodes, the cathode being made of the element to be determined or at least covered with it. When a suitable

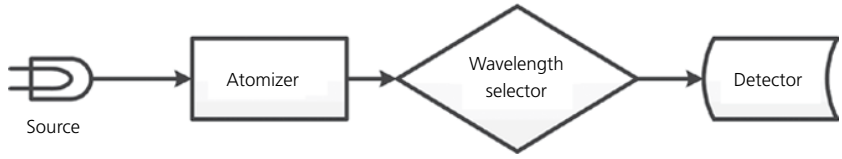


Figure 10.3 Simplified scheme of a basic AAS instrument.

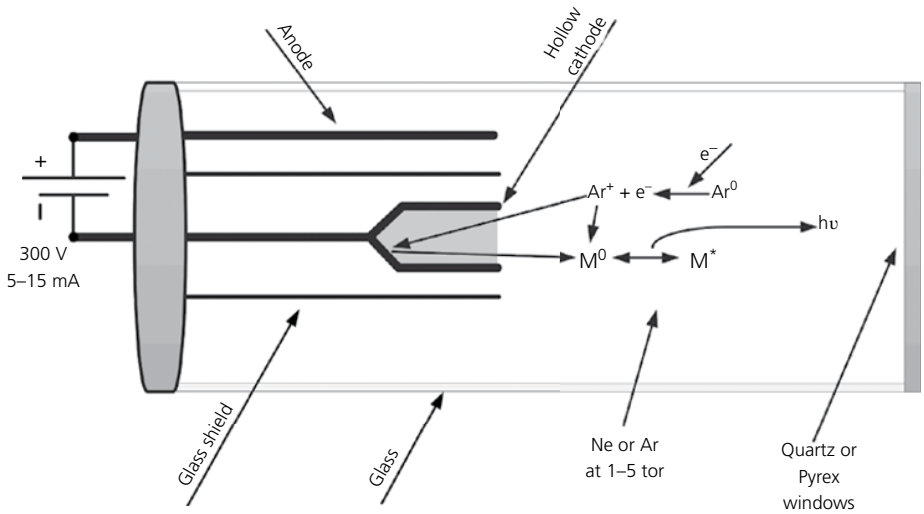


Figure 10.4 Scheme of the hollow cathode lamp.

voltage (300–400 V) is applied between the electrodes, the gas is ionized and the positively charged ions move towards the cathode. The impact of these accelerated ions on the surface of the cathode causes the analyte atoms to be ejected. An atomic vapour of analyte is thus produced in the neighbourhood of the cathode, and relaxation to the ground state results in an emission line, centred in wavelength with respect to the absorption line but narrower. The cathode is moulded as a cylinder or cup to focus the light beam and facilitate the deposition of the atoms on the surface of the electrode once the lamp is switched off. In some cases the cathode contains several metals, giving rise to multi-element lamps.

For some elements, especially As, Se and Sb, the hollow cathode lamps may have a reduced lifetime and provide low intensities, so that better performance may be obtained using another source that provides very narrow emission lines, the electrodeless discharge lamp (EDL). In this case, a small amount of the element, or one of its salts, together with an inert gas at a low pressure are placed in a closed bulb. There are no electrodes but ionization of the gas is achieved by submitting the bulb to the action of appropriate microwaves or radiofrequencies. The intensity of the light beam produced is higher than that obtained with HCLs, the drawback being that the device is much more expensive.

On the other hand, the atomizer operates at a high temperature and contains atoms, ions and excited molecules that themselves emit polychromatic radiation. This means that despite using a monochromator (see Figure 10.3), part of the radiation reaching the detector at the wavelength selected for the quantitative measurement comes from the atomizer itself. The simplest way to distinguish this radiation from that coming from the source is to modulate the latter by means of a mechanical chopper working at a fixed frequency. The system easily discriminates the continuous signal from the atomizer and the discontinuous, modulated radiation from the source that is to be measured.

10.6 The flame atomizer

In a relatively simple way, the flame atomizer transforms the solutions into a cloud containing the atoms of the analyte at the fundamental energetic level. For this, the device uses a flame, that is, a spatial volume in which a highly exothermic oxidation reaction (combustion) takes place in such a way that the energy released sustains the reaction. The flame is formed by a fuel, usually an organic compound, and an oxidant. A large number of oxidant–fuel combinations have been studied for FAAS but, based on the main characteristics of the flames, only two of them are commonly used, namely air–acetylene and nitrous oxide–acetylene flames. In the first, the chemical reaction

$$C_2H_2 + O_2 + N_2 \rightarrow 2CO + H_2 + N_2 \qquad (10.3)$$

allows temperatures of 2100–2400 °C to be reached, while the latter reaction

$$3N_2O + C_2H_2 \rightarrow 2CO + 3N_2 + H_2O \qquad (10.4)$$

results in in a hotter medium with temperatures ranging from 2600 to 2800 °C. The nitrous oxide–acetylene flame is particularly suitable for the determination of Sr, Ba, V, Al, Si and Ti, among others. However, since this flame is much hotter, with the corresponding drawbacks, most routine laboratories use almost exclusively the colder air–acetylene flame, reserving that based on nitrous oxide for cases where it is strictly necessary.

Leaving aside that an excessive temperature will result in undesired ionization of the atoms, the most serious concern with this hotter flame is due to its burning velocity. Flames are only stable for appropriate flow rates of the gases involved. If the burning velocity is too high and exceeds the rate at which the gas exits the slit of the burner head, the flame will propagate inside the burner (flashback) and an explosion may occur. The burning velocities of air–acetylene and nitrous oxide–acetylene flames are 160 and 286 cm/s, respectively, which means that the first flame is 'safer' than the second, and involves a relatively low consumption of gas. Whatever the case, commercially available FAAS spectrometers nowadays incorporate sufficient safety protections to permit this flame to be used safely. The selection of the flame depends on the analyte. Figure 10.5 shows the flame recommended for the most commonly determined elements.

The flames are not isothermal media, and relevant temperature gradients exist both in height over the burner head and along the axis from the edge of the flame to its centre. For this reason, the position of the flame with respect to the light beam that traverses it must be optimized. In modern instruments this adjustment is made automatically via software but the simplest models require manual adjustment.

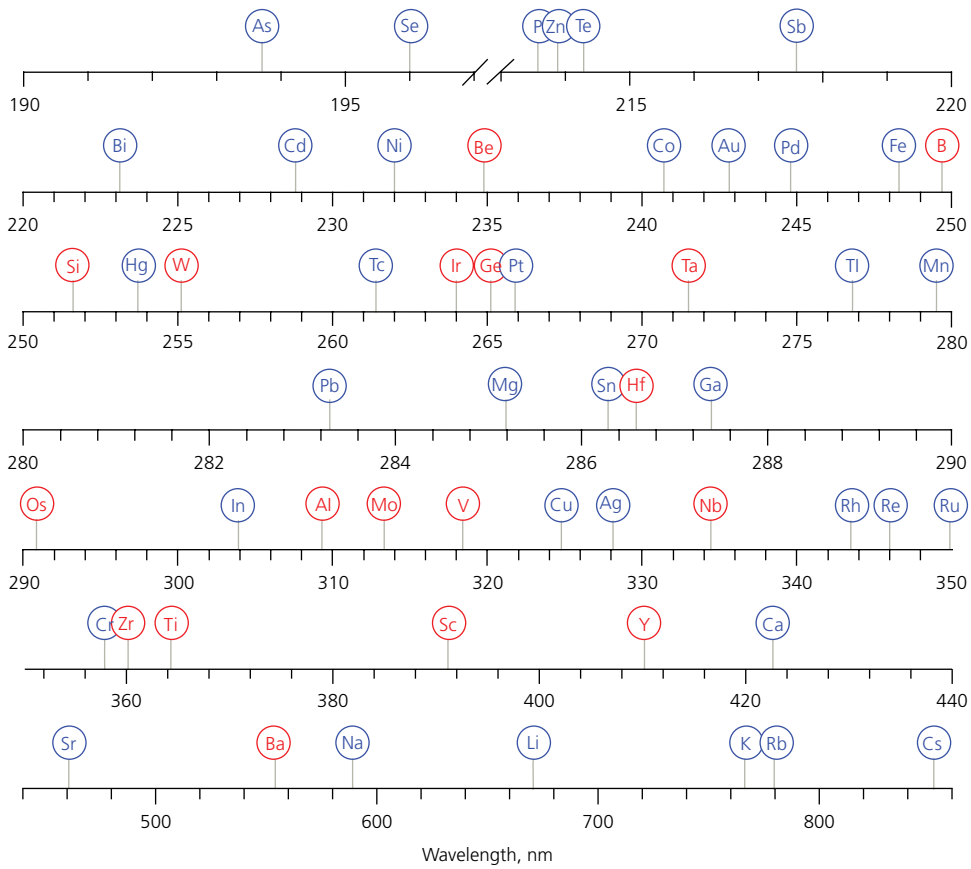


Figure 10.5 Wavelengths used for FAAS purposes with indication of the flame recommended: N_2O–C_2H_2 (red); air–C_2H_2 (blue). (See insert for colour representation of the figure.)

The most common way of introducing the liquid sample into a flame to obtain reproducible atomic clouds suitable for FAAS is to use an atomic nebulizer, a scheme of which is given in Figure 10.6. The nebulizer works on the basis of the Venturi effect. The entry of air through a small opening causes a reduced pressure at the tip of the tube, which is concentric with the capillary tube that is immersed in the solution. As a consequence, there is a continuous suction of the liquid sample, which is fractionated into droplets to form a spray (mist) at the exit of the device. The droplets are transferred to the atomizer (the burner), where they are desolvated. The analyte is then volatilized to finally obtain neutral atoms, which are irradiated with the light beam that coming from the source traverses the flame. The nebulizer is placed inside the spray chamber as shown in Figure 10.7. However, the operation is more complex than appears at a first sight, since the droplets have a wide size distribution. Large droplets are not suitable since their transport by the gas towards the burner is inefficient, and desolvation and atomization might be incomplete. Small droplets are much more appropriate, and so the spray generated at the exit of the nebulizer is first fractionated by means of an impact bead and then forced to cross a chamber (spray chamber) with several obstacles obstructing the way (flow spoilers). As a result of multiple collisions, a finer spray of low (a few micrometres in diameter) and homogeneous droplet size results. Note that only a small

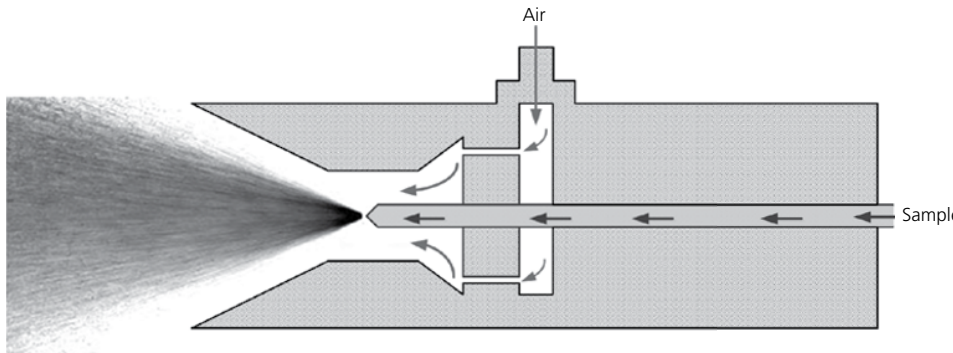


Figure 10.6 Scheme of the most common nebulizer for sample introduction in FAAS.

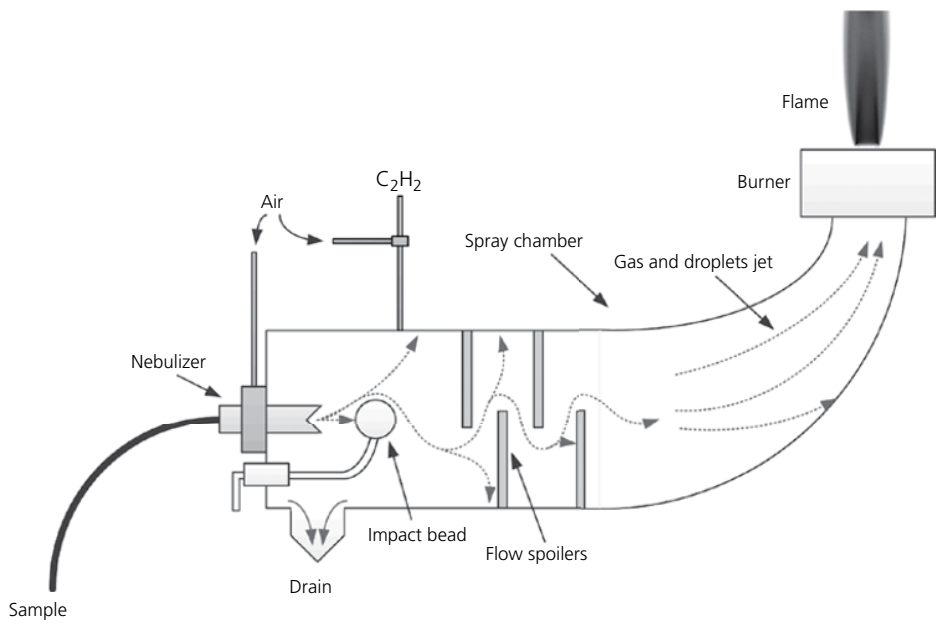


Figure 10.7 Schematic view of the spray chamber.

fraction (10–15%) of the liquid sample that enters the spray chamber reaches the atomizer, while the rest of the liquid is drained from the system. Despite the low transport efficiency, sensitive atomic absorption signals can be obtained for a large number of elements.

The performance of the pneumatic nebulizer depends on the physicochemical properties (viscosity, surface tension, temperature) of the solution which is being aspirated, the depth the capillary tip is immersed in the solution and the exact flow of the gases. All this has to be borne in mind by the analyst in order to use solutions of similar properties for calibration.

10.7 Detection of the radiation

To obtain the fraction of the electromagnetic radiation that is absorbed by the atoms of the analyte in the atomizer a detector, a suitable wavelength selector (monochromator) and electronic devices for the transduction of electrical signals are necessary. The ideal detector should have a high sensitivity, a high signal-to-noise ratio and a constant response within a wide range of wavelengths. In addition to these essential characteristics, the response should be obtained in a short time and be proportional to the impinging radiation, and the signal should be null in the absence of radiation. The most widely used detectors for FAAS purposes are photonic transducers, a general term that includes a number of devices such as photovoltaic cells (common in low-cost or old instruments), phototubes and photomultipliers, conductivity transducers (the absorption of radiation results in electron vacancies and so in a change in conductivity), silicon photodiodes and charge transfer transducers. Figure 10.8 shows the scheme of a photomultiplier tube. It basically consists of a photocathode, several additional electrodes (dynodes) and an anode. A voltage (900 V) is applied between anode and cathode so that when the photons strike the photocathode and electrons are released (photoelectric effect), the electrons are accelerated towards the set of dynodes (additional electrodes to which increasing voltages are applied) and a cascade effect is produced. The amplification is so great that finally about 10^7 electrons are obtained by each photon. The photomultiplier tube is an efficient, commonly used device but multichannel detectors also represent an interesting alternative [10, 12, 13].

A wavelength selector is placed between the burner and the detector. Figure 10.9 shows a a grating monochromator based on the Czerny–Turner configuration, which illustrates the separation of two wavelengths. The polychromatic radiation entering the slit results in a beam of parallel radiation which, when reaching the diffraction grating, changes direction, depending on the wavelength. The radiation is reflected in a concave mirror that focuses the wavelengths separated onto the focal plane. Depending on the orientation of the diffraction grating, a narrow wavelength interval may be isolated through the exit slit. The echelle arrangement, which combines a coarse grating and a prism in a Czerny–Turner configuration, is also used in atomic spectrometry. This configuration is advantageous, especially when combined with continuum radiation sources, as discussed elsewhere [10, 13].

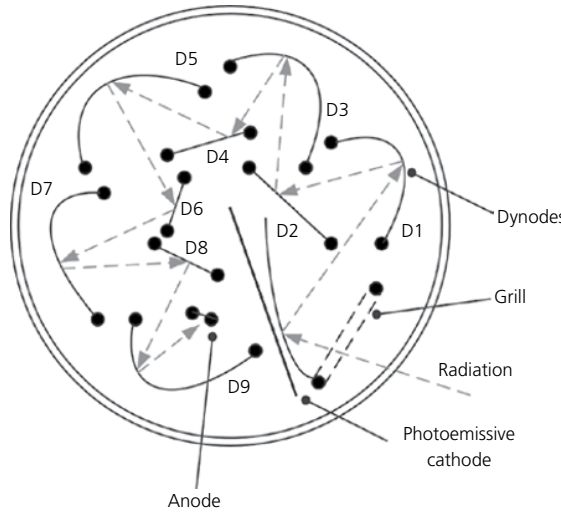


Figure 10.8 Photomultiplier tube.

10.8 Spectrometer configuration: correction devices

The simple configuration given in Figure 10.3 corresponds to a single-beam spectrometer. It is obvious that, in such an arrangement, any change in the intensity of the source will affect the detector reading, distorting the analytical results. The instrument also requires a sufficiently long warm-up time to ensure constant emission by the source. The drawbacks are overcome using a double-beam configuration, such as that shown in Figure 10.10, which includes a beam splitter. In this arrangement, part of the beam passes through the

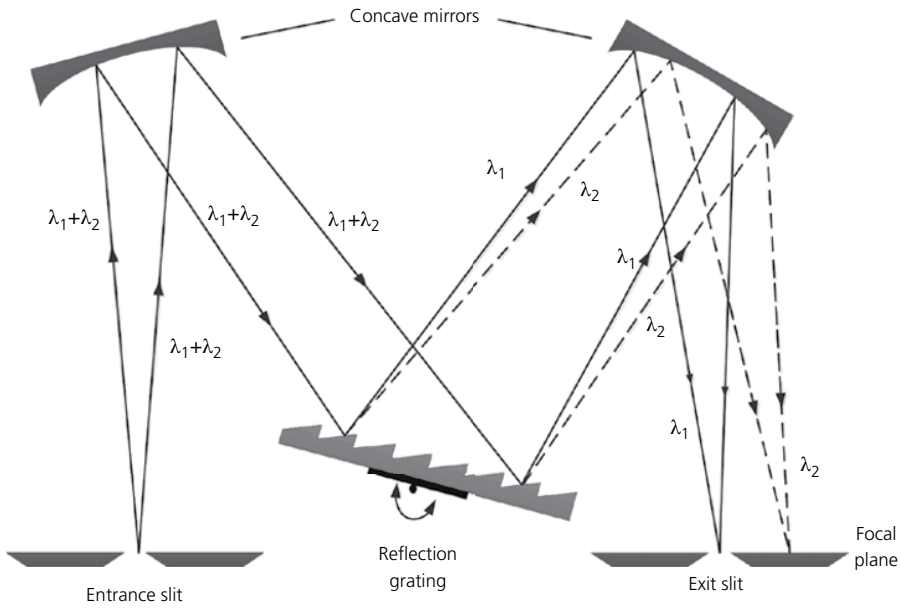


Figure 10.9 Grating monochromator.

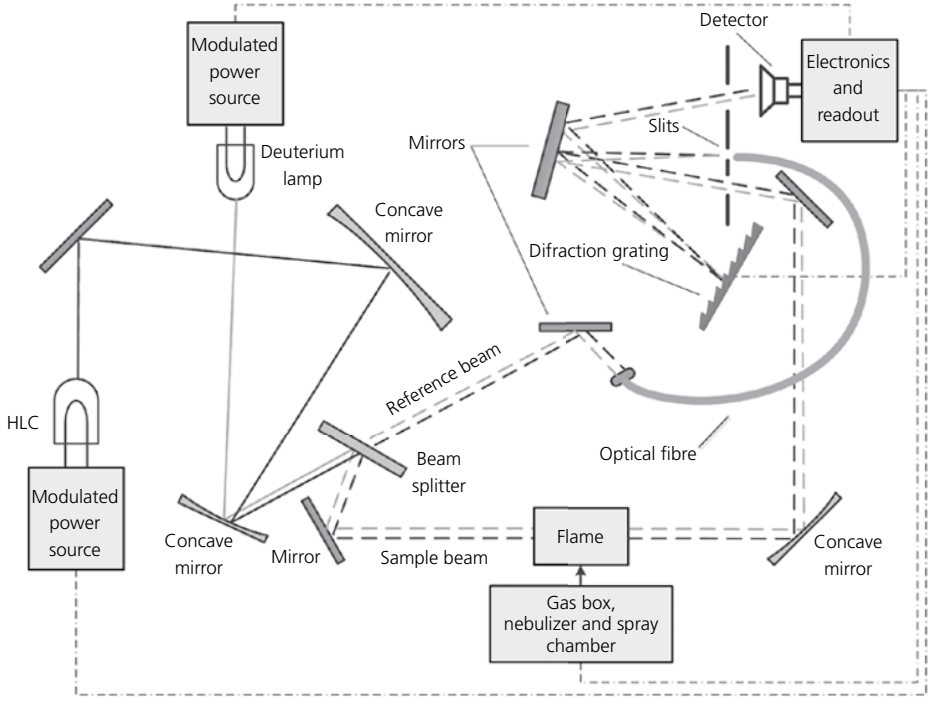


Figure 10.10 Scheme of a double-beam spectrometer provided with the common deuterium lamp for correction.

atomizer, while the other is used as a reference. Both beams converge in an asynchronous way before entering the monochromator and detector. In this way, the detector measures alternately during short intervals of time the light coming directly from the HCL and that traversing the flame. A comparison of both signals allows reliable correction of the fluctuations in the HCL light intensity.

On the other hand, it is important to note that in addition to the neutral atoms of the analyte inside the atomizer there is a large variety of molecules or ions coming from the sample itself or related with the combustion reaction. These chemical species may absorb a part of the radiation emitted by the source (background absorption), giving rise to erroneous analytical signals. The most common and relatively inexpensive way to overcome the drawback is to use, in addition to HCL, a continuum source, such as a deuterium lamp arranged as indicated in Figure 10.10. The atomizer is irradiated alternately with light from each source. In the case of the light coming from the HCL the total absorbance is measured, while when radiation is coming from the continuum source only the background is measured, which allows for easy correction. Advantage is taken of the fact that the bandwidth of the wavelength selector is greater than that of the analytical line so that the absorbance due to the analyte is negligible compared with the absorption measured in the case of the deuterium lamp. This simple, efficient correction device is common nowadays in flame spectrometers and should be present even in low-cost models.

There are other systems for background correction, the most important being that based on the application of a pulsed magnetic field (Zeeman corrector) although this device is more suitable for the electrothermal atomization mode.

10.9 Calibration

As already indicated (eqn 10.2), the analytical signal is directly related to the analyte concentration. The proportionality constant depends on a large number of instrumental parameters apart from the characteristics of the analyte itself. Provided all the instrumental parameters are kept constant, a number of solutions of the analyte with different concentrations (C_1, C_2, …, C_n) can be measured, giving a set of absorbance values (A_1, A_2, …, A_n). The absorbance–concentration data pairs can be fitted to an equation such as

$$A = \alpha + \beta \cdot C \tag{10.5}$$

where the coefficients α and β have a confidence interval (CI) given by

$$\mathrm{CI}(\alpha) = \alpha \pm t \cdot s_\alpha = \alpha \pm t \cdot s_e \sqrt{\frac{\sum_{i=1}^{n} C_i^2}{n \cdot \sum_{i=1}^{n} \left(C_i - \bar{C}\right)^2}} \tag{10.6}$$

$$\mathrm{CI}(\beta) = \beta \pm t \cdot s_\beta = \beta \pm t \cdot \frac{s_e}{\sqrt{\sum_{i=1}^{n} \left(C_i - \bar{C}\right)^2}}$$

where n is the number of absorbance–concentration data pairs, t is the value of the parameter corresponding to the Student distribution for $n - 2$ degrees of freedom at a given confidence level (usually 95%), $\bar{C}$ is the mean of the C values and s_e is a parameter that estimates the uncertainity of the absorbance readings (residual standard deviation of the regression). This is calculated from the differences between the absorbance readings (A_i) for each concentration and those estimated from the regression line ($A_{i,est}$) by means of:

$$s_e = \sqrt{\frac{\sum_{i=1}^{n} \left(A_i - A_{i,est}\right)^2}{n - 2}} \tag{10.7}$$

This parameter allows the detection limit to be calculated by means of an alternative criterion rather than using the procedure based on a blank assay [14]. Following this criterion the limit of detection (LOD) is defined as the analyte concentration corresponding to an absorbance value of $3 s_e / \beta$.

A typical calibration graph obtained using measurements with random errors is given in Figure 10.11. Note that the solid line (the regression line) is affected by an uncertainty due to the random errors, producing a regression band. This means that the average absorbance (A_x) obtained when a solution of unknown concentration is measured provides a confidence interval (CI) in the concentration calculated given by

$$\mathrm{CI}(C_x) = C_x \pm t \cdot \frac{s_e}{\beta} \cdot \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{\left(\bar{A}_x - \bar{A}\right)^2}{\beta^2 \sum_{i=1}^{n} \left(C_i - \bar{C}\right)^2}} \tag{10.8}$$

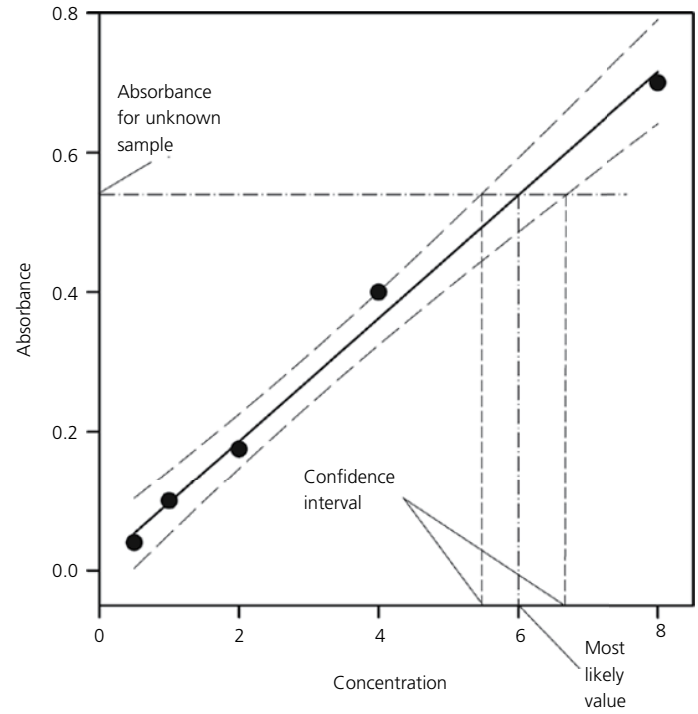


Figure 10.11 Typical calibration graph showing the confidence interval of the regression line.

where m is the number of absorbance measurements taken.

To check the quality of the fitting to a straight line, the quality coefficient (QC) [15, 16] can be used:

$$\mathrm{QC} = 100 \cdot \sqrt{\frac{\sum_{i=1}^{n}\left(\frac{A_i - A_{i,est}}{A_i}\right)^2}{n-1}} \qquad (10.9)$$

If the QC calculated is below a defined threshold (5% is a commonly accepted value), the line is considered as suitable for calibration. If not, the presence of outliers should be investigated, the range of concentrations covered should be lessened or fitting to a polynomial function of a higher order should be checked [17].

Most dealers of FAAS instruments provide tables for the characteristic concentration (c_0) values of common analytes. This parameter corresponds to the concentration of an analyte solution giving an absorbance reading of 0.0044 and is of practical use in routine work to check whether the measurements are being carried out in the optimal conditions for the instrumentation used. Some of the values given by a FAAS manufacturer [18] for a particular instrument and experimental conditions are shown in Table 10.1.

Sometimes other chemical species that are also present in the sample solution affect the analyte response. In such cases, calibration can be carried out by means of the standard additions method (SAM). For this purpose, different amounts of a standard solution are added to n solutions containing the same amount of sample, and all the solutions are made up to the same volume and then measured. The absorbance readings are plotted against the analyte concentration added, and the regression line is extrapolated to the intersection with the x-axis (Figure 10.12). The concentration at which the intersection occurs is that of the unknown sample solution. As occurs in direct calibration, the unavoidable uncertainty of the measurements results in an uncertainty for the concentration calculated. The extrapolated concentration and its confidence interval are given now by

$$C_{x,ext} = \left|\frac{-\alpha}{\beta}\right| \pm t \cdot \frac{s_e}{\beta}\sqrt{\frac{1}{n} + \frac{\bar{A}^2}{\beta^2 \sum_{i=1}^{n}\left(C_i - \bar{C}\right)^2}} \qquad (10.10)$$

Table 10.1 Characteristic concentrations (c_0) of several elements by FAAS.

Element	Wavelength (nm)	c_0 (mg/L)
Air–acetylene flame		
Ag	328.1	0.055
As	193.7	0.99
Au	242.8	0.33
Bi	223.1	0.44
Ca	422.7	0.088
Cd	228.8	0.033
Co	240.7	0.154
Cr	357.9	0.088
Cs	852.1	0.22
Cu	324.8	0.088
Fe	248.3	0.11
Hg	253.7	4.4
In	303.9	0.77
Ir	264	11
K	766.5	0.044
Li	670.8	0.044
Mg	285.2	0.0066
Mn	279.5	0.055
Na	589	0.011
Ni	232	0.154
Pb	283.3	0.44
Pd	244.8	0.22
Pt	265.9	2.2
Rb	780	1.1
Rh	343.5	0.198
Ru	349.9	0.66
Sb	217.6	0.55
Se	196	0.66
Tc	261.4	2.2
Te	214.3	0.44
Tl	276.8	0.66
Zn	213.9	0.022
Nitrous oxide–acetylene flame		
Al	309.3	1.1
B	249.7	13.2
Ba	553.6	0.44
Be	234.9	0.033
Ga	287.4	1.32
Ge	265.1	2.2
Hf	286.6	9.9
Mo	313.3	0.66
Nb	334.4	15.4
Os	290.9	0.99
P	213.6	308
Re	346	14.3
Sc	391.2	0.33
Si	251.6	2.2
Sn	286.3	3.3
Sr	460.7	0.11
Ta	271.5	12.1
Th	432.6	6.6
Ti	364.3	1.76
V	318.4	1.98
W	255.1	9.9
Y	410.2	1.76
Zr	360.1	6.6

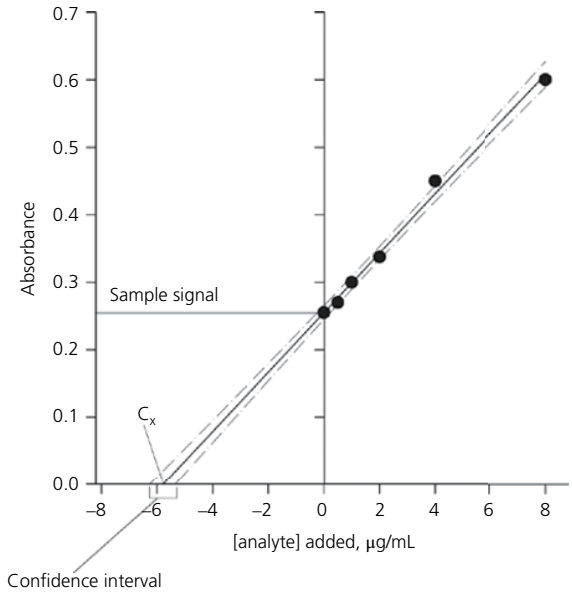


Figure 10.12 Standard additions method.

where t is the value for the Student distribution at a given confidence level for $n - 2$ degrees of freedom, C_i are the concentrations for the standard added and $\overline{C}$ and $\overline{A}$ are the mean values for the standard concentrations and absorbances obtained in the calibration, respectively.

It is important to point out that SAM does not correct all the interference effects but only those related with the modification of the analyte response factor. Other interferences are described in section 10.10. SAM is time-consuming, and so it is useful to have some criterion to hand that helps decide whether, when dealing with a particular type of sample, SAM can be avoided and simple direct calibration used instead. Let β_1 and β_2 be the slopes of the calibration lines obtained in the simplest direct way and in SAM, respectively. The question is to decide whether these slopes significantly differ for a given confidence interval. Assuming, for simplicity, that the number of degrees of freedom is the same for each regression ($n - 2$), the t value is calculated as

$$t = \frac{\beta_1 - \beta_2}{\sqrt{s_{\beta_1}^2 + s_{\beta_2}^2}} \qquad (10.11)$$

where s_β are the standard errors of the slopes calculated for the minimum square fitting. This t value is compared with the tabulated value (t_{tab}) of the Student distribution for a given confidence interval (usually 95%) and $2n-4$ degrees of freedom. If $t \leq t_{tab}$ the null hypothesis is satisfied, which means that there is no significant difference between both slopes and so the tedious SAM procedure is unnecessary.

10.10 Interferences

Compared with other analytical techniques, FAAS is relatively selective, but interferences occur as a consequence of the presence of other species in the atomizer and the flame composition. The main causes of error may be of a spectral or non-spectral type.

Spectral interferences are those caused by other species that absorb or emit radiation at wavelengths close to that of the analyte, so that there is an overlap with the atomic line measured within the spectral bandwidth selected in the spectrometer. Thus, for example, the light dispersion produced by solid particles or microdroplets of solvent inside the flame will result in a positive analytical error. The introduction in the atomizer of solutions with a high salt content can produce high levels of non-dissociated or partially unsolvated salts inside the flame and, consequently, wide absorption bands, an effect that is particularly severe at wavelengths below 300 nm. This molecular absorption is easily corrected by a deuterium device. Another way to overcome the interference is to measure at two wavelengths. First, the absorbance at the wavelength of the analyte is obtained, a measurement that corresponds to the analyte signal plus the interference effect. Then, a second measurement is obtained at a different wavelength at which the analyte does not absorb, but the interference species, which presents a wide band, does. The corrected analytical signal to be used for quantification is then obtained by difference.

Spectral interference also occurs when a line corresponding to another element is very close to the line of the analyte, so that there is an overlap. The deuterium device may fail to overcome this problem and overcompensation [19] may result. However, this type of interference is not frequent since atomic lines are narrow. The best way to solve the difficulty is to use a secondary line of the analyte instead of the resonance line.

Non-spectral interferences may have several causes. For example, some elements, especially alkaline and alkaline earths, may be partially ionized in a flame which reduces the atom population and consequently sensitivity. Since this effect is not usually proportional to the analyte concentration, the best solution is to incorporate an easily ionizable element at a relatively high concentration in the sample solution. In this way, this element (ionization suppressor) is ionized in the flame and ionization of the analyte is inhibited. This is what happens, for example, when barium is determined in a nitrous oxide–acetylene flame. The ionization is suppressed by incorporating a large amount (2 g/L) of potassium chloride in the solution to be measured.

On the other hand, some elements originate refractory oxides that may even react with other chemical species inside the flame, giving rise to thermally stable compounds that do not dissociate at the temperature of the flame used. The obvious consequence is a lower than expected absorbance value. This is the case for the interferences caused by aluminium, silicon and phosphate in the determination of alkaline earths using an air–acetylene flame. The commonest way to overcome this problem is to add to the sample solution a chemical that 'releases' the analyte from the refractory combination so that the expected atomic population is restored. A clear example is the use of a lanthanum salt to overcome the interference of phosphate in the determination of calcium. The scientific literature recommends that specific releasing agents be used for each particular case. On the other hand, it is important to note that use of the SAM cannot overcome these interferences, and the recommendations provided in the literature have to be strictly followed for reliable results to be obtained.

Interferences may also be solved by selecting a suitable temperature or flame. This is the case, for example, of chromium determination in an air–acetylene flame with a low proportion of fuel. The presence of iron decreases the sensitivity of the chromium measurement but the deleterious effect disappears if the proportion of acetylene is increased or the more energetic nitrous oxide–acetylene flame is used instead. Similar observations can be made for the determination of titanium, molybdenum and vanadium, which form thermally stable oxides in flames. The use of an appropriate nitrous oxide-based flame solves the drawback.

Finally, problems may appear due to differences in the physicochemical properties of the sample solution and the solutions used for calibration. These problems, which are often referred to as physical interferences, are mainly caused by the way in which the sample solution is introduced in the flame. Since the analytical response depends on the atomic population in the flame, it is obvious that differences in density, viscosity and surface tension, among other properties, that affect the nebulizer uptake rate must affect the analytical signal. Common sense must be applied and the physicochemical properties of the solutions to be used for calibration must be as close as possible to those of the sample solution. Most of these problems are also overcome by using a propelling device such as a peristaltic pump to introduce the sample solution, rather than the conventional aspiration mode.

To summarize, FAAS is selective enough for analytical purposes provided that the procedure recommended in the specialized literature is strictly followed in each case. The analyst should bear in mind that the flame to be used, possible releasing agents or other points of the exact protocol are the consequence of prior studies carried out to overcome practical difficulties and to ensure the reliability of the results.

10.11 Alternative ways of sample introduction in FAAS

The common way of introducing the sample solution in FAAS is the continuous aspiration mode which is made possible by the suction caused by the Venturi effect at the entry of a pneumatic nebulizer. However, while being simple, this has several drawbacks. For example, the relatively high consumption of sample solution may be a serious inconvenience when the volume of solution available for measurement is low. The minimum volume required for the determination is 3–5 mL, depending on the experimental conditions and the particular instrument used. In addition, problems due to capillary clogging may arise when solutions with a high salt content are continuously aspirated [20].

To overcome these drawbacks, procedures which involve introducing a microvolume of sample solution in the air stream at the entry of the nebulizer (discrete nebulization) or, better still, in a liquid carrier moved by a propelling device have been proposed. The latter (flow injection, FI) [21] has the advantages of a reduced consumption of sample and increased tolerance when dealing with highly saline solutions. Figure 10.13 shows a basic manifold constructed for this purpose. The peristaltic pump is used both to propel the carrier and to load the sample loop, while the nebulizer uptake rate is adjusted in the usual way to achieve maximum sensitivity. The difference between the pumping flow rate and the nebulizer uptake rate is compensated by the entry of solvent or air [22]. The peak height or area of the transitory signal obtained is used for quantification purposes. The volume of sample is low (50–100 μL) and high sampling frequencies (more than 100 samples per hour) are obtained. In addition, the FI–FAAS combination permits a controlled dispersion, which facilitates calibration and the on-line dilution of samples that are too concentrated to be measured directly [23, 24].

The advantages of FI–FAAS also include the possibility of introducing suspensions (slurries) prepared from finely powdered samples directly into the flame This is an interesting alternative in the case of trace analysis because complete dissolution of the sample is avoided, reducing the risk of contamination or analyte loss [25]. However it cannot be considered as a

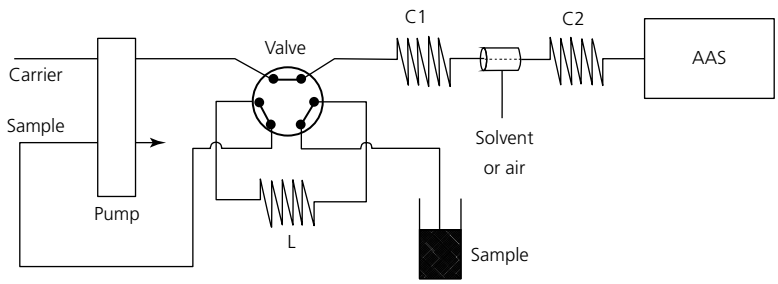


Figure 10.13 Basic FI manifold for FAAS purposes.

general modus operandi since several restrictive conditions need to be met. In addition to the direct use of suspensions, the FI–FAAS approach also facilitates the handling of samples such as fuels, cosmetics and edible oils [26].

Finally, it should be noted that if the sample solution is continuously pumped towards the nebulizer, rather than being aspirated, interesting advantages appear. If a stock standard solution is pumped at a linearly increasing flow rate from zero up to the nebulizer uptake rate using a simple T-piece immersed in pure water for flow compensation, an on-line concentration gradient is obtained, which facilitates calibration, on-line dilution and the practice of SAM [27, 28]. This methodology, based on computer-controlled flow rates combined with FAAS, has proved suitable for automatic calibration, giving rise to a smart system for the automatic measurement of solutions with minimal intervention on the part of the operator [29]. The pulsations in the flow caused by the rollers of the peristaltic pumps can even be used to obtain additional information [30].

10.12 Techniques involving vapour generation

Some analytes are introduced into the atomizer in the form of a vapour. In this case, the element may be handled as a gaseous cloud of neutral atoms (cold vapour) or as a volatile molecule that decomposes to release the atoms inside the atomizer (hydride generation).

10.12.1 Cold vapour technique

The cold vapour technique (CV-AAS) is, in practice, exclusively applied to the determination of mercury, which has a high vapour pressure and can be readily isolated from the sample solution. First, a reducing agent is added to obtain the elemental form:

$$Hg^{2+} + 2e^- \rightarrow Hg^0 \qquad (10.12)$$

and then the atoms are transferred by a carrier gas (usually argon) to the atomizer which, in this case, is a simple cell with the ends open, as depicted in Figure 10.14. Since the element is already in the atomic form, there is no need for heating to achieve atomization, which is the characteristic that gives its name to the technique. Despite this, it is convenient for the atomizer to be slightly heated to avoid the condensation of small droplets of water on its internal face since any falling droplet would disturb the light beam coming from the source.

The reducing agent can be tin (II) chloride or sodium tetrahydroborate. The reaction with the last agent is faster although the resulting atomic gas is mixed with hydrogen:

$$Hg^{2+} + 2BH_4^- + 2H^+ \rightarrow Hg^0 + 2H_2 + B_2H_6 \quad (10.13)$$

While there are no spectral interferences in this determination, a few chemical interferences due to the chemical matrix may arise [19]. Thus, some species could react with the reducing solution, decreasing its concentration, while others could amalgamate with mercury, hindering transport by the carrier gas. In any case, the interferences are scant and, with the exception of that posed by silver, may be overcome by replacing one reducing agent with the other. The most severe problem that arises is that of the high sensitivity of the technique. Extreme precaution is recommended to avoid contamination by the reagents and glassware used as well as by the atmosphere of the chemical laboratory.

In addition to the inorganic form, mercury may be present in the samples as organomercury species (methylmercury, dimethylmercury, ethylmercury and phenylmercury). Tin (II) chloride cannot reduce these compounds but tetrahydroborate can, which opens up the possibility of speciation.

The sensitivity of CV-AAS is further increased (LOD 0.1 ng/L) by using an on-line atom-trap device, in which all the mercury released from the sample solution is first retained and then released again over a short period simply by heating the device. Traps based on copper, silver and, preferably, gold [31] have been used but such extreme sensitivity is rarely required in practice.

The relevance and practical usefulness of CV-AAS has moved several manufacturers to market AAS instruments specifically devoted to this purpose. On the other hand, very small quantities of mercury may also be determined by atomic fluorescence spectrometry and instruments for this single aim also exist in the analytical market. There is no a clear reason to recommend atomic absorption or fluorescence for mercury determination, the final decision depending on the planning of each laboratory.

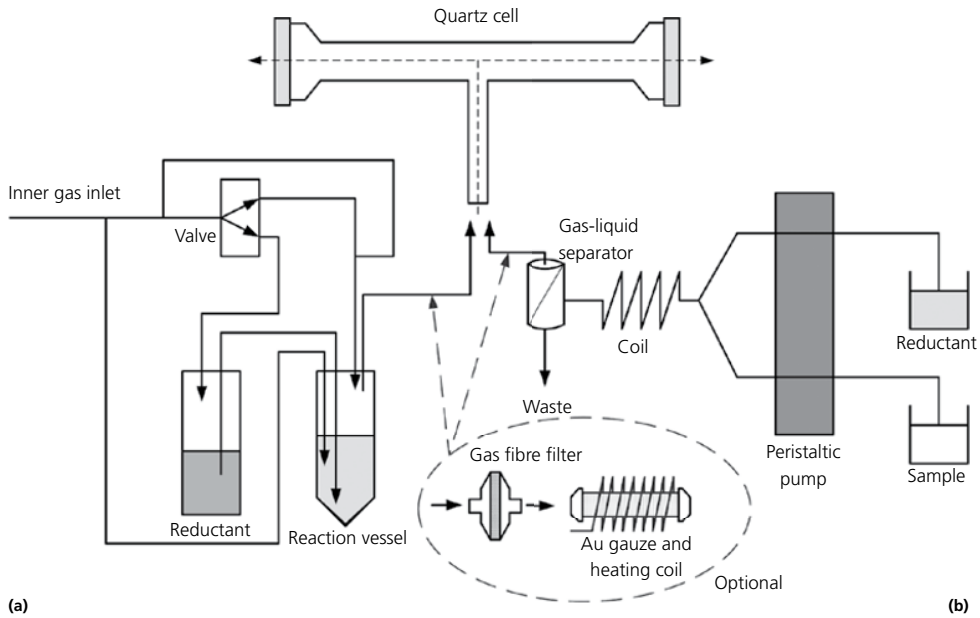


Figure 10.14 Techniques involving vapour generation: (**a**) batch mode; (**b**) continuous operation mode. The insert shows an atom trap for mercury. A cryogenic trap may be used in the case of hydrides.

10.12.2 Hydride generation technique

The determination of some elements by FAAS is not to be recommended due to insufficient sensitivity or to the fact their resonance lines are at low wavelengths, in a zone of the electromagnetic spectrum where even the gases involved in the flame reaction can pose interference problems. Some of these elements are also problematic when electrothermal atomization is carried out. The use of a volatile molecule to separate the elements of the sample solution is an interesting alternative choice. Although different volatile molecules could be used for the purpose, the generation of hydrides is by far the most useful. Nine elements (As, Bi, Ge, Pb, Se, Sn, Sb, Te and In) have been reported to form volatile hydrides appropriate for AAS measurements, and arsenic is without doubt the most representative and widely studied.

Three stages are necessary to carry out the determinations. Firstly, the hydride is generated in the solution which is being analysed. It must then be transferred to the atomizer, where it is decomposed by heating, originating the atomic vapour that absorbs the radiation coming from the source. This way of operating offers two advantages. The analyte is separated from the sample solution so that, provided there is no problem in generating the hydride, no interferences should appear in the following stages. In addition, sensitivity is greatly increased since the mass of analyte transferred to the atomizer is greater than that used in other atomization modes. Thus, for example, whereas the typical volume of sample in ETAAS is 20 µL, that used when HG-AAS is operated in the batch mode is 10–20 mL. Assuming high efficiency in hydride generation and transfer to the atomizer, the mass of analyte able to absorb radiation should be approximately three orders of magnitude higher. In practice, the difference in sensitivity is not so high since neither is the efficiency in generation and transport close to 100% nor is the density of the atomizer the same in both techniques. However, the improvement obtained with HG-AAS is still considerable [32].

The first stage in HG-AAS is to add a reducing agent to the sample solution to obtain the corresponding hydride. Classical reports for arsenic determination used metallic zinc in an acidic medium. However, obtaining arsine in this way is a slow process. Other reagents and reagent mixtures have been proposed and, nowadays, sodium tetrahydroborate is used almost exclusively. This reagent, when prepared in an alkaline medium and kept in the cold, is stable for 2–3 weeks, allows a rapid reduction reaction and is suitable for determining As, Bi, Ge, Pb, Se, Sn, Sb and Te.

As with CV-AAS, there are two operation modes. The classical mode is the batch mode but continuous operation involving the on-line mixing of the sample and reducing solutions (Figure 10.14) is much more suitable. To save sample solution, instead of relying on continuous pumping, an aliquot may be injected (FI-HG-AAS). For extremely diluted samples, an entrapment device can be included in the system but, given the high sensitivity of the technique, this will seldom be necessary.

The most commonly used atomizer is a quartz tube but graphite is also used. The atomizer has to be heated at 800–1000 °C to release the element from the hydride. Quartz atomizers can even be placed directly above a flame but this is not to be recommended. The best way is to use easily controlled electric heating.

As shown in Table 10.2, the sensitivity is much better than that of FAAS and even higher than that obtained in ETAAS, as already indicated. The values we give have to be considered only as orientative since they were taken from manufacturers' protocols and some articles consulted, and correspond to direct systems with no pre-concentration or trapping devices but in the optimal conditions.

Although the technique is selective, interferences may appear for several reasons. Differences may exist in the analytical signal depending on the oxidation state of the analyte, which affects the kinetics and extent of hydride generation. Thus, for example, the sensitivity reached for Sn(IV) is much higher than that for Sn(II). Similarly, for lead determination a strong oxidant is required before the reduction stage since the maximum sensitivity corresponds to Pb(IV). In contrast, a pre-reduction stage is necessary for selenium and tellurium since the hexavalent species do not form hydrides. Differences exist between the tervalent and pentavalent forms of arsenic and antimony, and a prior reduction to As(III) and Sb(III), respectively, is recommended. This can be achieved by using L-cysteine or a mixture of potassium iodide plus ascorbic acid.

Table 10.2 Atomic absorption spectrometric detection limits (μg/L).

Element	FAAS	Vapour-AAS
As	150	0.03
Bi	30	0.03
Ge	300	1.2
Hg	300	0.009
Sb	45	0.15
Se	100	0.03
Te	30	0.03

If the analytes are present in the sample in the form of organic compounds, a prior decomposition stage is necessary to release the inorganic forms. Sometimes the compounds are resistant to decomposition and energetic treatments are required. This should be taken into account, especially when dealing with arsenic or selenium compounds in biological samples. The case of arsenobetaine is of particular note. This quaternary arsonium compound, which is widely distributed in marine organisms, is relatively non-toxic and so discrimination of the more toxic inorganic arsenic is important in food analysis. Arsenobetaine does not form the volatile arsenic hydride and so it has to be oxidized by a strong oxidant such as peroxydisulfate while heating.

Matrix effects can also affect the efficiency of hydride generation but they can usually be solved by using SAM. Other difficulties arising during transport from the vessel to the atomizer or originated during the hydride decomposition stage are less frequent and can be overcome by strictly following the recommended procedures [33].

To summarize, the most significant feature of HG-AAS is its extreme sensitivity, which, in addition to its easy implementation in continuous operation mode, makes it suitable as detector in chromatographic separations. Its ability to deal with very low concentrations of arsenic is its most relevant feature. The negative side is the low number of elements to which the technique may be applied.

10.13 Electrothermal atomization

FAAS is a simple technique useful for determining large numbers of elements at low concentrations but it has two drawbacks. The first refers to the consumption of sample solution, a severe inconvenience when dealing with biological samples or valuable substances of which only a low volume is available. The second disadvantage concerns FAAS sensitivity, which is good but not always sufficient for many practical situations. Both difficulties can be overcome using ETAAS. In this case, the sample solution is directly introduced into the atomizer instead of being aspirated, and the atomic cloud obtained

remains exposed to the light beam coming from the source for several seconds. The consequence of using ETAAS is a large increase in sensitivity accompanied by minimal consumption of sample.

10.13.1 Types of atomizer

The most common type of atomizer consists of a graphite tube (typically 2–3 cm long, 0.5–1 cm diameter) with a hole for introducing small volumes (10–20 μL) of sample. The atomizer is heated by passing an electrical current through the tube, which at the same time is traversed by the beam of light coming from the excitation source. When mounted in the spectrometer, the graphite tube is prevented from reacting with the oxygen of the air by means of a gas shield, usually argon, which also flows through the tube in order to expel the volatile products that appear during the heating cycle. The device is also provided with a water-cooled system that permits the temperature to be rapidly decreased when heating is finished. Ordinary graphite (electrographite) is not suitable for ETAAS because of its porosity and reactivity, which would result in the analyte diffusing through the pores, poor reproducibility and a short useful lifetime. For these reasons, commercial atomizers are made of pyrolytic graphite, which is obtained by treating hydrocarbons at high temperature and low pressure. At the very least, electrographite should be coated with pyrolytic graphite (Figure 10.15).

The most conventional graphite atomizers use longitudinal heating, that is, electrical contactors are placed at each end of the tube. In this way, the centre of the tube is heated more rapidly, and the resulting temperature gradient means that isothermal conditions are not easily reached in the gas inside the tube. In transversely heated atomizers, the electrical current passes through the tube in such a way that the atomizer is heated across its circumference, which facilitates isothermal heating (Figure 10.16). Irrespective of the exact design, the gas inside the atomizer is heated more slowly than the wall, and a transitory temperature gradient inevitably appears. The lack of isothermal conditions may produce condensation and matrix interference problems, especially when dealing with volatile elements. To minimize this deleterious effect, a small pyrolytic platform can be inserted in the tube (see Figure 10.15) so that an aliquot of the sample is dispensed onto the platform instead of the tube wall. Because the surface contact between wall and platform is small, the latter is heated by convection,

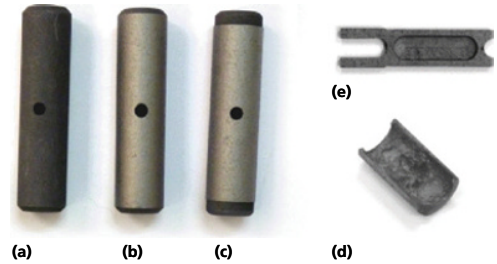


Figure 10.15 Graphite tubes for longitudinal heating: (**a**), (**b**) and (**c**) correspond to non-pyrolytic, pyrolytic and pyrolytically coated atomizers, respectively; (**d, e**) two platform models.

and atomization takes place in an isothermal medium. The platform atomization mode is advantageous when dealing with volatile elements, while wall atomization is recommended for elements requiring high atomization temperatures.

10.13.2 Heating program

For ETAAS measurements to be carried out, an aliquot of the sample is introduced into the atomizer by means of an autosampler, and a suitable heating program is applied. The program involves several steps, each characterized by three parameters: the temperature to be reached, the time necessary to reach this temperature (the ramp time) and the time the temperature is maintained (hold time). Figure 10.17 shows a typical program. As a first step, the temperature is increased slowly (typical ramps 10°C/s) until it reaches 110–120°C so that the solvent (usually water) is evaporated. The water vapour is expelled from the tube by the continually flowing purge gas. The ramp time of this stage must not be too short to prevent spattering inside the atomizer, which would result in a decrease in reproducibility. The temperature is then increased as high as possible to volatilize most of the sample matrix without premature loss of analyte. Typical temperature values for this ashing or pyrolysis step are in the 700–1000°C range, although they may exceed this depending on the matrix and analyte. Optimization of the exact conditions to carry out this step is essential to reduce the background signal obtained during atomization. Once the pyrolysis step is finished, the internal purge gas flow is switched off and the temperature is again raised, this time rapidly to produce the atomic cloud so that absorbance can be measured (atomization step). A typical absorbance–time profile obtained in this way is also shown in Figure 10.17.

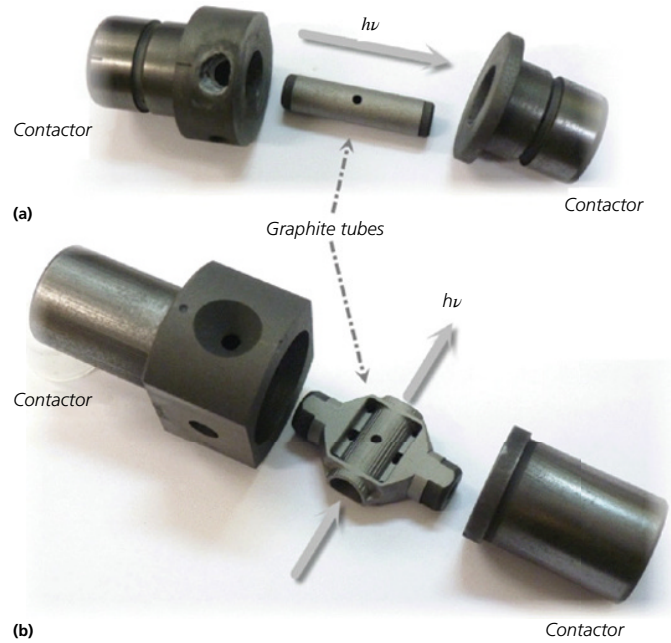


Figure 10.16 Atomizers for longitudinal (**a**) and transversal (**b**) heating showing the position of the contactors.

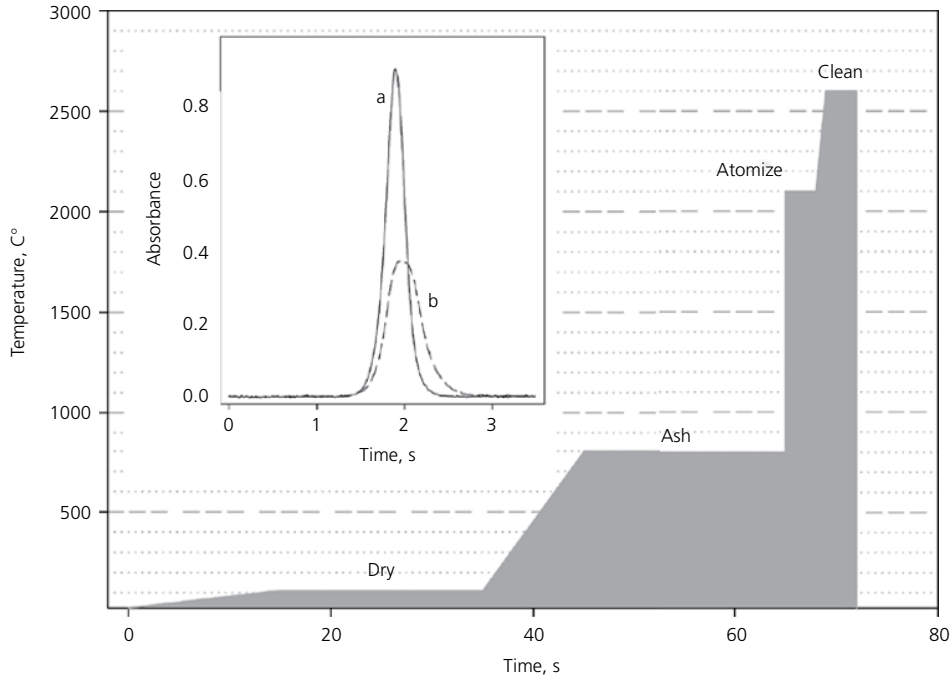


Figure 10.17 Typical four-stage heating program. The insert shows a background-corrected atomization profile (a) and the background signal (b).

The area of the peak profile is used as the analytical signal. Finally, when this stage is finished (the hold time is usually 3–5 s), the temperature is again increased to expel the residues. This cleaning stage is very short (2–3 s) but efficient since it is usually carried out at the maximum temperature allowable in the instrument. When the power is switched off, the temperature of the atomizer decreases rapidly to room temperature due to the water-cooling system. The atomizer is now clean and ready to carry out the next firing.

10.13.3 Chemical modifiers

Interference effects are more severe in ETAAS than in FAAS. Most of the difficulties arise from the complexity of the matrix since, during atomization, in addition to the analyte atomic cloud, other species still remaining in the matrix are also volatilized and can absorb or scatter the electromagnetic radiation, giving rise to a background signal that must be subtracted from the total absorbance to obtain the true signal. The relevance of the pyrolysis step is clear since its purpose is, as indicated, to simplify the matrix to minimize volatilization of concomitants during atomization and so decrease the background. Other difficulties may arise due to the presence in the matrix of chemicals, for example chloride ions, that may induce a premature loss of analyte before the atomization stage.

To minimize these and other similar inconveniences, most ETAAS procedures make use of chemical modification by incorporating a suitable chemical (the modifier) in the aliquot of the sample that facilitates the removal of the matrix during the pyrolysis step or stabilizes the analyte as a refractory compound so that the pyrolysis temperature can be increased without losing it. A well-known classical example of chemical modifier is ammonium nitrate, which is incorporated in the sample aliquot to overcome the deleterious effect of chloride ions. In this case, the displacement reaction:

$$NaCl + NH_4NO_3 \rightarrow NaNO_3 + NH_4Cl \uparrow \quad (10.14)$$

leads to most of the chloride being expelled from the atomizer in the form of volatile ammonium chloride, so that the deleterious effect is overcome. Another example of chemical modification which is particularly useful when dealing with samples with a high organic content, such as foods, is hydrogen peroxide. In such cases, the decomposition of the chemical releases a small amount of oxygen *in situ*, helping to eliminate the organic matter still present without harming the expensive pyrolytic material.

A large number of chemicals have been proposed for use as chemical modifiers. Palladium salts are recommended by many authors, and a mixture of magnesium and palladium salts has even been suggested as a 'universal modifier' to allow the temperature to be raised substantially, although there is not complete agreement in the literature as regards this universal benefit. Chemical modification has been revised in depth by several authors, and Volynskii [34] published a detailed study that is recommended for a thorough understanding of the subject. An interesting alternative in this respect involves prior treatment of the pyrolytic atomizer with the modifier (for example by coating, impregnation or electrolysis) so that this 'permanent modifier' can be used repeatedly for a high number of firings instead of being incorporated in each sample aliquot [35].

10.13.4 Background correction

Besides the need for a suitable pyrolysis step and chemical modification, the use of a background corrector system is indispensible in ETAAS. As indicated in Section 10.8, the most common correction device is a continuum source (deuterium lamp) that provides good correction at wavelengths below 350 nm. This background correction system is particularly useful for FAAS measurements although it performs less well in the case of ETAAS. While a large number of commercial instruments are equipped with this continuum source, it should be noted that its effectiveness may decrease as a result of the excessive complexity of the gaseous cloud obtained during atomization or due to the presence of a structured background, so that any overcorrection may have a distorting effect on the analytical signal. Background correction can also be carried out by means of a Zeeman-based device, in which case a strong magnetic field is applied. The most commonly used configuration applies an alternating magnetic field to the atomizer in a direction which is perpendicular to that of the light beam from the irradiation source. When the magnetic field is off, any signal is due to the analyte plus the background, while when the field is on, only the background signal is measured. The need to include a polarizer in the system decreases the intensity of the light beam, which worsens the signal-to-noise ratio and leads to an unavoidable decrease in sensitivity. This drawback

is largely compensated by the reliability of the correction, which is carried out exactly at the analyte wavelength.

To summarize, the Zeeman-based device has a wider range of applications and is more efficient for use in ETAAS in the case of structured backgrounds, although the sensitivity is decreased and the cost is greater that when the common deuterium corrector is used. Ideally, the instrument should be provided with both correction devices, using the deuterium system mainly in FAAS and reserving the Zeeman corrector for the most complicated ETAAS measurements.

10.13.5 Sample introduction

The usual way of introducing a small sample volume in the electrothermal atomizer is to use an autosampler. Manual injection by using a micropipette is also possible but not to be recommended. The autosampler plays an important role since, in addition to injecting the samples and the standard solutions for calibration, it can be programmed to incorporate the chemical modifier, to dilute samples if necessary, and to carry out other functions that increase the performance of the instrument. In some cases, in which sample pretreatment is carried out in a continuous flow system, despite the discontinuous mode of ETAAS operation, it can be combined with FI methodology. Several approaches have also been described [36, 37] that produce volatile analytes which are then introduced in the graphite atomizer, thus increasing sensitivity.

On the other hand, ETAAS is particularly useful for the direct analysis of solid samples, without the need for a previous dissolution stage. This is a clear advantage over other techniques because the risk of analyte loss or contamination during sample mineralization is avoided, accompanied by a saving of time and reagents.

It should be noted that when dealing with a solution, an aliquot of the sample is first dried inside the atomizer, so that a small solid residue is obtained on the wall or graphite platform. When dealing directly with solid samples, the situation is similar in that a small portion of the solid is placed on the surface of the atomizer. However, the main difference is that the solution is the result of a previous treatment of the sample with acids or other suitable chemicals, that is, the sample matrix has been destroyed or at least simplified during the treatment. In the case of the direct introduction of the solid sample, the matrix is intact, and the destruction/simplification role played by the dissolution process has to be assumed by the pyrolysis step. In addition, the efficiency of the chemical modifier might also decrease when dealing directly with the solid. Despite these differences, the abundant literature on the subject has demonstrated that an appropriate chemical modifier, together with an optimized heating program, provide reliable results with a reproducibility similar to that found when handling solutions [38].

There are two approaches to handling the solid samples for this purpose: direct introduction of the sample or using a suspension (slurry) prepared from the finely powdered sample. The first presents the advantages of speed, minimal manipulation and the possibility of using devices specifically sold for the purpose. On the other hand, practical problems include the need to weigh and introduce in the atomizer a small amount of sample (about 1 mg), which must be representative but which is frequently heterogeneous.

The slurry method is based on using a suspension that, when suitably prepared, can be handled similarly to a solution. The advantage of this approach is that there is no problem related with weighing or due to non-homogeneity, since a relatively large (up to 1 g) amount of sample is taken to prepare the suspension. One drawback, however, is the need for a low particle size that will permit a sufficiently stable suspension to be formed, sampled and injected into the atomizer. Stabilization can be aided by means of surfactants or by sonicating the samples for a short time.

The problems caused by the introduction of a relatively large amount of a non-digested biological sample can be solved by optimizing the heating program and, especially, through the action of appropriate modifiers. In this case, a build-up of carbonaceous residues would occur inside the atomizer and the background signal would be so high that it could not be compensated by the correction devices; moreover, the useful lifetime of the expensive pyrolytic material would decrease. Figure 10.18 shows the deterioration in the pyrolytic material caused when suspensions prepared from silica matrix (A) or baby food slurries (B and C) were atomized. These inconveniences can be overcome in a simple way by incorporating hydrofluoric acid (for the silica matrix) or hydrogen peroxide (for the food samples) in the slurries to act as a chemical modifier. Table 10.3 [39–41] provides some significant examples that demonstrate how traces of elements in complex

samples can be determined by this methodology, with no need for tedious preparation steps that are prone to the risk of contamination or loss of analyte.

Both methodologies, the direct use of solids and slurries, have proven useful for the analysis of foods, and are considered as mature well-established procedures for a variety of samples and relevant applications, as discussed in several reviews [40, 42, 43]. However, it is important to bear in mind that such approaches cannot be regarded as a panacea applicable to all foods since certain requirements must be satisfied, mainly the need for a low particle size, while the physicochemical characteristics of some foods can make this essential condition difficult to meet.

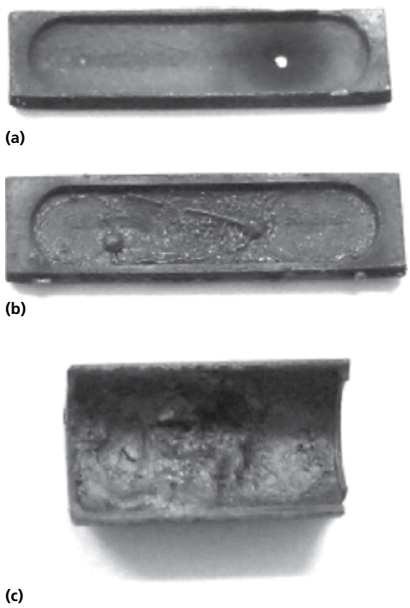


Figure 10.18 Deterioration of graphite platforms due to the introduction of slurries prepared from silica (**a**) and baby food samples (**b, c**). The deterioration is avoided by using appropriate modifiers.

10.14 Speciation

The exact chemical form in which an element is present in the sample affects its toxicity, and so speciation is a subject of clear relevance, especially when dealing with food analysis. In this regard, the direct use of AAS does not permit the different forms of an analyte to be discriminated since the response depends on the cloud of neutral atoms originated inside the atomizer, which is related to the total analyte concentration in the original sample. Consequently, speciation requires two steps, the first to separate the different chemical forms, the second to measure the amount of element present in each fraction.

There is a variety of separation techniques that can be coupled to FAAS, HG-AAS and CV-AAS, including those that operate in a continuous way (liquid chromatography, gas chromatography and some flow injection manifolds) and those based on a more or less automated batch operation. In the first case, the AAS instrument is the detector of the chromatographic or flow system, while in the latter, it is used as a conventional measuring instrument for the different fractions containing the chemical species isolated.

It should be noted that although FAAS operates in a continuous mode, the sensitivity is often insufficient for speciation purposes since the low total amount of the analyte is distributed through the different fractions. In contrast, the higher sensitivity of HG-AAS and CV-AAS render these atomization modes more suitable for speciation [44] but the number of elements that may be determined is limited.

10.14.1 Non-chromatographic procedures

Most such procedures use a solid phase arranged as a cartridge or minicolumn so that the analyte or one of its complexes can be selectively retained and then eluted [45, 46]. This separation usually results in a preconcentration effect that facilitates FAAS determination,

Table 10.3 Some examples of procedures involving direct use of the sample in ETAAS.

Analyte	Matrix	Modifier	Reference
Arsenic	Fish-based baby food	Palladium, cerium, tetramethylammonium hydroxide	[39]
Phosphorus	Honey, milk and infant formulas	Palladium, magnesium nitrate	[40]
Lead	Honey	Palladium, magnesium nitrate, dihydrogenphosphate	[41]

as demonstrated for example in the case of the on-line solid extraction of lead compounds in a functionalized cellulose sorbent, which allows differentiation between tetraalkyl lead and the sum of inorganic lead and organolead species containing a smaller number of alkyl groups [47]. Other representative examples deal with iron and chromium speciation [48].

The high sensitivity of CV-AAS allows methylmercury and inorganic mercury to be speciated. In this case, only methylmercury is retained in the minicolumn and it is then eluted with a small volume of acid solution, the manifold being designed in such a way that the entire procedure is carried out on-line [49]. On the other hand, it has to be remembered that in some cases, as occurs with arsenobetaine, arsenocholine, arsenosugars, selenomethionine and selenocysteine, the chemical forms are quite stable and require decomposition treatments, which prevents automation and hinders practical applications. However, this difficulty may be overcome by selecting appropriate ways for sample treatment. In this regard, interesting procedures based on photolysis and sonolysis-promoted redox reactions have been proposed for the non-chromatographic speciation of Se(IV), Se(VI), selenomethionine and selenocysteine using HG-AAS as the detection system [50].

Interest has grown in recent years in the coupling of solid-phase microextraction [51] and liquid-phase microextraction [52] techniques to FAAS, HG-AAS or CV-AAS [53–56] for speciation studies. However, the application to complex matrices of these methodologies, that require relatively large volumes of sample, is still infrequent since preliminary treatment steps are necessary and these may alter the original species of the analyte. Conversely, the high sensitivity showed by ETAAS (see Section 10.13) together with the fact that this technique only needs a few microlitres of liquid to be carried out, has resulted recently in procedures allowing non-chromatographic speciation from a new perspective.

10.14.2 Chromatographic procedures

The scientific literature reports procedures that use AAS as the detection system in gas chromatography. However, this coupling presents at last two drawbacks: first, a derivatization reaction is required to obtain volatile compounds suitable for gas chromatography and also, since the maximum volume to be injected in gas chromatography is limited, sensitivity is insufficient for speciation studies, especially when using FAAS. In this respect, other atomic or mass-based techniques are more suitable due to their higher sensitivity.

The combination of AAS with liquid chromatography is more advantageous since there is no need for derivatization, although the problems posed by insufficient sensitivity still remain in the flame atomization mode. Good results have been reported in the case of atomization based on vapour generation. For example, liquid chromatography with HG-AAS as the detection system was successfully applied to the separation and determination of inorganic tin, tributyltin, dibutyltin, monobutyltin, diphenyltin and monophenyltin in seafoods [57]. Similarly, good results have been reported for the speciation of As(III), As(V), dimethylarsinic acid and monomethylarsonic acid in baby foods [58]. These analytes were first separated by anion-exchange liquid chromatography and, after reacting with tetrahydroborate, arsenic was measured by HG-AAS. Arsenobetaine was also determined using on-line oxidation with peroxydisulfate before incorporating the reducing agent to the flow system.

Capillary electrophoresis has also been successfully applied for the separation of inorganic mercury and organomercuric compounds, using CV-AAS for the final measurement [59]. However, from a practical point of view, other detection systems are more suitable for this purpose.

10.15 Sample treatment

The high selectivity and easy availability of AAS in the different atomization modes here considered, together with reasonably good sensitivity, make this a versatile and widely used technique for food and beverage analysis. As already indicated, the common practice requires that the samples be in liquid form and that an appropriate sample dissolution stage be used when dealing with solids. Sample treatment depends on the final aim: total concentration of the analyte or its speciation, the first purpose being the most usual [60].

The first stage of a reliable analysis is sampling, and the analyst must be aware of the importance of this step. Good sampling requires a representative homogeneous portion of the sample to be obtained and kept in such conditions that no alteration occurs during transport and storage prior to analysis [61]. The size of samples and subsamples, the spatial temporal distribution of the

sampling process and the possible necessity of some prior treatment before homogenization are key points that need to be kept in mind [62].

When dealing with beverages, sample handling is minimal. Usually degasification and filtration, if required, together with suitable dilution to overcome matrix effects suffice for most practical cases. This is useful even for a complex sample such as honey, which, once mixed with water, may be aspirated directly into the flame for the determination of calcium, magnesium and zinc [63].

When dealing with solid samples, or some liquid samples such as edible oils, the situation is more complicated since it is necessary to decide whether a complete mineralization stage, wet or dry, is necessary or whether the analytes may be isolated from the sample by lixiviation with an appropriate reagent (see Figure 10.19 for a simplified scheme). Wet mineralization is commonly based on nitric and hydrochloric acids, although other reagents such as sulfuric and perchloric acids or hydrogen peroxide are also widely used. The decomposition stage can sometimes be carried out on a simple heating plate but, for practical reasons, which include improved reliability, automation and the possibility of treating a large number of samples simultaneously, the use of a microwave oven is highly recommended. A number of manufacturers market microwave ovens provided with temperature and pressure sensors and these are, without doubt, the best alternative [64].

The size of the sample aliquot to be placed in the microwave oven, the volume and concentration of the acids and the temperature program to be applied depend on the particular type of food. Thus, for foods with more than 50% water, such as meat products, fish, fruits, vegetables, juices, milk, yogurts, and liquors with a high sugar content, 1 g of sample can be sufficient. For foods with a lower water content such as cheese, cacao, dry spices, dry fruits, cereals and legumes, 0.5 g is recommended while, for fats and oils, the amount should be lowered to no more than 0.3 g to avoid gas overpressure. It is important to emphasize that these values and others mentioned in this section should only be considered as orientative, and the indications provided by the manufacturer of the particular microwave used must be respected. To use amounts of sample and reagents above those recommended in an attempt to improve the detection limit is bad practice and is to be rejected.

The EPA 3052 procedure for biological and botanical materials, in addition to a wide variety of other matrices, can be quoted as a representative example of wet digestion. This procedure is versatile, well proven and permits variations that make it suitable for the determination of a variety of elements. To the sample (no more than 0.5 g) is first added 9 mL of concentrated nitric acid; then, depending on the matrix, 3 mL of concentrated hydrochloric or hydrofluoric acid is incorporated. The addition of a small quantity (0.5–2 mL) of concentrated hydrogen peroxide helps bring about the complete

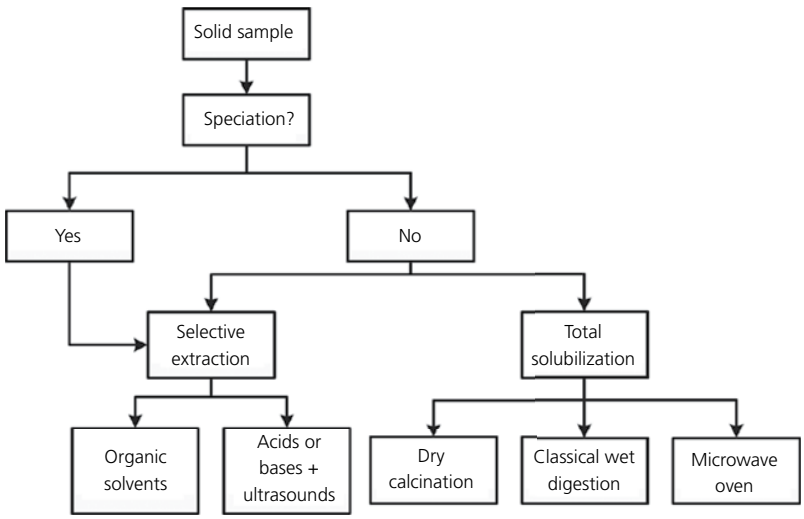


Figure 10.19 Flow diagram suggested when dealing with solid samples.

oxidation of the organic matter. The heating program consists of raising the temperature to 180°C in 5.5 min and then holding for 9.5 min.

As indicated, microwave treatment is preferable but alternatives exist. In the case of using open-air digestion, a reflux device may be necessary to avoid the loss of volatile elements. Again, the most common reagents are nitric and hydrochloric acids although hydrofluoric and sulfuric acids are also used, depending on the sample. For example, a mixture of nitric and hydrofluoric acids with hydrogen peroxide has been recommended for vegetables, while a simple mixture of nitric acid plus hydrogen peroxide is suitable for animal tissues [19].

Dry calcination at a high temperature is also possible. In this case, magnesium nitrate is incorporated in the sample and calcination is carried out at 450°C to obtain the ashes.

Complete digestion of the sample is not always necessary. For example, treatment with acids and ultrasound [65] allows many elements to be lixiviated from vegetables [66], with a saving of time with respect to total digestion. In spite of this, it should be borne in mind than lixiviation depends on the particular type of sample, particle size, the element to be measured and the exact experimental conditions. So, verification of the extent of the lixiviation achieved in each case is recommended.

Taking into account both the sensitivity of FAAS and the common concentrations found in foods, this atomization mode is suitable for the direct determination of potassium, sodium, calcium, magnesium, copper, iron, manganese and zinc using the air–acetylene flame. The applications extend to aluminium, chromium, nickel, silicon and tin when the nitrous oxide–acetylene flame is used, although some restrictions in sensitivity exist. For other elements pre-concentration steps are needed and a large number of reagents have been proposed. A classic example is the use of ammonium pyrrolidin dithiocarbamate to extract chelate compounds in methyl isobutyl ketone. Solid-phase extraction may also be used for the same purpose. The literature also reports a large number of procedures to increase automation by means of continuous flow or FI methodologies involving minicolumns, filters for retaining precipitates, knotted reactors, etc. [67, 68]. In this way, the sampling frequency increases but the enrichment factor decreases.

Liquid-phase microextraction uses very low volumes of acceptor phase and so large enrichment factors are reached. However, these microvolumes are incompatible with the conventional way of sample introduction in FAAS. To carry out the measurement, discrete nebulization or dilution up to 1–2 mL is necessary, meaning that the advantage obtained in the microextraction process is partially lost. Despite this, benefits include the saving of reagents and solvents [69, 70]. Ionic liquids [71, 72] and cloud point extraction [73] have also been recommended for pre-concentration before FAAS measurement. ETAAS is much more suitable for combination with liquid microextractions systems since only a low volume of sample is required. The conjunction of a very sensitive technique, such as ETAAS, and the high preconcentration factor inherent in the microextraction process results in unusually high sensitivities that in some cases exceed those achievable by ICP-MS.

For CV-AAS and HG-AAS, unless selective extraction is carried out, the sample must be digested completely since only in this way will the corresponding vapour be released by the action of the reducing agent. For mercury determination in foods, CV-AAS (or atomic fluorescence spectroscopy) is nowadays the best choice. If digestion is carried out in an open-air system, a well-cooled reflux device is necessary. The mixture of acids affects the efficiency of transformation of organomercuric compounds into inorganic mercury. Nitric acid plus sulfuric acid or hydrogen peroxide are recommended. However, the convenience of using microwave digestion in a closed system has to be emphasized. Note that, in addition to the saving of time and the lower risk of analyte loss, the amount of acids required is low, and so contamination problems are minimal.

As regards arsenic compounds in foods, HG-AAS is the most appropriate technique. Dry calcination is sometimes recommended in the literature but despite the incorporation of a large amount of magnesium salt, the risk of analyte loss is high, and this treatment is prone to error. Wet mineralization in a microwave oven is much better. In this case, perchloric acid (pay particular attention to safety precautions when handling this acid), in addition to nitric acid and sulfuric acid has been suggested. It must be remembered that some arsenic organic compounds (arsenobetaine, arsenocholine and oxy-bis-dimethylarsine) require particular energy conditions for the digestion process. The experimental conditions for the transformation of organic selenium compounds are even more severe. Similar considerations apply to other elements forming hydrides.

10.16 Conclusions

FAAS has become a well-established tool in the analytical laboratory for the routine determination of a large number of elements. The technique uses robust and reliable instrumentation and allows sensitive measurements to be carried out. The scant interferences are well documented in the literature and can be easily overcome. The cost of the instrument and maintenance is low, as is consumption of the relatively cheap gases involved. Similar considerations may be made as regards CV-AAS and HG-AAS. These latter techniques have a restricted scope since the number of elements that may be determined is low but they offer extreme sensitivity at a relatively low cost compared with other atomic or mass-based techniques. The analytical performance of AAS is greatly improved if the electrothermal atomization mode is used. In this case, the sensitivity is very high, and the possibility exists of directly using solid or undigested samples with a saving in time and reagents.

However, the main drawback of AAS is its operation in sequential mode, which affects sampling frequency. In addition, although sensitivity is sufficient for many routine direct determinations, FAAS, the most simple and economic instrument, is not suitable for use as a detector in chromatographic systems [74]. ETAAS has sufficient sensitivity but its discontinuous way of operation renders this atomization mode unsuitable for this purpose. These weaknesses have led many laboratories to replace AAS instruments by ICP-based techniques, mainly ICP-MS, due to the possibility of carrying out simultaneous detection and the very high sensitivity that is possible. However, these techniques should not be considered as incompatible but as complementary in the food analysis laboratory. The price of acquisition and maintenance of ICP-MS is much higher than that of AAS in the different atomization modes. Moreover, there is no need to use such powerful and expensive equipment to solve simple analytical problems. It seems more reasonable to use low-cost AAS instrumentation, which in addition does not require highly skilled operators for many common routine determinations, reserving ICP-MS for tasks in which its high performance is required.

On the other hand, the advances in instrumentation and new methodologies reported in the recent years have opened up new possibilities to improve AAS performance. In this regard, the development of instruments based on a continuum radiation source and a high-resolution double monochromator [75, 76] allows multi-elemental [77] determinations (including molecular species [78]) in FAAS with a high degree of automation. This new generation of instruments also permits simultaneous measurements to be made in ETAAS, although this interesting possibility is restricted in practice because the atomization conditions necessary for the elements to be determined must be similar. Only if this condition is met could the simultaneous determination be made using a single firing. This is the most significant advance in decades, and allows a different focus to the technique. Also the use of diode laser AAS [79] with modulation of the wavelength considerably improves sensitivity and permits the technique to be used for detection in chromatographic systems. However, the practical repercussion of this advance has been lower than that obtained by means of the continuum source.

Sample preparation is one of the most active subjects for innovation in AAS practice. Liquid and solid microextraction processes [11] allow the analytes to be concentrated from large sample volumes to a few microlitres of liquid, which may then be introduced in the atomizer of the AAS instrument. In this way, detection limits are lowered. Maximal benefits are obtained when electrothermal atomization is used but advantages also exist using FAAS [80] as we have mentioned.

To summarize, AAS in its different atomization modes (FAAS, HG-AAS, CV-AAS and ETAAS) continues to be a relevant analytical tool for the chemical analysis of foods. Versatility, low cost and good performance mean that its presence is essential in the routine analytical laboratory worldwide.

Abbreviations

AAS: Atomic absorption spectrometry
AES: Atomic emission spectrometry
AFS: Atomic fluorescence spectrometry
CI: Confidence interval
CV-AAS: Cold vapour technique
ED: Electrodeless discharge lamp
ETAAS: Electrothermal atomic absorption spectrometry
FAAS: Flame atomic absorption spectrometry
FI-HG-AAS: Flow injection hydride generation atomic absorption spectrometry

GC: Gas chromatography
HCL: Hollow cathode lamp
HG-AAS: Hydride generation atomic absorption spectrometry
ICP: Inductively coupled plasma
ICP-MS: Inductively coupled plasma mass spectrometry
LC: Liquid chromatography
LOD: Limit of detection
QC: Quality coefficient
SAM: Standard additions method

References

1 Welz B, Sperling M. The historical development of atomic absorption spectrometry. In: *Atomic Absorption Spectrometry*, 3rd edn. Wiley-VCH Verlag GmbH, Weinheim, 2007, pp. 1–61.
2 Agricola G. *De Re Metallica*, 1556, translated and reprinted by Dover Publications, New York, 1950. Available at http://ia600301.us.archive.org/28/items/deremetallica00agri/deremetallica00agri.pdf.
3 Newton I. *Opticks: or a Treatise of the Reflections, Inflections and Colours of Light*, 1704, 4th edn published in 1730. Reprinted by Dover Publications, New York, 1952. Available at http://ia700304.us.archive.org/11/items/opticksortreatis1730newt/opticksortreatis1730newt.pdf
4 Kirchhoff G, Bunsen R. XXIV. On chemical analysis by spectrum observations, *Quarterly Journal of the Chemical Society of London* 1861;**13**:270–289.
5 Thomsen V. A timeline of atomic spectroscopy. *Spectroscopy* 2006;**21**(10):32–42.
6 Walsh A. The application of atomic absorption spectra to chemical analysis. *Spectrochim Acta* 1955;**7**:108–117.
7 L'Vov BV. Fifty years of atomic absorption spectrometry. *J Anal Chem* 2005;**60**:382–392.
8 Hieftje GM. Atomic-absorption spectrometry: has it gone or where is it going. *J Anal Atom Spectrom* 1989;**4**:117–122.
9 Welz B. Atomic absorption spectrometry: pregnant again after 45 years. *Spectrochim Acta B* 1999;**54**:2081–2094.
10 Welz B, Becker-Ross H, Florek S, Heitmann U. *High-Resolution Continuum Source AAS*. Wiley-VCH, Weinheim, 2005.
11 Pena-Pereira F, Lavilla I, Bendicho C. Liquid-phase microextraction approaches combined with atomic detection: a critical review. *Anal Chim Acta* 2010;**669**:1–16.
12 Skoog DA, Holler FJ, Crouch SR. *Principles of Instrumental Analysis*, 6th edn. Thomson Brooks/Cole, Belmont, CA, 2007, pp. 220–221.
13 Sanz-Medel A, Pereiro R. *Analytical Atomic Absorption Spectrometry: An Introduction*. Coxmoor Publishing Co., Oxford, 2008.
14 Miller JN, Miller JC. *Statistics and Chemometrics for Analytical Chemistry*, 6th edn. Pearson Education, Ontario, 2010.
15 Knecht J, Stork G. Percentage and logarithmic procedures for calculation of calibration curves. *Fresenius Z Anal Chem* 1974;**270**:97–99.
16 Van Loco J, Elskens M, Croux C, Beernaert H. Linearity of calibration curves: use and misuse of the correlation coefficient. *Accredit Qual Assur* 2002;**7**:281–285.
17 Andrade-Garda JM, Carlosena-Zubieta A, Soto-Ferreiro RM, Teran-Baamonde J, Thompson M. Classical linear regression by the least squares method. In: Andrade-Garda JM (ed.) *Basic Chemometric Techniques in Atomic Spectroscopy*, 2nd edn. Royal Society of Chemistry, Cambridge, 2013, pp. 52–117.
18 Analytical methods for atomic absorption spectroscopy (1996). Perkin-Elmer Inc. Available at http://eecelabs.seas.wustl.edu/files/Flame%20AA%20Operating%20Manual.pdf
19 Welz B, Sperling M. *Atomic Absorption Spectrometry*, 3rd edn. Wiley-VCH, Weinheim, 1999.
20 Sneddon J (ed.) *Sample Introduction in Atomic Spectroscopy*. Atomic Spectroscopy Library, Vol. 4. Elsevier, Amsterdam, 1990.
21 Sanz-Medel A. (ed.) *Flow Analysis with Atomic Spectrometric Detectors*. Atomic Spectroscopy Library, Vol. 9. Elsevier, Amsterdam, 1999.
22 López-García I, Hernández-Córdoba M, Sánchez-Pedreño C. Flow-injection atomic-absorption spectrometry with air compensation. *Analyst* 1987;**112**:271–276.
23 López-García I, Viñas P, Campillo N, Hernández-Córdoba M. Use of submicroliter-volume samples for extending the dynamic range of flow-injection flame atomic absorption spectrometry. *Anal Chim Acta* 1995;**308**:85–95.
24 Fang ZL, Welz B, Sperling M. Expansion of dynamic-range of flame atomic-absorption spectrometry by an efficient flow-injection dilution system based on dispersion of microliter-volume samples. *Anal Chem* 1993;**65**:1682–1688.
25 Viñas P, Campillo N, López-García I, Hernández-Córdoba M. Rapid determination of calcium, magnesium, iron and zinc in flours using flow-injection flame atomic-absorption spectrometry for slurry atomization. *Food Chem* 1993;**46**:307–311.
26 Burguera JL, Burguera M. Pretreatment of oily samples for analysis by flow injection-spectrometric methods. *Talanta* 2011;**83**:691–699.
27 López-García I, Viñas P, Hernández-Córdoba M. Flow-injection dilution system for the analysis of highly concentrated samples using flame atomic-absorption spectrometry. *J Anal Atom Spectrom* 1994;**9**:553–561.
28 López-García I, Kozak J, Hernández-Córdoba M. Nonconventional semiautomated standard addition procedure based on membrane micropumps for flame atomic absorption spectrometry. *Spectrosc Lett* 2007;**40**:15–26.
29 López-García I, Sánchez-Merlos M, Hernández-Córdoba M. Automatic dilution system for use in flame atomic absorption spectrometry. *J Anal Atom Spectrom* 1998;**13**:551–556.
30 López-García I, Arroyo J, Viñas P, Hernández-Córdoba M. A manifold for the automatic dilution of concentrated solutions in flame atomic absorption spectrometry. *Anal Bioanal Chem* 2002;**372**:587–592.

31 Schroeder WH, Hamilton MC, Stobart SR. The use of noble metals as collection media for mercury and its compounds in the atmosphere. *Rev Anal Chem* 1985;**8**:179–209.

32 Dedina J, Tsalev DL. *Hydride Generation Atomic Absorption Spectrometry*. John Wiley & Sons Ltd, Chichester, 1995.

33 Tyson JF, Yourd E. Flame atomic absorption spectroscopy, including hydride generation and cold vapor techniques. In: Cullen M (ed.) *Atomic Spectroscopy in Elemental Analysis*. Blackwell Publishing, Oxford, 2004, pp. 239–280.

34 Volynskii AB. Chemical modifiers in modern electrothermal atomic absorption spectrometry. *J Anal Chem* 2003;**58**: 905–921.

35 Tsalev DL, Slaveykova VI, Lampugnani L, D'Ulivo A, Dimarco M, Georgieva R. Permanent modification in electrothermal atomic absorption spectrometry: advances, anticipations and reality. *Spectrochim Acta B* 2000;**55**:473–490.

36 Tseng WC, Sun YC, Yang MH, Chen TP, Lin TH, Huang YL. On-line microdialysis sampling coupled with flow injection electrothermal atomic absorption spectrometry for in vivo monitoring of extracellular manganese in brains of living rats. *J Anal Atom Spectrom* 2003;**18**:38–43.

37 Burguera JL, Burguera M. Flow injection-electrothermal atomic absorption spectrometry configurations: recent developments and trends. *Spectrochim Acta B* 2001;**56**:1801–1829.

38 Miller-Ihli N. Influence of slurry preparation on the accuracy of ultrasonic slurry electrothermal atomic absorption spectrometry. *J Anal Atom Spectrom* 1994;**9**:1129–1134.

39 López-García I, Briceño M, Hernández-Córdoba M. Non-chromatographic screening procedure for arsenic speciation analysis in fish-based baby foods by using electrothermal atomic absorption spectrometry. *Anal Chim Acta* 2011;**699**: 11–17.

40 López-García I, Viñas P, Romero-Romero R, Hernández-Córdoba M. Fast determination of phosphorus in honey, milk and infant formulas by electrothermal atomic absorption spectrometry using a slurry sampling procedure. *Spectrochim Acta B* 2010;**62**:48–55.

41 Rodriguez-Garcia JC, Barciela-Garcia J, Herrero-Latorre C, García-Martin S, Peña-Crecente RM. Comparison of palladium–magnesium nitrate and ammonium dihydrogenphosphate modifiers for lead determination in honey by electrothermal atomic absorption spectrometry. *Food Chem* 2005;**91**:435–442.

42 Cal-Prieto MJ, Felipe-Sotelo M, Carlosena A *et al*. Slurry sampling for direct analysis of solid materials by electrothermal atomic absorption spectrometry (ETAAS). A literature review from 1990 to 2000. *Talanta* 2002;**56**:1–51.

43 Vale MGR, Oleszczuk N, dos Santos WNL. Current status of direct solid sampling for electrothermal atomic absorption spectrometry: a critical review of the development between 1995 and 2005. *Appl Spectrosc Rev* 2006;**41**:377–400.

44 Long Z, Chen C, Hou X, Zheng C. Recent advance of hydride generation-analytical atomic spectrometry: Part I. Technique development. *Appl Spectrosc Rev* 2012;**47**:495–517.

45 Gonzalvez A, Armenta S, Cervera ML, de la Guardia M. Non-chromatographic speciation. *Trends Anal Chem* 2010;**29**: 260–268.

46 Vieira MA, Grinberg P, Bobeda CRR, Reyes MNM, Campos RC. Non-chromatographic atomic spectrometric methods in speciation analysis: a review. *Spectrochim Acta B* 2009;**64**: 459–476.

47 Naghmush AM, Pyrzynska K, Trojanowicz M. Flame AAS determination of lead in water with flow-injection preconcentration and speciation using functionalized cellulose sorbent. *Talanta* 1995;**42**:851–860.

48 López-García I, Moreno BM, Campillo N, Hernández-Córdoba M. On-line filtration system for determining total chromium and chromium in the soluble fraction of industrial effluents by flow injection flame atomic absorption spectrometry. *Anal Bioanal Chem* 2002;**373**:98–102.

49 Donard OFX, Ritsema R. Hyphenated techniques applied to the speciation of organometallic compounds in the environment. In: Barcelo D (ed.) *Environmental Analysis: Techniques, Applications and Quality Assurance*. Elsevier, New York, 1993, pp. 550–606.

50 Mendez H, Lavilla I, Bendicho C. Mild sample pretreatment procedures based on photolysis and sonolysis-promoted redox reactions as a new approach for determination of Se(IV), Se(VI) and Se(-II) in model solutions by the hydride generation technique with atomic absorption and fluorescence detection. *J Anal Atom Spectrom* 2004;**19**:1379–1385.

51 Mester Z, Sturgeon R. Trace element speciation using solid phase microextraction. *Spectrochim Acta B* 2005;**60**:1243–1269.

52 Pena-Pereira F, Lavilla I, Bendicho C. Miniaturized preconcentration methods based on liquid–liquid extraction and their application in inorganic ultratrace analysis and speciation: a review. *Spectrochim Acta B* 2009;**64**:1–15.

53 Baig JA, Hol A, Akdogan A *et al*. A novel strategy for chromium speciation at ultra-trace level by microsample injection flame atomic absorption spectrophotometry. *J Anal Atom Spectrom* 2012;**27**:1509–1517.

54 Basiri S, Moinfar S, Hosseini MRM. Determination of As(III) using developed dispersive liquid–liquid microextraction and flame atomic absorption spectrometry. *Int J Environ Anal Chem* 2011;**91**:1453–1465.

55 Martinis EM, Wuilloud RG. Cold vapor ionic liquid-assisted headspace single-drop microextraction: a novel preconcentration technique for mercury species determination in complex matrix samples. *J Anal Atom Spectrom* 2010;**25**: 1432–1439.

56 Zachariadis GA, Anthemidis AN, Daftsis EI, Stratis JA. On-line speciation of mercury and methylmercury by cold vapour atomic absorption spectrometry using selective solid phase extraction. *J Anal Atom Spectrom* 2005;**20**:63–65.

57 Viñas P, López-García I, Merino-Meroño B, Campillo N, Hernández-Córdoba M. Liquid chromatography-hydride generation-atomic absorption spectrometry for the speciation of tin in seafoods. *J Environ Monit* 2004;**6**:262–266.

58 Viñas P, López-García I, Merino-Meroño B, Campillo N, Hernández-Córdoba M. Speciation of arsenic in baby foods and the raw fish ingredients using liquid chromatography-hydride generation-atomic absorption spectrometry. *Chromatographia* 2003;**57**:611–616.

59 Deng B, Xiao Y, Xu X, Zhu P, Liang S, Mo W. Cold vapor generation interface for mercury speciation coupling capillary electrophoresis with electrothermal quartz tube furnace atomic absorption spectrometry: determination of mercury and methylmercury. *Talanta* 2009;**79**:1265–1269.

60 Dugo G, Di Bella G, Rando R, Saitta M. Sample preparation for the determination of metals in food samples. In: Pawliszyn J, Mondello L, Dugo P (eds) *Comprehensive Sampling and Sample Preparation*. Academic Press, Oxford, 2012, pp. 495–519.

61 Ahuja S, Diehl D. Sampling and sample preparation. In: Ahuja S, Jespersen N (eds) *Modern Instrumental Analysis*. Comprehensive Analytical Chemistry Vol. 47. Elsevier, Amsterdam, 2006, pp. 15–40.

62 Curren MSS, King JW. Sampling and sample preparation for food analysis. In: Pawliszyn J (ed.) *Sampling and Sample Preparation in Field and Laboratory*. Comprehensive Analytical Chemistry Vol. 37. Elsevier, Amsterdam, 2002, pp. 869–894.

63 López-García I, Viñas P, Blanco C, Hernández-Córdoba M. Fast determination of calcium, magnesium and zinc in honey using continuous flow flame atomic absorption spectrometry. *Talanta* 1999;**49**:597–602.

64 Ojeda CB, Rojas FS. Sample dissolution for elemental analysis. Microwave digestion. In: Alan PT, Colin P (eds) *Encyclopedia of Analytical Science*, 2nd edn. Elsevier, Oxford, 2005, pp. 153–163.

65 Tadeo JL, Sánchez-Brunete C, Albero B, García-Valcárcel AI. Application of ultrasound-assisted extraction to the determination of contaminants in food and soil samples. *J Chromatogr A* 2010;**1217**:2415–2440.

66 Wieteska E, Zioek A, Drzewinska A. Extraction as a method for preparation of vegetable samples for the determination of trace metals by atomic absorption spectrometry. *Anal Chim Acta* 1996;**330**:252–257.

67 Miró M, Hansen EH. On-line sample processing involving microextraction techniques as a front-end to atomic spectrometric detection for trace metal assays: a review. *Anal Chim Acta* 2013;**782**:1–11.

68 Lemos VA, de la Guardia M, Ferreira SLC. An on-line system for preconcentration and determination of lead in wine samples by FAAS. *Talanta* 2002;**58**:475–480.

69 Anthemidis AN, Mitani C. On-line liquid phase microextraction based on drop-in-plug sequential injection lab-at-valve platform for metal determination. *Curr Anal Chem* 2013;**9**:250–278.

70 Dadfarnia S, Shabani AMH. Recent development in liquid phase microextraction for determination of trace level concentration of metals: a review. *Anal ChimActa* 2010;**658**: 107–119.

71 Martin-Calera A, Pino V, Afonso AM. Ionic liquids as a tool for determination of metals and organic compounds in food analysis. *Trends Anal Chem* 2011;**30**:1598–1619.

72 Martinis EM, Berton P, Monasterio RP, Wuilloud RG. Emerging ionic liquid-based techniques for total-metal and metal-speciation analysis. *Trends Anal Chem* 2010;**29**: 1184–1201.

73 Bosch-Ojeda C, Sánchez-Rojas F. Separation and preconcentration by cloud point extraction procedures for determination of ions: recent trends and applications. *Microchim Acta* 2012;**177**:1–21.

74 Tsalev DL. Vapor generation or electrothermal atomic absorption spectrometry? Both! *Spectrochim Acta B* 2000;**55**: 917–933.

75 Welz B. High-resolution continuum source AAS: the better way to perform atomic absorption spectrometry. *Anal Bioanal Chem* 2005;**381**:69–71.

76 Welz B, Vale MGR, Florek S, Okruss M, Huang MD, Becker-Ross H. High-resolution continuum source atomic absorption spectrometry: theory and applications. In: Meyers RA (ed.) *Encyclopedia of Analytical Chemistry*. John Wiley & Sons Ltd, 2010. doi: 10.1002/9780470027318.a9124.

77 Oliveira SR, Gomes Neto JA, Nóbrega JA, Jones BT. Determination of macro- and micronutrients in plant leaves by high-resolution continuum source flame atomic absorption spectrometry combining instrumental and sample preparation strategies. *Spectrochim Acta B* 2010;**65**: 316–320.

78 Huang MD, Becker-Ross H, Florek S, Heitmann U, Okruss M. Determination of halogens via molecules in the air–acetylene flame using high-resolution continuum source absorption spectrometry: Part I. Fluorine. *Spectrochim Acta B* 2006;**61**:572–578.

79 Zybin A, Koch J, Wizemann HD, Franzke J, Niemax K. Diode laser atomic absorption spectrometry. *Spectrochim Acta B* 2005;**60**:1–11.

80 Sánchez-Rojas F, Bosch-Ojeda C, Cano-Pavón JM. Dispersive liquid–liquid microextraction combined with flame atomic absorption spectrometry for determination of cadmium in environmental, water and food samples. *Anal Methods* 2011;**3**:1652–1655.

CHAPTER 11

Elemental composition analysis of food by FAES and ICP-OES

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Abstract: Foods are an extremely diverse group of products that are often supplemented with additional items and sometimes contaminated with harmful substances. The concept of food analysis has grown in complexity as a result of international regulations and legislation, consumer demand, and globalization of the food market. Thus it has become an absolutely necessary task to trace a detailed description of a food, in terms of its nutritional value, chemical composition, bioactivity and toxicological aspects. Food analysis pushes research towards more advanced, sensitive and environmentally friendly methodologies. Sample preparation is a crucial part of the analysis. A successful food analysis cannot be achieved without an appropriate, convenient and reliable sample preparation strategy. On the other hand, in the last few years, a considerable variety of analytical methods applied to food analysis has been made available at the same rate as the increasing concern about food safety. The choice of the analytical methodology depends on various factors, such as nature of sample, goal of the analysis, and the availability of human and instrumental resources. In this chapter, current atomic emission-based sampling methodologies and sample preparation techniques used for food analysis and the advantages and drawbacks of each one are discussed. In addition, two elemental spectrometric determination techniques, flame atomic emission spectrometry (FAES) and inductively coupled plasma optical emission spectrometry (ICP-OES), their fundamentals, operational and experimental considerations regarding elemental analysis in food samples, and applications will be described.

Keywords: elemental analysis, food, emission spectrometry, sampling, sample preparation

11.1 Sampling and sample preparation for FAES and ICP-OES determination

Except for drinking water, food samples are complex composites with components that differ chemically from each other. In general terms, food samples are complex non-homogeneous mixtures of a staggering range of chemical substances. This makes it hard to isolate and determine analytes of interest, even with the advanced techniques of separation and identification available. Roughly speaking, it is not often possible to analyse food without manipulation. Before the analytical determination, food samples go through several stages after sampling, i.e. sample preparation through filtration, homogenization, dissolution, trace enrichment, and removal of interferents. For the preparation of an appropriate number of samples, the methods have to be selected not only on the basis of the expected concentration of the analytes in the solution that results from the decomposition process, but also according to general requirements such as multi-element analysis and dynamic range of the determination, number and mass of samples, laboratory equipment, and the experience of the analytical staff [1].

From the above, it is evident that overall analysis time and costs will be strongly influenced by these procedures and, what is more, they will contribute highly to the error generation through incomplete dissolution of the matrix or volatilization of the analytes and contamination from reagents or vessels [2, 3]. The more sensitive and accurate analytical techniques have become, the more attention has been given to sample clean-up. The detection limit of advanced analytical techniques is sometimes practically established by the attainable blank value, which is dependent on the sample preparation stages [3, 4].

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11.1.1 Sampling

Sampling is the most important step in the analytical process. In order to obtain reliable analytical results, it is essential that the samples are taken from the lot in a representative way [5]. Therefore, sampling strategy and design, sampling technique, and sample size must be harmonized optimally. There exist general guidelines for sampling [6–10] as well as special instructions for food and related products [11].

The general demand on sampling is that it has to be carried out in a representative way, to be precise, representative with regard to both the properties of the material and the analytical problem. This twofold representativeness of the sample means that sampling strategies are different for average analysis (bulk analysis) and for testing homogeneity within a lot of n batches.

Sampling is a crucial step in the analytical process, particularly in cases where there are large differences between the material under investigation and the test sample (laboratory sample) with regard to both amounts and properties, especially grain size, fluctuations in quality and inhomogeneities. In the case of food samples, sampling involves the random selection of a certain representative portion, number of container, and product units from a particular lot of the same food. It takes place where any article of food is manufactured, stored for sale, etc. Adequate sample size and suitable containers for sampling (or appropriate preservatives) to prevent spoilage/damage before analysis are needed to made the sampling process successful. The number of units that comprise a representative sample from a selected lot of a food product must be statistically significant. The proper statistical sampling procedure, according to whether the food is solid, semi-solid, viscous or liquid, must be determined at the time of sampling.

Clean, dry, leak-proof, wide-mouthed, sterile and metal-free containers of a size suitable for sample of the product must be used. Before sampling, a sampling plan must be written considering not only the sample type and available amount, but also the hazard to human health and adulteration among others.

Sampling tools

Samples collected from bulk packages or unpackaged foods must be placed in suitable containers for storage and handling to be presented for laboratory analysis. Common tools such as pliers, spoon, screwdriver and knife are useful for opening containers or cutting bags of food products. They must be coated by, or made of, a metal-free material such as polytetrafluoroethylene (PTFE) or polypropylene. Glassware may also be used (special care must be taken when analysing Na, B, Al and Si, and strict procedural blanks should be prepared). Unless the sampling tool is in an unopened, metal-free, sealed container, it must be soaked in nitric acid overnight, washed with ultrapure water (resistivity higher than 18.2 MΩ·cm) and left to dry under ISO 5 (Class-100) laminar flow hood, and then stored in hermetically sealed plastic bags (e.g. Ziploc bags).

Sampling containers

The sample containers must fit the requirements of the sample type. Typically, for liquids, clean dry containers of appropriate waterproof and leak-proof polyethylene or polypropylene plastic material are recommended. The containers must have a secure closure comprising rubber or plastic stoppers, or a screw-cap of plastic, coated with an insoluble non-absorbent. For solids or semi-solids, clean, dry, wide-mouthed, cylindrical receptacles should be used. Special containers are used in particular cases. For instance, to sample butter, suitable wide-mouthed jars should be used. The butter must not be allowed to come into contact with paper or any water- or fat-absorbing surface.

Whenever possible, samples should be collected from previously unopened boxes or crates, unopened retail packages, packages, and unopened bulk containers such as sealed big containers. As with sampling tools, sample containers must not have contact with other materials that could generate cross-contamination. Preferably, they must be within sealed plastic bags; if not, they must be washed with nitric acid and ultrapure water, and left to dry within ISO 5 (class 100) laminar flow hoods.

11.1.2 Food treatment for elemental analysis by atomic emission spectrometry

It is very common that elemental analysis by atomic emission spectrometry (ICP-OES and FAES) is carried out with instruments equipped with liquid-sample introduction systems, usually pneumatic nebulizers (Figure 11.1) of the following types: concentric (Meinhard), cross-flow or V-groove. These devices are interfaced with the atomizer by means of a suitable spray chamber that cuts off the solution drops beyond a certain diameter, classified into

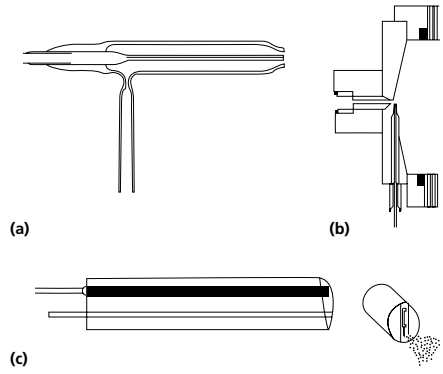


Figure 11.1 Meinhard **(b)**, cross-flow **(b)** and V-groove **(c)** nebulizers for ICP-OES.

three general types: double pass (Scott-type), single pass (for high solids) and cyclonic. Some instruments are furnished with sample introduction systems equipped with desolvation devices (e.g. jacketed spray chambers allowing heating/cooling of the spray stream) to enhance sample input, but lowering the amount of solvent that reaches the atomizer (ICP or flame).

It is usual for FAES measurements to be carried out with flame atomic absorption spectrometers (FAAS), which in turn are equipped with cross-flow nebulizers and single-pass spray chambers (premixing chamber). Detailed and fundamental discussions on sample introduction systems for atomic spectrometry can be found in the books by Montaser and Golightly [12] and Broekaert [13].

Depending on the selected sample introduction system, the composition of the sample to be analysed must meet certain requirements regarding total amount of dissolved solids (TDS), organic compounds (TOC) and acid concentration. For instance, concentric (Meinhard) nebulizers with double-pass (Scott-type) spray chambers perform adequately when TDS, TOC and acid concentration are kept as low as possible (typically levels below 5% p/v, 3% v/v and 10% v/v, respectively, are recommended). Specific applications based on ICP-OES involve high-efficiency introduction systems such as ultrasonic nebulization (USN) [14], microconcentric nebulizers (MCN) [15, 16], electrothermal vaporization (ETV) [17–19] or laser ablation [20–22].

A comparative study of the performance of conventional pneumatic nebulization and USN both coupled to ICP-OES can be found in the *Elemental Analysis Manual for Food and Related Products* of the US Food and Drugs Administration (Table 11.1) [23]. Replicate analysis precision (measured as relative standard deviation) was 15% or less for all element concentration measurements above limit of quantification (LOQ), except for chromium in mussel (85%) and soy powdered infant formula (52%), and selenium in whole egg powder (22%). Extremely high variability was observed for chromium and many divergent results were excluded from performance assessment.

It remains for the analyst to choose the appropriate preparation methodology in relation to the sample type, the available equipment and any imperatives of the analysis and indeed of the whole study. In general, aqueous samples can be introduced to analysis directly and without any previous special pretreatment, i.e. total

Table 11.1 Comparison of the analytical performance in ICP-OES of a Meinhard nebulizer and an ultrasonic nebulizer.

Element	Pneumatic nebulizer		Ultrasonic nebulizer	
	LOD* (mg/kg)	LOQ* (mg/kg)	LOD* (mg/kg)	LOQ* (mg/kg)
Al	0.8	2	0.1	0.3
As	2	4	0.03	0.08
Ba	0.05	0.2	0.2	0.4
B	0.3	0.8	0.05	0.2
Cd	0.3	0.9	0.005	0.02
Ca	8	30	20	40
Cr	2	5	0.05	0.2
Co	0.3	0.8	0.005	0.02
Cu	0.1	0.3	0.009	0.03
Fe	0.2	0.3	0.05	0.2
Pb	3	6	0.04	0.2
Mg	2	6	7	20
Mn	0.2	0.4	0.001	0.003
Mo	0.4	1	0.02	0.03
Ni	0.9	3	0.02	0.06
P	2	6	0.06	0.2
K	20	40	0.7	2
Se	3	9	0.2	0.4
Na	2	5	20	40
Sr	0.03	0.07	0.4	1
Tl	2	6	0.04	0.2
V	0.2	0.5	0.006	0.02
Zn	0.3	0.8	0.02	0.05

*Based on $10 \times \sigma$ of method blanks, 4-g analytical portion and 50-mL analytical solution.

or partial decomposition, as long as measured concentrations using spectrometric methods are reliable and satisfactory while possible interferences are under control. In most cases only very little sample preparation is required and the easiest way is simple sample dilution. The dilution factor used in this case depends on the concentrations of analytes and main matrix components; knowledge about sample composition could be very helpful. Such an approach certainly reduces the analysis time and sample handling. It leads to low reagent consumption and generation of minimal residue or waste. To minimize possible matrix interferences, standard additions and matrix-matched standards are proposed for calibration. Direct determinations from liquid samples (e.g. waters, beverages) with minimal sample treatment such as dilution, degassing or matrix components evaporation provide a viable alternative to digestion as a mean of sample preparation. In ICP-OES, several types of organic liquids can be introduced, but an increase in the radiofrequency (RF) power is required to maintain the stability of the plasma [24].

In most cases, preparation of solid samples involves several stages: drying (e.g. air, laboratory oven), homogenization (e.g. mixing, crushing), grinding (e.g. mills, mortars), followed by mineralization and dissolution of a subsample. The solution so obtained is ultimately diluted to a proper volume. Ideally, the organic fraction of the sample has been decomposed and completely eliminated during these preparation steps and only dissolved inorganic compounds constitute the residue to be analysed [25].

Sample digestion techniques

For more than 100 years, procedures have been described for the destruction of organic samples and the formation of aqueous solutions of the residues [25]. A large number of approaches, and their modifications, have been described but fall into two main classes, dry ashing and wet digestion.

Dry ashing

The first step of dry ashing ensures the decomposition of organic matter by heating the sample to a relatively high temperature with atmospheric oxygen serving as the oxidation agent. Different reagents (ashing aids) may sometimes be added to assist this process. Solubilization of the resultant ash using an appropriate acid or mixture of acids is the following step. This procedure ensures total destruction of the organic matter; the associated elements are generally transformed to carbonate or oxide forms. At present it is performed using fully programmable muffle furnaces with precise and accurate computer-based control of thermal programs. Several applications in liquid samples require intermediate evaporation to dryness, and it is usually carried out on sand baths or on hot plates. Typical procedures for dry ashing have been described recently (Table 11.2) and involve similar conditions for a number of dried (solid and liquid) food samples [26, 27].

Wet digestion

Wet digestion in closed systems, with the addition of reagents to solubilize and/or oxidize organic samples, is a well-known strategy; it was introduced in 1834 when Henry and Zeise determined sulfur in organic matrices [4, 5]. Wet digestions require much lower temperatures than those applied for dry ashing, and liquid conditions are maintained throughout. It is one of the most well-used techniques for disaggregating food and obtaining free ionic species [6]. Generally speaking, closed decomposition systems have been found better than open ones, reducing systematic errors and leading to correct analytical results. Over the years, detection limits have been improved by reducing the analytical blank (with the use of pure reagents and advanced vessel materials), by improving the signal-to-noise ratio (with more efficient sample oxidation) and by diluting the decomposition residue as little as possible.

A true quality advance in sample digestion was the introduction of pressurized dissolution combined with microwave enhanced chemistry [1]. Many fundamental mechanistic differences separate microwave heating from other heating methods. Solutions are heated so efficiently that reaction time-scales are dramatically reduced and the process control offered by microwave-assisted heating is better than that of any other heating method. As a result, microwave oven digestion (MOD) is highly amenable to standardization and automation.

This technique is strictly dependent on food composition. Thus carbohydrates are easily mineralized with nitric acid at 180 °C, while fats, proteins and amino acids are incompletely digested due to the relatively low oxidation potential of nitric acid at 200 °C, and require the addition of hydrogen peroxide.

Typical analytical portions range from 0.5 to 2 g [23]. High-fat foods require a lower mass while a larger mass can be used with high-water content foods. Often the

Table 11.2 Application of dry ash digestion for multi-element analysis of food samples by ICP-OES.

Sample	Amount	Temperature program	Diluting agent	Determined elements
Plum Fig Mulberry Grape Apricot Apple NIST SRM 1515 Apple leaves	1.0 g	Ramp to 450 °C (slow) Hold on 450 °C (16 h)	Dissolution in 5 mL HNO_3 (25% v/v), the solution filtered through Whatman filter paper and then the sample made to 10 mL	Ba, Pb, Cd, Mn, Cr, Co, Ni, Cu, Mn, Zn, Sr and Fe
Tomato sauce Rice Chickpea Bulgur Walnut Hazelnut White, black and pumpkin seed Garlic Pistachio Biscuit Mushroom Beef	1.0 g	Ashing at 450–500 °C for 16 h	Dissolution in 1–2 mL of concentrated HNO_3 and filtered through blue band filter paper. The sample was diluted to 10 mL with distilled water	Al, Mn, Fe, Cu, Zn and Se*
Chicken meat Milk Red wine Beer	1.0 g	Drying at 105 °C for 24 h. Ashing at 450–500 °C for 16 h	Dissolution in 1–2 mL of concentrated HNO_3 and filtered through blue band filter paper. The sample was diluted to 10 mL with distilled water	Al, Mn, Fe, Cu, Zn and Se*
NIST SRM 1515 Apple leaves	0.2 g	Ashing at 450–500 °C for 16 h	Dissolution in 1–2 mL of concentrated HNO_3 and filtered through blue band filter paper. The sample was diluted to 10 mL with distilled water	Al, Mn, Fe, Cu, Zn and Se*

*Recoveries lower than 50% were found for Se.

maximum analytical portion may be determined using a sample's known energy (i.e. caloric) content as an indicator of pressure produced during digestion. For some products (e.g. juice concentrates) the energy content may not be known. Maximum energy release permitted for 600 and 800 psi digestion vessels (90-mL capacity) has been empirically determined as 3 and 6 kcal, respectively [23]. This maximum vessel energy prevents a digestion from reaching the vessel's maximum operating pressure before digestion is complete. The energy of an analytical portion must not exceed these values for safe operation. An analytical portion should not exceed 5 g even if calculation indicates that a larger portion could be taken. This maximum mass will prevent excess dilution of the nitric acid used for digestion and ensure a complete digestion. For samples of unknown composition, a maximum of 0.5 g dry weight should be analysed for safety concerns. Table 11.3 shows different amounts for selected samples when digested with acids and microwave heating. If the maximum pressure attained for this unknown is less than the vessel limit, then a greater mass may be analysed. In many cases the maximum analytical mass will not be necessary or should not be used, for example the high level of salt in some foods may interfere with analysis [28]. The food energy content is available from many sources [29].

Extraction procedures

Ultrasonic-assisted extraction

The application of ultrasonic-assisted extraction (UAE) has accelerated sample preparation and reduced reagent consumption [30]. Ultrasound speeds up sample preparation

by diminishing solvent gradient concentrations in the solid–liquid interface, liberates unstable species into the irradiated medium, and sometimes increases sample surface area due to solid erosion. Ultrasound has been employed for sample preparation in order to improve analytical throughput [31]; however, the chemical signal of samples submitted to ultrasonic irradiation can be severely compromised since collapse of the cavitation bubble results in a strong local temperature increase and free radical production [32], which could provoke analyte loss and gross analytical errors. Analyte losses were also observed in spectrometric determinations of various metals in food samples pretreated with ultrasound devices compared with other sample preparation techniques and certified materials [33–36].

Table 11.3 Typical analytical portion mass for selected foods when using 800 psi microwave digestion vessels.*

Food	Portion (g)
American cheese	1.2
Beef liver	2.4
Peaches, canned	5
Spaghetti, canned	5
Dill pickles	4.0†
Spinach	5
Fruit cocktail, canned	5
Lettuce, Iceberg	5
Nuts, mixed	0.8
Peanut butter	0.8
Raisin bran cereal	1.6
Tomato catsup	3.0*
Yellow mustard	3.0*
White bread	1.8

*From Türker [38].

†Recommended portion is less than the calorie limit of the digestion vessel due to the interference produced by the high-salt content.

Ultrasonication can be applied in analytical chemistry in two ways: directly to the sample, or indirectly through the walls of the sample container using a water bath, which is the most available and cheapest source of ultrasound irradiation. However, there are other more efficient designs, for example horns (indirect or direct) or ultrasonic probes (direct to the sample), able to develop higher ultrasound power (Figure 11.2).

Typically, UAE lasts several minutes (<10 min), requires low sample mass (100 mg) and diluted reagents (1% HNO_3). Overall, UAE is a fast (15 min including centrifugation time) and simple approach for the extraction of a number of metals prior to their determination by ICP-OES. Precision better than 10% can be achieved for many elements in multiple extractions. Parameters affecting extraction, such as extractant concentration, sonication time and ultrasound amplitude, temperature and leaching reagents, are variables accounting for a successful extraction procedure.

Elemental speciation using ultrasonication has been done in different ways, for example ultrasonication has been used for boosting enzymatic kinetics in order to degrade organic matter in such a way that elemental

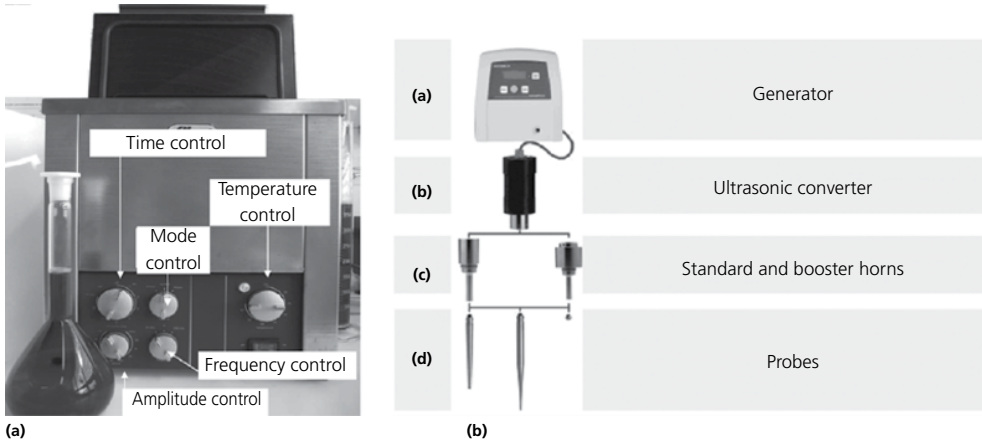


Figure 11.2 Direct and indirect ultrasound application. **(a)** Modern ultrasonic bath incorporating the following advances: dual frequency, variable amplitude and three different modes of sonication. From Fietkau *et al.* [54]. **(b)** An ultrasonic probe: (a) RF generator (20 kHz); (b) converter; (c) standard and booster horns; (d) probes. The design is crucial for good performance. Adapted from Bandelin Electronic GmbH & Co. KG.

organic species are extracted into solution without further degradation [37].

Solid-phase extraction

Perhaps a less widespread approach in the field of sample preparation for elemental analysis by emission techniques is solid-phase extraction (SPE) (and its alternative solid-phase microextraction, SPME). However, when a separation technique must be applied to reduce matrix effects, separate analytes or species of an analyte (speciation) and/or to increase analyte concentration (preconcentration), SPE has shown many advantages over other separation techniques such as (i) reduced solvent usage, (ii) low disposal costs, (iii) short extraction times, (iv) high efficiency, (v) ecologically safe, (vi) elimination of some of the glassware, (vii) isolation of analytes from large volumes of sample with minimal or zero evaporation losses, (viii) reduced exposure of analytes to organic solvents, and (ix) more reproducible results [38, 39].

A wide range of solid materials is available for the column SPE technique, which can be easily automated and coupled to emission determinative techniques. A typical instrumental set-up is shown in Figure 11.3. It describes an on-line SPE method for lead quantification in infusions (maté tea) by ultrasonic nebulization and ICP-OES. Polyurethane foam was used as sorbent material, which was packed in a conical minicolumn [14, 40]. A number of different sorbents have been used in similar approaches including activated carbon for Pb determination in bee honey [41, 42]. Another particular SPE methodology is that carried out with by means of knotted reactors, where the analyte ions are retained as an organic precipitate within a knotted PTFE tubing [43].

The mechanism of retention of analytes on solid phases depends on the nature of the solid phase and the species to be retained. The retention process usually involves adsorption of the metal ions at the surface of the sorbent via interactions with various functional groups, and ion exchange, chelation and ion-pairing formation processes. It also depends on the experimental conditions, such as pH, flow rates and metal ion/organic reagent concentration ratios.

Quality assurance and error sources in the sample preparation process

A major source of error is frequently related to the material used for the vessels involved in the sample dissolution process and in solution storage, even if the ratio of sample weight to vessel surface is as reduced as possible.

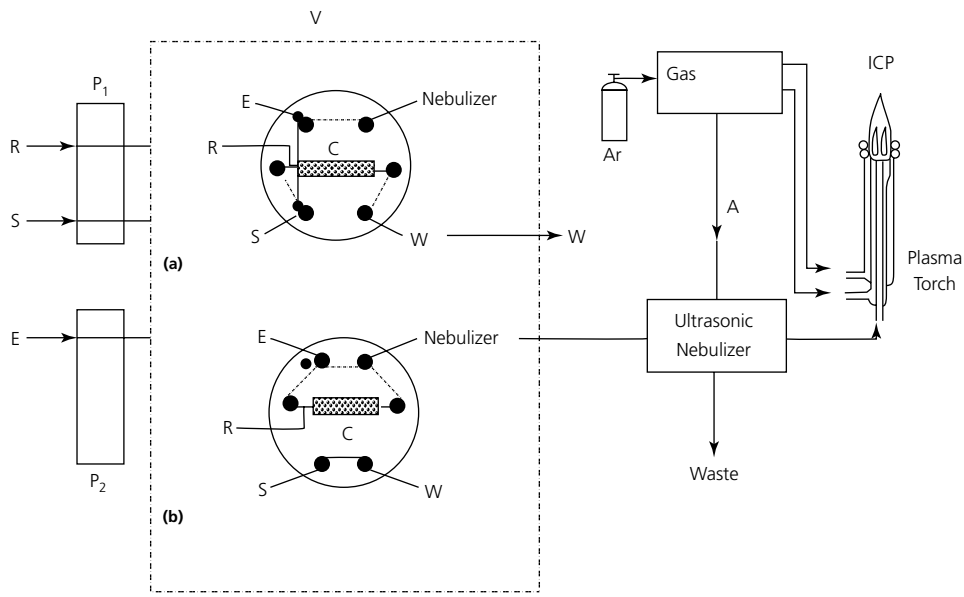


Figure 11.3 Schematic diagram of the instrumental set-up. S, sample (flow rate 10 mL/min); R, reagent (flow rate 5.0 mL/min); E, eluent (flow rate 1.5 mL/min); A, Ar (flow rate 0.7 L/min); W, waste; P1, P2, peristaltic pumps; C, minicolumn packed with polyurethane foam; V, injection valve. Valve positions: **(a)** sample loading and **(b)** injection. From Marchisio *et al.* [14]. Reproduced with permission of Elsevier

The use of non-disposable sampling equipment creates a potential for cross-contamination between samples. To eliminate the risk of cross-contamination, all non-disposable labware must be decontaminated, and blank samples must be analysed to document the effectiveness of decontamination. This procedure must also be carried out on any device that is not precleaned by the manufacturer. The best way to avoid contamination is to use precleaned disposable equipment. This includes sample containers, plastic or metal spoons, scoops, mixing bowls and other specific devices for food sampling. All these tools are available in a variety of disposable, inexpensive, single-use kits.

General considerations when weighing, handling and preparing food samples for analysis by FAES and ICP-OES

Weighing plays a crucial role in most analytical methodologies. Due caution should be exercised when weighing test samples that are hygroscopic [44, 45]. Calibration of analytical balances against standard weights of appropriate classes should be conducted on schedule. Weighing is preferably performed in a room that fulfils International Organization of Legal Metrology (OIML) requirements for calibration of weights of class E2 (temperature change ±0.7 °C per hour with a maximum of ±1 °C every 12 hours; range of relative humidity 40–60% with a maximum of ±10% every 4 hours) [45].

Procedural and reagent blanks are strongly dependent on laboratory cleanliness. The air quality is thus a matter of study. The use of clean rooms is recommended for the determination of sub-ppm levels. They are classified according to the size and the number of particles permitted per volume of air [46]. To minimize the risk of airborne contamination, clean rooms of ISO Class 4, 5 or 6 (US FED STD 209E equivalent: Class 10, 100 or 1000, respectively) are suitable for the analysis of samples having analyte concentrations at trace levels. Other activities (e.g. drying of acid-washed Teflon/HDPE bottles, polyethylene syringes and pipette tips, and preparation of sub-boiled nitric acid and ultrapure water) are often conduced in clean rooms.

If the food in question is hygroscopic (e.g. powdered milk, coffee, sugar, flour), moisture determination will be necessary. Oven drying at a temperature of 85 ± 2 °C is usually the procedure commonly recommended [47].

Final remarks

The quality of the analytical determination itself, data manipulation and subsequent report are strongly influenced by the operations described in the previous sections. In this context, sampling procedures (and sampling plan), transport, storage, manipulation, and treatment of samples to prepare analytical portions should be carefully carried out in order to avoid representativeness issues due to bad sampling, sample loses or analyte contamination. Metal content in the environment, sample container composition, and any other labware in contact with foods and food derivatives must be taken into consideration and, in this way, strict procedural blanks should be obtained along with the samples prior to trace elemental analysis by FAES and ICP-OES.

11.2 Inorganic analysis of major, minor and trace elements in food samples

The determination of inorganic elements in food substances is critical for assessing nutritional composition and identifying sources of food contamination. The measurement of inorganic elements is challenging because there is a wide variety of edible substances [48]. As a result, highly diverse matrices must be analysed accurately. The different matrices can lead to numerous interferences in the measurement of inorganic elements of interest and these interferences can vary with matrix composition. A key consideration in food analysis is the complex nature of the matrix, both in composition and form. Food substances can be composed of a wide variety of components including fats, proteins and sugars. Additionally, most foods are in solid form. The combination of complex matrices and solid samples means that most food substances have to be digested to break down the matrices and convert the samples to liquids, which are more easily analysed by inorganic techniques than solids. Other complexities involve the inorganic elements of interest, which can be divided into two main classes: nutritional and toxic. Nutritional elements (e.g. Mg, P and Fe) are present at high levels (mg/kg), while toxic elements (e.g. Pb, Hg and Cd) should only be present at trace levels (ng/kg or µg/kg) [49].

11.2.1 Flame emission techniques for analysis of food samples

The application of emission techniques to the analysis of different elements in food samples depends on the level of energy applied through the excitation source. When flame emission is applied to elements such as the alkaline and alkaline earth metals, which can be excited at low temperatures, simple emission spectrums with defined and intense lines, free of interferences, can be obtained. Substitution of flame by more powerful sources like plasma increases the number of elements that can be analysed, including the metallic and non-metallic elements. This section describes emission methods employing flame as the excitation source.

For the majority of emission instruments, flame is the atomization source so samples must be in solution in order to be introduced as an aerosol by nebulization. Although a wide variety of flames has been used over the years, essentially only two are currently in use for analytical measurements: air–acetylene and nitrous oxide–acetylene. With these two flames, appropriate analytical conditions can be achieved for most analytes. Choice of flame and detection limits for most elements are depicted in Table 11.4. It is worth considering that any atomic absorption spectrometer can be converted into an emission spectrometer by turning off the hollow cathode lamp and replacing the logarithm amplifier.

When an atomic absorption spectrometer is employed for emission analysis, some of the characteristics of atomic absorption are retained, such as security, elevated reproducibility, specificity and application possibilities. These characteristics are achieved by the use of high-resolution monochromators, photomultipliers and burners with gas control units (GCUs) for different flames. GCUs are important when nitrous oxide–acetylene flames are necessary for the atomization of elements with tendencies to form refractory oxides as depicted in Table 11.4, especially bearing in mind the explosive character of this type of flame.

Table 11.4 Flame choices and detection limits reported for emission spectrometry.

Element	Wavelength (nm)	Detection limit (μg/mL)
Air–acetylene		
Ca	422.7	0.005
K	766.4	0.0005
Na	589.0	0.0005
Rb	780.0	0.001
Nitrous oxide–acetylene		
Ag	328.1	0.02
Al	396.1	0.005
Au	267.6	0.5
Ba	553.5	0.001
Cd	326.1	2
Co	345.3	0.05
Cr	425.4	0.005
Cu	327.4	0.01
Fe	371.9	0.05
In	451.1	0.002
Li	670.7	0.00003
Mg	285.1	0.005
Mn	403.1	0.005
Mo	390.3	0.1
Ni	341.4	0.03
Pb	405.7	0.2
Sn	284.0	0.5
Sr	460.7	0.0001
Ti	399.8	0.2
V	437.9	0.01
W	400.8	0.5
Zn	460.1	3

Flame photometry

An emission spectrometer involving a simple configuration of air–propane flame with a nebulizer, a spray chamber, low-resolution filters (5 nm) and photoelectric cells or phototubes as detectors instead of photomultipliers is defined as flame photometry (Figure 11.4). Since the air–propane flame is a 'cold' flame reaching 1725 °C (compared with air–acetylene at 2250 °C), application of these systems is limited to a few elements, mainly Na, K, Li and Ca. These characteristics turn flame photometry into a popular, simple and low-cost technique suitable for routine analysis.

Application of flame photometry to food analysis

Flame emission methods applied to food analysis have been described in the literature for many decades. In the early 1960s, flame emission spectrometry was applied to the analysis of several elements when the instruments were equipped with a quartz prism as monochromator.

As mentioned previously, the most used version of atomic emission spectrometry in food analysis with flame as atomization source is flame photometry. Despite the fact that instruments are simple and easy to use, a number of factors must be considered by operators

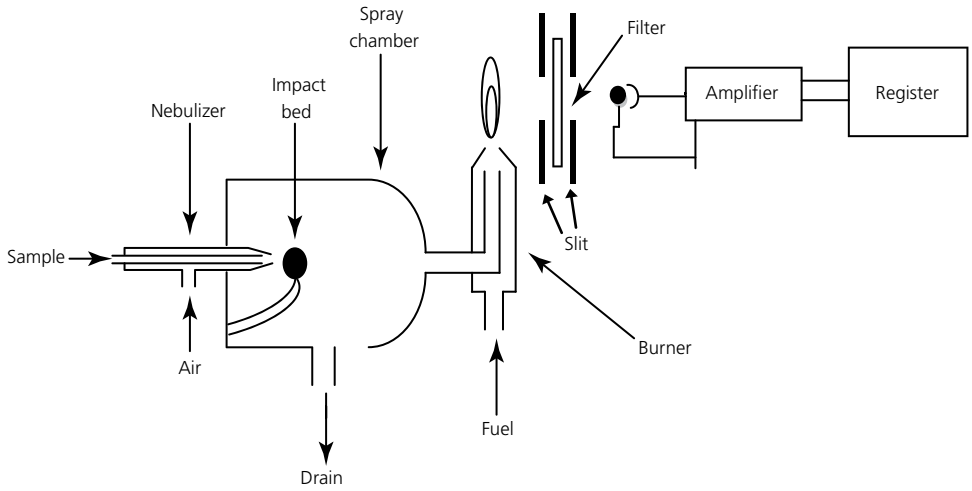


Figure 11.4 Schematic design of a flame photometer.

in order to obtain observable and reliable results. Before starting any analyte measurements, burner and flame height giving the smallest background plinths must be selected [50]. Samples must be filtered to avoid clogging in the nebulizer, spray chamber and burner [51].

Sodium and potassium determinations

These elements are major electrolytes in body fluids, with adequate intakes of 1.5 and 4.7 g/day for sodium and potassium, respectively [52], from which the importance of their determination in food can be inferred. Sodium and potassium concentrations vary depending on the food sample. Accordingly, some consideration must be given to self-absorption effects, interferences, ionization and detection limits.

The effects of fluctuations in aspiration rate and flame temperature can be countered by introducing lithium as internal standard. To this end, the common flame photometer configuration has been introduced (Figure 11.5). Two channels emit for sodium and potassium, respectively, with a third channel for lithium radiation (used as internal standard). The measurement of lithium is influenced in the same way as for sodium and potassium, causing the ratio of their spectral intensities to be constant at given concentrations [53]. Each signal is isolated by a different filter with a phototube as detector and a signal amplifier. Thus sodium and potassium signals are registered in relation to the lithium signal. Because of the high concentration of sodium and potassium in some food samples, like hot dogs, the well-known

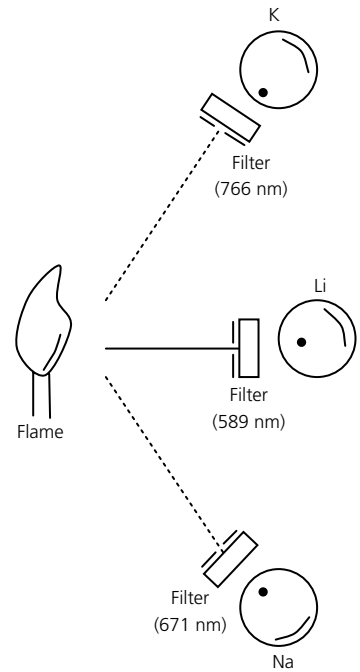


Figure 11.5 Three-channel photometer for Na, Li and K emission monitoring.

phenomenon of self-absorption is of concern along with the corresponding curvature of the calibration plot toward the concentration axis. One method to minimize this problem is to dilute the sample by a known factor. However, sample dilution can produce exaggerated

sodium and/or potassium contamination, ionization and volumetric errors [54].

The ionization effect is normally more pronounced at low analyte concentrations and high flame temperatures [54], such as with the air–acetylene flame. Using this flame, it can be seen that an ionization suppressant like caesium has little or no effect on sodium, but enhances potassium emission strongly at both low and high levels of this element. As expected, the ionization effect in air–acetylene is worse at low potassium concentrations. In sharp contrast, in the cooler air–propane flame, potassium emission, at levels above 10 mg/L, is not significantly affected by an excess of caesium addition. In the same type of flame, sodium emissions are not affected by an excess of caesium addition at either high or low sodium levels. For all practical purposes, the ideal 'no-effect' situation with respect to ionization can therefore be achieved in this cold flame.

Chemical interferences occur when the element of interest combines with another species from the food matrix in the flame, thus altering the number of atoms available for emission. Solutions to these issues are based on the application of separation techniques and/or addition of a competitive reagent with the interfering agent, for example addition of Sr(II) or La(III) to overcome the interference of PO_4^{3-} or SO_4^{2-} over Ca(II) determination in milk samples.

Matrix also interferes when the physical characteristics (viscosity, surface tension, etc.) of the sample differ considerably from standard solutions. This may occur when samples contain large amounts of dissolved salts or acids and when different solvents are used for samples and standards. A well-documented matrix effect is the signal excess error for potassium determination when high amounts of sodium are present, as in the case of drinking waters. In this fashion, ionization of sodium (usually 50–200 times higher than potassium) suppresses potassium ionization, favouring the occurrence of non-ionized potassium and consequently enhancement of its emission. To control this, samples and standards must be matrix matched or the sample must be diluted until any matrix effect becomes significant. Alternatively, these interferences can be solved by the standard addition method [55].

Spectral interferences can be of several different types, but in stoichiometric or fuel-lean flames, and with water as the solvent or suspension medium, they almost universally derive from major inorganic constituents of food samples, like meat for instance, rather than trace or organic constituents [51]. Interferences from calcium and potassium slightly interfere with sodium determination employing a 10-nm bandpass filter photometer at highly exaggerated levels (1000 mg/L) of interference. For potassium determination, no significant spectral interference from Na, Ca or Mg has been detected. No spectral line or background interference corrections appear to be required for either sodium or potassium content at the usually large sodium and potassium levels encountered in food samples.

Finally, flame emission provides good detection limits for sodium and potassium of 0.05 µg/L for both elements (in aqueous solution, measured in air–acetylene premixed flames formed by 5- to 10-cm slot burners) [56].

Calcium and lithium

Calcium interferences from sodium, potassium and proteins in the matrix are a major difficulty with the flame emission method. In order to compensate for the matrix interferences encountered, standards used in calcium analyses must be prepared in a matrix comparable to that of the samples [57].

Lithium determinations can be interfered by spectrum lines of calcium, and for this reason spectral line or background interference corrections must be considered [58]. Cereals, meat, fish, fruit, vegetables and milk show a constant positive effect from sodium in solution [50]. This effect has been solved by dilution and by the application of the standard addition method.

11.2.2 ICP-OES applied to food analysis

As mentioned, atomic emission spectrometry uses quantitative measurement of the optical emission from excited atoms to determine the studied analyte concentration. Analyte atoms in solution are aspirated into the excitation region where they are desolvated, vaporized and atomized by a flame, discharge or plasma. The high-temperature atomization sources provide the proper energy to promote the atoms into high-energy levels. Thus the atoms decay back to lower levels by emitting light. Since the transitions are between distinct atomic energy levels, the emission lines in the spectra are narrow. The spectra of multi-elemental samples can be very congested, and spectral separation of nearby atomic transitions requires a high-resolution spectrometer. Since all atoms in a sample are excited simultaneously, they can be detected simultaneously, and is one of the

major advantages of atomic emission spectrometry. ICP-OES is one of the most commonly used radiation sources in atomic emission spectrometry. ICP-OES has increasingly attracted the interest of analytical chemists and is widely used for routine elemental analysis since its commercial introduction in 1974. This large acceptance can be explained by some excellent analytical characteristics such as high precision, long-term stability, high selectivity and high sensitivity as well as low limit of detection (LOD), a large linear dynamic range and low matrix effects, particularly when ICP is compared with alternative methods such as atomic absorption spectrometry. However, matrix effects can be observed, even if their magnitude is usually low [59].

One of the more important exposure routes for humans from potentially harmful contaminants including metals or metalloids is oral ingestion. The form and the nature of the metal is an important consideration [60, 61]. It is relatively easy to determine total metal content of a sample either directly using analytical techniques (e.g. X-ray fluorescence spectroscopy) or after a digestion procedure and measurement using atomic spectrometry (e.g. FAES, ICP-OES). However, determination of the chemical form of the element requires additional sample pretreatment and/or separation (e.g. different metal-oxidation states could be determined using ion-exchange media, whereas organometallic forms may require separation using reversed-phase chromatography directly coupled with the detector). The chemical form and nature of a metal can influence whether the metal is beneficial to humans (i.e. important in *in-vivo* processes) or detrimental (i.e. toxic) [61]. As mentioned, inorganic food analysis involves the determination of nutritional and toxic elements and species. Thus nutritional elements are best determined by ICP-OES because of the technique's advantages and ability to measure high concentrations accurately. However, ICP-OES is not so useful for toxic element determination because it does not have the sensitivity necessary to measure low and trace levels [60]. In order to overcome this drawback, toxic elements are usually pre-concentrated (see section 11.1.2) prior to ICP-OES analysis.

Food analysis and advantages of ICP-OES

Spectrometers for ICP-OES can measure up to 70 elements simultaneously in a single sample analysis [61]. Dual viewing of the plasma offers LODs down to parts-per-billion (ppb) levels (especially axial viewing) and extends the working ranges for percentage concentration measurements (radial viewing). The choice of alternative wavelengths for the analytes of interest allows analytical results free from spectral interferences. Solid-state detectors (e.g. segmented-array charge-coupled device detectors [62]) usually provide uniform resolution at all wavelengths. Simultaneous background correction can be performed adjacent to the analytical wavelengths. The technical difficulty of direct analysis of samples containing organic solvents has recently been solved by using a special sample introduction system [63–65].

Food analysis and limitations of ICP-OES

ICP-OES is unsuitable for the analysis of some elements (e.g. As and Se) because of their high ionization potentials, thus resulting in poor LOD. However, when hydride generation systems are linked to ICP-OES, the determination of these elements becomes possible. On the other hand, inter-element interferences may not be completely resolved, even using the sophisticated detection system. Elements that might cause interference because of their potential levels in food and their spectral characteristics include phosphorus, calcium, iron, zinc, aluminium and titanium. These six elements should be included in the analyte list even if quantitative results are not needed. Interference effects must be evaluated for each instrument. Appropriate corrective measures (e.g. inter-element correction equations and multi-component spectral fitting) should be adopted as far as feasible [63, 66, 67]. In ICP-OES emphasis is given to the problems created by the matrix on the emission measurements of sample elements. Thus, particularly in the case of food analysis where complex samples are analysed, the matrix composition could strongly affect the analytical signal [60].

Operational parameter selection for ICP-OES

Additional efforts have been made to characterize the mechanisms responsible for the observed interferences. Thus different systems and operating conditions have been employed in studies on this subject in order to develop more efficient methods for overcoming them, such as (i) selection of an appropriate sample introduction system; (ii) use of a robust plasma; (iii) proper selection of the plasma observation zone; and, when necessary, (iv) use of an auxiliary method (i.e. internal standard, standard additions) [59, 60].

The studies carried out confirm that under non-robust conditions, most matrix effects result from complex processes that occur in the plasma so that the effects that originate in the spray chamber are masked. In marked contrast, with use of robust conditions (i.e. high RF power, low carrier gas flow rate, and large internal diameter of the injector), plasma conditions are not changed when the matrix concentration is significantly modified regardless of the observation mode (lateral or axial), and matrix effects can be minimized to almost the same extent, regardless of the elements, line characteristics and observation mode. The residual, depressive effect is assigned to aerosol transport and filtering, which emphasizes the crucial role of the spray chamber. The effects are enhanced when low carrier gas flow rates and solution uptake rates are used, which corresponds to the present trend in ICP-OES.

In summary, selection of the nebulizer and spray chamber should be conducted by keeping in mind not only their efficiency and results in terms of precision, but also their behaviour relative to the matrix effects. It might be questioned whether there is a need to keep a spray chamber. The spray chamber is the major source of flicker noise, and some remaining large droplets cause a significant perturbation of the plasma during the processes of evaporation and atomization. Moreover, it does not seem very logical to reject into a drain up to 98% of the solution. To solve this drain problem, micronebulizers with uptake rates in the range 10–100 mL/min may be used. Although they are still associated with a spray chamber, their efficiency is significantly better than that of conventional pneumatic nebulizers. This type of sample introduction is certainly a promising research direction.

Matrix effect correction strategies

The use of an internal standard or a multi-element internal standard (based on Sc, Mn, Se, In, Sr, Y, Co, Zr and Mo) or other variations of it, such as the parameter-related internal standard method (PRISM), the proportional correction method is able to partially compensate the matrix effect in most cases [63, 68]. Additionally, matching of matrix elements is a suitable approach for overcoming non-spectroscopic interferences. However, this approximation is not advised when analysing large numbers of samples with different matrices. The presence of percentage levels of heavy metals in a sample matrix often causes difficulties in the determination of elements of interest at ppm levels. In order to compensate for strong matrix effects, standard solutions used for calibration should be fortified (spiked) with the same matrix elements at levels similar to those of the sample. On the other hand, sample dilution is the simplest way to reduce matrix effects, being more comparable with the slopes from the standard addition method and from the external calibration curve [69]. However, a high dilution factor has a detrimental effect on the LOD and might introduce mistakes in calculations of final concentrations [70]. There are many other methodologies based on empirical modelling or on the use of multivariate calibration techniques, such as multiple linear regression (MLR), principal component regression (PCR) and partial least square regression (PLS), to minimize matrix effects [60]. Flow injection analysis (FIA) systems, where the concentration of the matrix is reduced by dilution of a small sample volume in a carrier stream, can also be applied successfully to overcome matrix effects [60, 71]. Another possible method to reduce these effects is the use of an ionization buffer, such as a caesium solution [72]. Caesium acts as a plasma buffer and allows the ICP system to accept changes in the matrix composition without significant changes to the plasma excitation conditions. When other strategies are not able to eliminate or to correct for matrix effects it is necessary to separate the analyte from the matrix sample (as mentioned in section 11.1.2). Precipitation is a classic way of separating analytes. Different solvent extraction methods have also been employed for the separation and/or pre-concentration of analytes from the matrix prior to the analysis. In recent years, liquid–liquid extractions (based on cloud point and ionic liquids systems, among others), chelating resins, and methods based on solid–liquid interactions have also been employed for overcoming non-spectroscopic interferences in ICP techniques applied to food analysis [48, 73, 74].

Sample introduction approaches for food samples

A sample introduction system is used to transport a sample into the central channel of the ICP as a gas, vapour, aerosol of fine droplets, or solid particles. A wide variety of sample introduction methods have been developed, such as nebulization, hydride generation (HG), slurry, ETV and laser ablation [75].

Regardless of the nature of the food sample, they are most commonly introduces as liquids. The considerations

made in selecting the liquid introduction system include TDS, presence of suspended solids, presence of hydrofluoric acid or caustic, detection limit needs, precision, sample load, sample size limitations and operating budget. It is the analyst who has the task of choosing the best introduction components. The different nebulization strategies used for ICP analysis have been described previously in this chapter. Among the most novel approaches, ETV has been used to solve problems associated with pneumatic nebulization. The major advantage of ETV as a means of sample introduction is that the transportation efficiency is dramatically improved over a pneumatic nebulizer, from less than 5% to over 60% [75]. Consequently, the LODs are improved by at least an order of magnitude. Some difficult to analyse samples, those with high TDS for example, can be introduced by ETV. ETV devices have been improved in order to deal with the formation of carbide species and memory effects [75]. As an example, vanadium species present in water samples have been separated by hollow-fibre liquid-phase microextraction and the vanadium in the post-extraction organic phase has been injected into the graphite furnace for ETV/ICP-OES detection [76].

On the other hand, the combination of the separation power of chromatography and the detection capability of atomic emission spectrometry results in many advantages. One of them is the ability to obtain speciation information. Both gas chromatography (GC) and high-performance liquid chromatography (HPLC) can be coupled with ICP-OES [77]. The successful combination of these two techniques is realized through the use of chromatographic couplers that require few, if any, modifications to be adapted to the commercial ICP-OES. To improve the transport efficiency and to minimize the influence of organic solvents on the stability of the ICP, USN, water-cooled thermospray chambers and glass frit nebulizers have been utilized for sample introduction in HPLC/ICP-OES [75]. The solvent load on the plasma can also be decreased by aerosol thermostating, increasing the incident RF power, application of a condenser, or by use of a micro-HPLC column. Recently, Terol *et al*. [77] reported for the first time a high-temperature liquid chromatographic (HTLC) method coupled to ICP-OES for the simultaneous determination of carbohydrates and metals in food samples. In order to accomplish the proposed aim, the interface between both techniques was studied and optimized so as to obtain the best separation efficiencies and analytical figures of merit. The developed coupling was applied to the analysis of magnesium, calcium, sodium, zinc, potassium and boron at ppm levels in milk, cream, candy, isotonic beverages and beer. The same group developed another methodology based on HPLC/ICP-OES for the joint determination of several water-soluble vitamins and Cr, Mo, Se, Mn, Zn, Fe, Mg, Ca and K in multivitamin complexes [78]. The coupled techniques allowed determination of the mentioned analytes in a period of time not much higher than that required for the simple determination of the minerals, and it was less sensitive to interferences in trace quantities. This research group also proposed the rapid analytical method for the determination of organic and inorganic species in tomato samples also through HPLC/ICP-OES [79]. In this work, a high-efficiency nebulizer associated to a low inner volume cyclonic spray chamber was used to interface both techniques. By registering the intensity at the sulfur and phosphorus emission wavelengths, the content of anions such as sulfate and phosphate was determined. The huge amount of information obtained made possible the discrimination among different samples of native tomato cultivars. Additionally, an HPLC/ICP-OES methodology was validated for the analysis of organic and inorganic constituents in food samples such as non-alcoholic beverages, wines and a certified non-fat milk powder sample [80]. On the other hand, laser ablation and thermospray, two other important sample non-conventional introduction methods, have also been used for ICP-OES analysis of food samples such as powdered infant formula and water samples [81–83]. Each of these has its advantages and disadvantages. For example, laser ablation can be used to vaporize any solid samples into the ICP, but generally it has poor reproducibility and high cost.

ICP-OES as a tool for food commodities analysis

The determination of geographic origin of commodities and food products is becoming an increasingly active research area. There is growing enthusiasm among consumers for high-quality food with a clear regional identity. The determination of geographic origin and adulteration practices through chemical analysis coupled with sophisticated data classifying techniques is timely [84–87].

The inorganic elemental analysis by atomic absorption and emission spectrometries, often combined with chemometrics, has enabled the determination of the

geographical origin of various food products. Multi-element and isotopic analyses have been applied to a range of foodstuffs to develop methods that will permit their geographical origins to be determined with varying degrees of certainty [86]. The use of minerals and trace elements is powerful because trace elements are significantly more stable in commodities than some other types of organic compounds.

As mentioned, the combination of atomic absorption and emission techniques has allowed the determination of the geographical origin of several foodstuffs, among them orange juice, nuts [88, 89], and potatoes [90]. Chemical profiling by ICP-OES has distinguished the geographical growing origins of coffee [91, 92] and fruits [93, 94]. Furthermore, ICP-OES has also been successfully combined with ICP-MS to classify onions [95, 96], tea [98–101], olive and argan oils [102, 103], rice [104, 105] and honeys [106–108].

Wine

The determination of geographic origin of wines has been an active area of research for some years [109–111]. These studies involve the chemical determination of organic and inorganic compounds. In wine analysis, when simultaneous multi-element quantification in a broad range of concentrations is required, ICP-OES is a more appropriate technique. The possibility of using both axial and radial viewing modes provides a dynamic range from 0.01 to 1000 mg/L, which also allows the determination of elements at ultratrace levels. Thus, for instance, Li, Fe, Mn, Cu, Zn, Se, Ba, Pb, Bi, V and even rare earth elements have been successfully determined by ICP-OES [108–111]. When operating in ICP-OES with conventional pneumatic nebulization of liquids, the presence of ethanol may cause both spectral and non-spectral interferences and degrade analytical performance by introducing noise. Desolvation systems are useful for reducing ethanol-based matrix effects. In ICP-based techniques, analysis after digestion is commonly employed to check accuracy and matrix effects for direct wine analysis.

Tea

Information about levels of elements in tea leaves, made teas and their infusions, including those regarded as micronutrients or toxics, increasingly attracts the attention of tea consumers and producers. Total concentrations of elements in the mentioned matrices are primarily determined using atomic and mass spectrometries, including ICP-OES, which is particularly effective in the multi-element analysis of tea within wide concentration ranges. Tea samples require to be digested at the sample preparation stage before measurements. Analysis of the resultant sample solutions and quantification of elements are commonly carried out versus the standard external calibration. However, external calibration with matrix-matched standard solutions cannot give correct results due to the existence of vast matrix effects. Calibration by standard additions is preferred to compensate for the effect of the matrix resulting from the digestion of tea samples. The analysis of certified reference materials of tea is quite often made to guarantee the reliability of results obtained. In the determination of elements present at extremely low levels in tea samples and their infusions (Cd and Pb), an additional enrichment step is recommended. Such an on-line preconcentration procedure has been proposed for measurements of Cd by ICP-OES with ultrasonic nebulization [112].

Juice

ICP-based methods have been applied to quantify major and trace elements in juice samples from different origins [113]. Pattern-recognition analysis and a proper selection of target elements such as Ba, B, Gd, Mn and Rb have been used as discriminators of geographic origin [114]. Early investigations used atomic absorption to determine elemental concentrations. The introduction of ICP methodologies allowed a wider range of elements to be analysed economically. Using this larger number of trace and ultratrace elements in juice, and commercially available chemometric software packages, investigators have been able to further identify geographic origin and detect pulpwash addition to juice [87, 89]. In addition, since different species of citrus have individual profiles [115], trace element determination could be potentially useful for controlling citrus products for truth-in-labelling.

Rice

Rice does not only share important systemic relationships with other cereal species, but it is also the principal mineral element source for over half the world's population. Each year, hundreds of millions of metric tonnes of rice are consumed. Asian cultivated rice has been moved across the globe with migrating human populations.

Rice cultivation can now be found on all continents except Antarctica [47]. Variation pattern and traits associated with mineral element contents in rice have been studied for many years and by several determinative techniques including ICP-OES [104]. Data have provided an understanding of the agricultural, environmental and evolutionary effects of classification traits, thus assisting with the optimization of rice quality.

Coffee

Research work has principally focused on demonstrating the feasibility of elemental analysis to differentiate the geographical growing regions of coffee beans. Elemental analysis based on the study of K, Mg, Ca, Na, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, S, Cd, Pb and P levels of coffee bean samples has been performed using only ICP-OES [91].

Conclusions regarding ICP-OES applied to food analysis

ICP-OES is a powerful tool for the determination of the content of trace, minor and major elements in a great variety of food sample matrices. This type of analysis has gained in strength and significance during the last years. One of the reasons is the fact that single or multi-element measurements can be performed for up to 70 elements. In addition, analytical performance of ICP-based systems is competitive with most other inorganic analysis techniques, especially with regard to sample throughput and sensitivity. The optimized procedures reported in the literature are normally applied to evaluate the intrinsic quality of foodstuffs and to establish its food safety with respect to existing regulatory requirements.

11.3 Conclusions and future trends

Situations related to contamination, adulteration, quality control and quality assurance (taste, smell, composition, protected denomination of origin, etc.) of foods or food derivatives define the analytical problem. The final goal of the analytical chemist is to obtain true and ready information to solve the demand. Accuracy, representativeness, precision, sensitivity, selectivity, adequate sampling, rapidity, robustness, personal considerations (safety, ergonomic operations), laboratory conditions and available equipment and others are the milestones of the quality of the generated information.

Assured information about the elemental concentration in food samples is essential from the nutritional, technological, toxicological and societal points of view. Atomic and inorganic mass spectrometric techniques, after appropriate sample preparation, are most frequently used in order to obtain required and reliable information about metals in foods, particularly at trace levels. In this perspective, the integrity of chemical information is strongly dependent on the prior analytical steps and adequate selection of a sample preparation procedure is of capital importance. It has been observed that different sample preparation procedures have been successfully applied for determination of a wide variety of elements in diverse food samples and the trends are to minimize sample handling and reagent consumption in order to reduce sample contamination and to improve analytical throughput. On the other hand, many new and innovative approaches to elemental food analysis continue to appear in the literature, driven by regulatory considerations, technological advances, the trend toward speciation and the constant push for lower detection limits and the demand for increased laboratory efficiency. Thus well-established techniques such as FAES and ICP-OES are the tools of choice for the quantitative evaluation and monitoring of nutritional and toxic elements in food samples.

Abbreviations

AA: Atomic absorption
ETV: Electrothermal vaporization
FAAS: Flame atomic absorption spectrometry
FAES: Flame atomic emission spectrometry
FIA: Flow injection analysis
GC: Gas chromatography
GCUs: Gas control units
HG: Hydride generation
HDPE: High density polyethylene
HPLC: High-performance liquid chromatography
HTLC: High temperature liquid chromatography
ICP-MS: Inductively coupled plasma mass spectrometry
ICP-OES: Inductively coupled plasma optical emission spectrometry
LA: Laser ablation
LOD: Limit of detection
LOQ: Limit of quantification
MCN: Microconcentric nebulizer

MLR: Multiple linear regression
MOD: Microwave oven digestion
OIML: International Organization of Legal Metrology
PC: Proportional correction method
PCR: Principal component regression
PLS: Partial least square regression
PRISM: Parameter-related internal standard method
PTFE: Polytetrafluoroethylene
RF: Radiofrequency
SPE: Solid-phase extraction
SPME: Solid-phase microextraction
TDS: Total dissolved solids
TOC: Total organic compounds
UAE: Ultrasonic-assisted extraction
USN: Ultrasonic nebulization
WD: Wet digestion

References

1 Buldini PL, Ricci L, Sharma JL. Recent applications of sample preparation techniques in food analysis. *J Chromatogr A* 2002;**975**:47–70.
2 Stoeppler M. *Sampling and Sample Preparation*. Springer-Verlag, Heidelberg, 1997.
3 Wilson ID, Adlard TR, Poole CF, Cooke M. *Encyclopedia of Separation Science*. Academic Press, San Diego, CA, 2000.
4 Peris M. Present and future of expert systems in food analysis. *Anal Chim Acta* 2002;**454**:1–11.
5 Danzer K. *Analytical Chemistry*, Springer-Verlag, Heidelberg, 2007.
6 Kraft G. Probennahme an festen Stoffen. In: Kienitz H, Bock R, Fresenius W, Huber W, Tolg G (eds) *Analytiker-Taschenbuch, Bd. 1*. Springer, Heidelberg, 1980.
7 Gy PM. *Sampling of Particulate Materials: Theory and Practice*. Elsevier, Amsterdam, 1982.
8 Horwitz W. IUPAC Analytical Chemistry Division: Nomenclature for sampling in analytical chemistry. IUPAC Recommendations 1990. *Pure Appl Chem* 1990;**62**: 1193–1208.
9 Gy PM. *Sampling of Heterogeneous and Dynamic Material Systems: Theories of Heterogeneity, Sampling and Homogenizing*. Elsevier, Amsterdam, 1992.
10 Wegscheider W. Proper sampling: a precondition for accurate analyses. In: Gunzler H (ed.) *Accreditation and Quality Assurance in Analytical Chemistry*. Springer, Heidelberg, 1996.
11 NMKL (Nordic Committee on Food Analysis). Procedure No. 12: Guide on sampling for analysis of foods, 2002. Available at http://www.nmkl.org/index.php?lang=en
12 Montaser A, Golightly DW. *Inductively Coupled Plasmas in Analytical Atomic Spectrometry*, 2nd edn. Wiley-VCH, Weinheim, 1992.
13 Broekaert JAC. *Analytical Atomic Spectrometry with Flames and Plasmas*. Wiley-VCH, Weinheim, 2006.
14 Marchisio PF, Sales A, Cerutti S, Marchevski E, Martinez LD. On-line preconcentration/determination of lead in Ilex paraguariensis samples (mate tea) using polyurethane foam as filter and USN-ICP-OES. *J Hazard Mater* 2005;**124**:113–118.
15 Todolí JL, Maestre S, Mora J, Canals A, Hernandis V. Comparison of several spray chambers operating at very low liquid flow rates in inductively coupled plasma atomic emission spectrometry. *Fresenius J Anal Chem* 2000;**368**: 773–779.
16 Todolí JL, Mermet JM. Sample introduction systems for the analysis of liquid microsamples by ICP-AES and ICP-MS. *Spectrochim Acta B* 2006;**61**:239–283.
17 Resano M, Vanhaecke F, de Loos-Vollebregt MTC. Electrothermal vaporization for sample introduction in atomic absorption, atomic emission and plasma mass spectrometry: a critical review with focus on solid sampling and slurry analysis. *J Anal Atom Spectrom* 2008;**23**:1450–1475.
18 Detcheva A, Barth P, Hassler J. Calibration possibilities and modifier use in ETV ICP OES determination of trace and minor elements in plant materials. *Anal Bioanal Chem* 2009;**394**:1485–1495.
19 Chen S. Electrothermal vaporization in inductively coupled plasma atomic emission spectrometry for direct multielement analysis of food samples with slurry sampling. *J Anal Chem* 2005;**60**:254–258.
20 Knápek J, Komárek J, Novotný K. Determination of cadmium, chromium and copper in high salt samples by LA-ICP-OES after electrodeposition: preliminary study. *Microchim Acta* 2010;**171**:145–150.
21 Gilon N, El-Haddad J, Stankova A *et al*. A matrix effect and accuracy evaluation for the determination of elements in milk powder LIBS and laser ablation/ICP-OES spectrometry. *Anal Bioanal Chem* 2011;**401**:2681–2689.
22 Hansen TH, de Bang TC, Laursen KH, Pedas P, Husted S, Schjoerring JK. Multielement plant tissue analysis using ICP spectrometry. *Methods Mol Biol* 2013;**953**:121–141.
23 US Food and Drud Administration. *Elemental Analysis Manual (EAM) for Food and Related Products*. Available at http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006954.htm
24 Hoenig M, de Kersabiec AM. Sample preparation steps for analysis by atomic spectroscopy methods: present status. *Spectrochim Acta B* 1996;**51**:1297–1307.
25 Hoenig M. Dry ashing. In: Mester Z, Sturgeon R (eds) *Sample Preparation for Trace Element Analysis*. Elsevier, Amsterdam, 2003.
26 Demirel S, Tuzen M, Saracoglu S, Soylak M. Evaluation of various digestion procedures for trace element contents of some food materials. *J Hazard Mater* 2008;**152**:1020–1026.
27 Altundag H, Tuzen M. Comparison of dry, wet and microwave digestion methods for the multi element determination in some dried fruit samples by ICP-OES. *Food Chem Toxicol* 2011;**49**:2800–2807.

28 Mindak WR, Cheng J, Jacobs RM. Analytical portion to analytical solution. In: *Elemental Analysis Manual (EAM) for Food and Related Products*. US Food and Drug Administration. Version 1.1, 2010. Available at http://www.fda.gov/Food/FoodScienceResearch/aboratoryMethods/ucm195383.htm
29 US Department of Agriculture. National Nutrient Database for Standard Reference, Release 26. Nutrient Data Laboratory Home Page. Available at http://www.ars.usda.gov/Services/docs.htm?docid=8964
30 Andrade Korn MG, da Boa Morte ES, Batista dos Santos DC *et al*. Sample preparation for the determination of metals in food samples using spectroanalytical methods: a review. *Appl Spectrosc Rev* 2008;**43**:67–92.
31 Santos Júnior D, Krug FJ, Pereira MG, Korn M. Currents on ultrasound-assisted extraction for sample preparation and spectroscopic analytes determination. *Appl Spectrosc Rev* 2006;**41**:305–321.
32 Luque de Castro MD, Capote FP. *Analytical Applications of Ultrasound*. Elsevier, Amsterdam, 2007.
33 Melo MHA, Costa ACS, Nobrega JA, Ferreira SLC. The use of water soluble tertiary amine reagent for solubilization and metal determination in fish muscle tissue. *J Braz Chem Soc* 2005;**16**:69.
34 Manutsewee N, Aeungmaitrepirom W, Varanusupakul P, Imyim A. Determination of Cd, Cu, and Zn in fish and mussel by AAS after ultrasound-assisted acid leaching extraction. *Food Chem* 2007;**101**:817–824.
35 Maduro C, Vale G, Alves S *et al*. Determination of Cd and Pb in biological reference materials by electrothermal atomic absorption spectrometry: a comparison of three ultrasonic-based sample treatment procedures. *Talanta* 2006;**68**:1156–1161.
36 Balarama Krishna MV, Arunachalam J. Ultrasound-assisted extraction procedure for the determination of major, minor and trace elements in lichen and mussel samples by ICP-MS and ICP-AES. *Anal Chim Acta* 2004;**522**:179–187.
37 Peña-Farfal C, Moreda-Piñeiro A, Bermejo-Barrera A, Bermejo-Barrera P, Pinochet-Cancino H, de Gregori-Henríquez I. Speeding up enzymatic hydrolysis procedures for the multi-element determination in edible seaweed. *Anal Chim Acta* 2005;**548**:183–191.
38 Türker AR. Separation, preconcentration and speciation of metal ions by solid phase extraction. *Sep Purif Rev* 2012;**41**:169–206.
39 Türker AR. New sorbents for solid-phase extraction for metal enrichment. *Clean Soil Air Water* 2007;**35**:548–557.
40 Lemos VA, Ferreira SLC. On-line preconcentration system for lead determination in seafood samples by flame atomic absorption spectrometry using polyurethane foam loaded with 2-(2-benzothiazolylazo)-2-p-cresol. *Anal Chim Acta* 2001;**441**:281–289.
41 Cerutti S, Orsi RF, Gásquez JA, Olsina RA, Martinez LD. On-line preconcentration/determination of lead traces in bee honey by inductively coupled plasma optical emission spectrometry (ICP-OES) using a conical minicolumn packed with activated carbon. *J Trace Microprobe Tech* 2003;**21**:421–432.
42 Pyrzynska K, Kilian K. On-line sorption-based systems for determination of cadmium with atomic spectrometry detectors. *Water Res* 2007;**41**:2839–2851.
43 Lara RF, Wuilloud RG, Salonia JA, Olsina RA, Martinez LD. Determination of low cadmium concentrations in wine by on-line preconcentration in a knotted reactor coupled to an inductively coupled plasma optical emission spectrometer with ultrasonic nebulization. *Fresenius J Anal Chem* 2001;**371**:989–993.
44 Yip YC, Lam JCW, Tong WF. Commonly used methodologies for inorganic analysis in international key comparisons. *Trends Anal Chem* 2009;**28**:214–236.
45 Organisation Internationale de Métrologie Légale. Weights of Classes E1, E2, F1, F2, M1, M1_2, M2, M2_3 and M3 Part 1: Metrological and Technical Requirements. Available at http://www.oiml.org/en/files/pdf_r/r111-1-e04.pdf
46 International Standards Organization. Cleanrooms and associated controlled environments. Part 1: Classification of air cleanliness by particle concentration. ISO 14644-1:1999. Available at http://www.iso.org/iso/catalogue_detail.htm?csnumber=53394 (1999)
47 Sasaki T, Burr B. International rice genome sequencing project: the effort to completely sequence the rice genome. *Curr Opin Plant Biol* 2000;**3**:138–141.
48 Martin-Calero A, Pino V, Afonso AM. Ionic liquids as a tool for determination of metals and organic compounds in food analysis. *Trends Anal Chem* 2011;**30**:1598–1619.
49 Neubauer K. Determination of inorganic elements in foods. *Spectroscopy Europe* 2007;**19**:20–22.
50 Evans WH, Read JI. Determination of lithium, rubidium and strontium in foodstuffs. *Analyst* 1985;**110**:619–623.
51 Wichman MD, Fietkau R, Fry RC. Simplified slurry method for direct flame emission determination of dietary salt in processed meat. *Appl Spectrosc* 1986;**40**:233–238.
52 Institute of Medicine. *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate*. National Academies Press, Washington, DC, 2005.
53 Feldman FJ. Internal standardization in atomic emission and absorption spectrometry. *Anal Chem* 1970;**42**:719–724.
54 Fietkau R, Wichman MD, Fry RC. Slurry atomization direct flame emission determination of Na and K in hot dogs. *Appl Spectrosc* 1984;**38**:118–124.
55 Harris DC. *Quantitative Chemical Analysis*. W.H. Freeman, New York, 2003.
56 Pickett EE, Koirtyohann SR. Emission flame photometry: a new look at an old method. *Anal Chem* 1969;**41**:28A–42A.
57 Berman E. Biochemical applications of flame emission and atomic absorption spectroscopy. *Appl Spectrosc* 1975;**29**:1–10.
58 American Society for Testing Materials. Symposium on Flame Photometry: presented at the 54th Annual Meeting of the American Society for Testing Materials, Atlantic City, NJ, 19 June 1951. Available at https://openlibrary.org/works/OL1164215W/Symposium_on_flame_photometry

59 Mermet JM. Revisitation of the matrix effects in inductively coupled plasma atomic emission spectrometry: the key role of the spray chamber. *J Anal Atom Spectrom* 1998;**13**: 419–422.

60 Todoli JL, Gras L, Hernandis V, Mora J. Elemental matrix effects in ICP-AES. *J Anal Atom Spectrom* 2002;**17**:142–169.

61 Intawongse M, Dean JR. *In-vitro* testing for assessing oral bioaccessibility of trace metals in soil and food samples. *Trends Anal Chem* 2006;**25**:876–886.

62 Mermet JM, Ivaldi JC. Real-time internal standardization for inductively coupled plasma atomic emission spectrometry using a custom segmented-array charge coupled device detector. *J Anal Atom Spectrom* 1993;**8**:795–801.

63 Yip Y-C, Chung-wah J, Wai-fong Tong L. Commonly used methodologies for inorganic analysis in international key comparisons. *Trends Anal Chem* 2009;**28**:214–236.

64 Grindlay G, Maestre S, Gras L, Mora J. Introduction of organic solvent solutions into inductively coupled plasma-atomic emission spectrometry using a microwave assisted sample introduction system. *J Anal Atom Spectrom* 2006;**21**: 1403–1411.

65 Grindlay G, Gras L, Mora J, Hernandis V. Design and evaluation of an improved microwave-based thermal nebulizer for liquid sample introduction in inductively coupled plasma atomic emission spectrometry. *J Anal Atom Spectrom* 2008;**23**: 129–139.

66 US Environmental Protection Agency. Method 6010B. Inductively coupled plasma atomic emission spectrometry. Available at http://www.caslab.com/EPA-Methods/PDF/EPA-Method-6010B.pdf

67 Yang X, Wei J, Liu H, Tang B, Zhang Z. Direct determination of trace elements in tungsten products using an inductively coupled plasma optical emission charge coupled device detector spectrometer. *Spectrochim Acta B* 1998;**53**: 1405–1412.

68 Salit ML, Turk GC, Lindstrom AP, Butler TA, Beck II CM, Norman B. Single-element solution comparisons with high-performance ICPOES. *Anal Chem* 2001;**73**:4821–4829.

69 Mok WJ, Senoo S, Itoh T, Tsukamasa Y, Kawasaki K, Ando M. Assessment of concentrations of toxic elements in aquaculture food products in Malaysia. *Food Chem* 2012;**133**:1326–1332.

70 Ruiz-de-Cenzano M, Beser U, Cervera ML, de la Guardia M. Fast determination of fish mineral profile. Application to Vietnamese panga fish. *Ecotoxicol Environ Saf* 2013;**95**:195–201.

71 Vickers GH, Ross BS, Hieftje GM. Reduction of mass-dependent interferences in inductively coupled plasma-mass spectrometry by using flow-injection analysis. *Appl Spectrosc* 1989;**43**:1330–1333.

72 Stepan M, Musil P, Poussel E, Mermet J-M. Matrix-induced shift effects in axially viewed inductively coupled plasma atomic emission spectrometry. *Spectrochim Acta B* 2001;**56**: 443–453.

73 Vaezzadeh M, Shemirani F, Majidi B. Microextraction technique based on ionic liquid for preconcentration and determination of palladium in food additive, sea water, tea and biological samples. *Food Chem Toxicol* 2010;**48**: 1455–1460.

74 Yücel Y, Sultanoğlu P. Characterization of Hatay honeys according to their multi-element analysis using ICP-OES combined with chemometrics. *Food Chem* 2013;**140**:231–237.

75 Hou X, Jones BT. Inductively coupled plasma/optical emission spectrometry. In: Meyers RA (ed.) *Encyclopedia of Analytical Chemistry*. John Wiley & Sons Ltd, Chichester, 2000, pp. 9468–9485.

76 Li L, Hu B. Hollow-fiber liquid phase microextraction for separation and preconcentration of vanadium species in natural waters and their determination by electrothermal vaporization-ICP-OES. *Talanta* 2007;**72**:472–479.

77 Terol A, Paredes E, Maestre SE, Prats S, Todolí JL. High-temperature liquid chromatography inductively coupled plasma atomic emission spectrometry hyphenation for the combined organic and inorganic analysis of foodstuffs. *J Chromatogr A* 2010;**1217**:6195–6202.

78 Paredes E, Prats MS, Maestre SE, Todolí JL. Development of an analytical method for the combined determination of water-soluble vitamins and minerals through high-performance liquid chromatography–inductively coupled plasma atomic emission spectrometry hyphenation. *Food Anal Method* 2012;**5**:897–908.

79 Paredes E, Maestre SE, Prats MS, Todolí JL. Rapid analytical method for the determination of organic and inorganic species in tomato samples through HPLC-ICP-AES coupling. *Food Chem* 2008;**111**:469–475.

80 Paredes E, Maestre SE, Prats S, Todolí JL. Simultaneous determination of carbohydrates, carboxylic acids, alcohols and metals in foods by high-performance liquid chromatography inductively coupled plasma atomic emission spectrometry. *Anal Chem* 2006;**78**:6774–6782.

81 Hola M, Krásenský P, Kanický V. UV and IR laser ablation study of natural organic powdered materials. An example: ICP-OES with laser ablation of spiked infant formula cast pellets. *J Anal Atom Spectrom* 2006;**21**:974–977.

82 Gilon N, El-Haddad J, Stankova A *et al*. A matrix effect and accuracy evaluation for the determination of elements in milk powder LIBS and laser ablation/ICP-OES spectrometry. *Anal Bioanal Chem* 2011;**401**:2681–2689.

83 Teixera Tarley CR, Figuereido E, Matos GF. Thermospray flame furnace-AAS determination of copper after on-line sorbent preconcentration using a system optimized by experimental designs. *Anal Sci* 2005;**21**:1337–1342.

84 Luykx DMAM, van Ruth SM. An overview of analytical methods for determining the geographical origin of food products. *Food Chem* 2008;**107**:897–911.

85 Drivelos SA, Georgiou GA. Multi-element and multi-isotope ratio analysis to determine the geographical origin of foods in the European Union. *Trends Anal Chem* 2012;**40**:38–51.

86 Kelly S, Heaton K, Hoogewerff J. Tracing the geographical origin of food: the application of multi-element and multi-isotope analysis. *Trends Food Sci Technol* 2005;**16**:555–567.

87 Cordella C, Moussa I, Martel AC, Sbirrazzuoli N, Lizzani-Cuvelier L. Recent developments in food characterization and adulteration detection: technique-oriented perspectives. *J Agric Food Chem* 2002;**50**:1751–1764.
88 Schwartz RS, Hecking LT. Determination of geographic origin of agricultural products by multivariate analysis of trace element composition. *J Anal Atom Spectrom* 1991;**6**:637–642.
89 Simpkins WA, Louie H, Wub M, Harrison M, Goldberg D. Trace elements in Australian orange juice and other products. *Food Chem* 2000;**71**:423–433.
90 Rivero RC, Hernandez PS, Rodriguez EMR, Martin JD, Romero CD. Mineral concentrations in cultivars of potatoes. *Food Chem* 2003;**83**:247–253.
91 Anderson KA, Smith BW. Chemical profiling to differentiate geographic growing origins of coffee. *J Agric Food Chem* 2002;**50**:2068–2075.
92 Valentin JL, Watling RJ. Provenance establishment of coffee using solution ICP-MS and ICP-AES. *Food Chem* 2013;**141**:98–104.
93 Perez AL, Smith BW, Anderson KA. Stable isotope and trace element profiling combined with classification models to differentiate geographic growing origin for three fruits: effects of subregion and variety. *J Agric Food Chem* 2006;**54**:4506–4516.
94 Matos-Reyes MN, Simonot J, López-Salazar O, Cervera ML, de la Guardia M. Authentication of Alicante's Mountain cherries protected designation of origin by their mineral profile. *Food Chem* 2013;**141**:2191–2197.
95 Ariyama K, Aoyama Y, Mochizuki A, Homura Y, Kadokura M, Yasui A. Determination of the geographic origin of onions between three main production areas in Japan and other countries by mineral composition. *J Agric Food Chem* 2007;**55**:347–354.
96 Ariyama K, Horita H, Yasui A. Chemometric techniques on inorganic elements composition for the determination of the geographic origin of Welsh onions. *Anal Sci* 2004;**20**:871–877.
97 Chope GA, Terry LA. Use of canonical variate analysis to differentiate onion cultivars by mineral content as measured by ICP-AES. *Food Chem* 2009;**115**:1108–1113.
98 Moreda-Piñeiro A, Fisher A, Hill SJ. The classification of tea according to region of origin using pattern recognition techniques and trace metal data. *J Food Compos Anal* 2003;**16**:195–211.
99 Fernandez-Caceres PL, Martın MJ, Pablos F, Gonzalez AG. Differentiation of TEA (Camellia sinensis) varieties and their geographical origin according to their metal content. *J Agric Food Chem* 2001;**49**:4775–4779.
100 Szymczycha-Madeja A, Welna M, Pohl P. Elemental analysis of teas and their infusions by spectrometric methods. *Trends Anal Chem* 2012;**35**:165–181.
101 Marcos A, Fisher A, Rea G, Hill SJ. Preliminary study using trace element concentrations and a chemometrics approach to determine the geographical origin of tea. *J Anal Atom Spectrom* 1998;**13**:521–525.
102 Zeiner M, Juranovic-Cindric I, Kevin DS. Characterization of extra virgin olive oils derived from the Croatian cultivar Oblica. *Eur J Lipid Sci Technol* 2010;**112**:1248–1252.
103 Gonzalvez A, Ghanjaoui ME, El Rhazi M, de la Guardia M. Inductively coupled plasma optical emission spectroscopy determination of trace element composition of argan oil. *Food Sci Technol Int* 2010;**16**:65–71.
104 Zengl Y, Wang L, Du J *et al*. Elemental content in brown rice by inductively coupled plasma atomic emission spectroscopy reveals the evolution of Asian cultivated rice. *J Integr Plant Biol* 2009;**51**:466–475.
105 Gonzalvez A, Armenta S, de la Guardia M. Geographical traceability of 'Arròs de Valencia' rice grain based on mineral element composition. *Food Chem* 2011;**126**:1254–1260.
106 Yucel Y, Sultanoglu P. Characterization of Hatay honeys according to their multi-element analysis using ICP-OES combined with chemometrics. *Food Chem* 2013; **140**:231–237.
107 Lachman J, Kolihová D, Miholova D, Košata J, Titěra D, Kult K. Analysis of minority honey components: possible use for the evaluation of honey quality. *Food Chem* 2007;**101**:3973–3979.
108 Fernandez-Torres R, Perez-Bernal JL, Bello-Lopez MA, Callejon-Mochon M, Jimenez-Sanchez JC, Guiraum-Perez A. Mineral content and botanical origin of Spanish honeys. *Talanta* 2005;**65**:686–691.
109 Brescia MA, Kosyir IJ, Caldarola V, Kidricy J, Sacco A. Chemometric classification of Apulian and Slovenian wines using 1H NMR and ICP-OES together with HPICE data. *J Agric Food Chem* 2003;**51**:21–26.
110 Danzer K, Carcia DDC, Thiel G, Reichembacher M. Classification of wine samples according to origin and grape varieties on the basis of inorganic and organic trace analyses. *Am Lab* 1999;**31**:29–34.
111 Alvarez M, Moreno IM, Pichardo S, Cameán AM, González AG. Mineral profile of 'fino' wines using inductively coupled plasma optical emission spectrometry methods. *Food Chem* 2012;**135**:309–313.
112 Marchisio PF, Sales A, Cerutti S, Marchevsky E, Martinez LD. On-line preconcentration of cadmium in commercial tea samples using polyurethane foam as filter associated with ultrasonic nebulization-inductively coupled plasma optical emission spectrometric detection. *Instrum Sci Technol* 2005;**33**:449–459.
113 Simpkins WA, Louie H, Wu M, Harrison M, Goldberg D. Trace elements in Australian orange juice and other products. *Food Chem* 2000;**71**:423–433.
114 Bayer S, McHard JA, Winefordner JD. Determination of the geographic origins of frozen concentrated orange juice via pattern recognition. *J Agric Food Chem* 1980; **28**:1306–1307.
115 Nikdel S, Barros SM. Citrus juice trace mea by automated sequential multielement ICAP-AES. *Proc Florida State Horticultural Soc* 1984;**97**:79–81.

CHAPTER 12

New developments in food analysis by ICP-MS

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Abstract: Inductively coupled plasma mass spectrometry (ICP-MS) has become one of the most popular analytical techniques for elemental analysis in all types of samples including food and beverages. This is due to its well-known analytical characteristics: very low detection limits, multi-element capabilities, the possibility of measuring isotope ratios and its easy coupling with chromatographic techniques. Nowadays, it is not uncommon to find ICP-MS instruments in laboratories dedicated to the routine analysis of food and beverages. We present a thorough review of new developments in food and beverage analysis by ICP-MS including a brief description of the technique. First, spectral interpretation is discussed for qualitative analysis including the different ways described for the removal of spectral interferences. Then, the quantification modes which can be applied with ICP-MS are introduced, including semi-quantitative analysis using the response curve, calibration graphs with internal standards and isotope dilution mass spectrometry. Next, the applications of ICP-MS for total element analysis and speciation analysis of food and beverages are reviewed. In this section, information about legal requirements, sample preparation procedures, validation of analytical methodologies with reference materials, bioavalability studies and the use of isotope dilution for both certification of reference materials and routine analysis is included. Finally, the use of ICP-MS in provenance studies is highlighted.

Keywords: ICP-MS, fundamental theory, total elemental analysis, speciation analysis, provenance studies

12.1 Inductively coupled plasma as ion source for mass spectrometry

Atmospheric pressure inductively coupled plasma (ICP) has been employed as excitation source for atomic emission spectrometry (AES) since 1964. Many of the useful emission lines were obtained from excited ions indicating that this source was also an efficient ionization source. The coupling of ICP as ion source for mass spectrometry (MS) was first described in 1980 [1] and the first commercial ICP-MS instrument appeared in 1983. Now, more than 30 years later, ICP-MS is clearly the dominant technique in trace elemental analysis. In this chapter we will summarize its main features and applications for the determination of trace elements and elemental species in food and beverages.

The atmospheric pressure ICP source employed in MS follows the typical design of atomic emission sources. The energy in the ICP is obtained from a three-turn radiofrequency (RF) induction coil made of copper which is cooled internally by a water flow. The argon plasma is formed in the inner space of the induction coil and is separated from the coil by a dielectric barrier made of vitrified quartz and constitutes what is called the 'torch'. The inner diameter of a typical plasma torch is 18 mm and is formed by three concentric tubes in which pure argon is flowing continuously at atmospheric pressure. The hottest part of the plasma (about 10,000 K) occurs in the vicinity of the induction coil while the centre of the plasma is relatively cooler (about 6000–7000 K). A high-velocity flow of argon can be introduced in the centre of the plasma creating a flow-through channel for sample introduction. The sample aerosol, typically a wet aerosol, is desolvated, the particles are vaporized and all chemical bonds are broken. After atomization the main ionization reaction is electron impact but other mechanisms such as Penning ionization or charge transfer have also been postulated. So, the ionization process in the plasma is mainly governed by the Saha equation. At the typical temperatures of the argon plasma,

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elements with first ionization potentials lower than 7 eV are ionized almost quantitatively in the ICP. These include alkalis, alkaline earth elements, most of the transition elements and the lanthanides and actinides. The ionization degrees for other elements decrease with increasing ionization potential. Still, mercury (10.5 eV) can be ionized about 10% in the argon ICP. Except for fluorine and some of the noble gases (He, Ne) most of the elements of the periodic table can be ionized to a certain degree in the ICP and hence can be detected by MS. Most of the elements form monopositive atomic ions, which simplifies the interpretation of the mass spectra obtained by ICP-MS.

12.1.1 Sample introduction into the ICP

Liquid samples are normally introduced after pneumatic nebulization while gaseous samples can be introduced directly. Solid samples are usually introduced after laser ablation. The most important requirement of any sample introduction device is the formation of particles of small size which can be transported quantitatively to the plasma. Most pneumatic nebulizers show a very wide particle size distribution in the primary aerosol, which requires a spray chamber to eliminate the larger particles. The efficiency of sample transport to the plasma by nebulization is thus less than quantitative. Additionally, the introduction of solid, liquid or gaseous samples in the plasma may affect their properties. For example, reduction in the ionization degrees of the elements has been observed when organic modifiers (e.g. methanol, acetonitrile) are nebulized. Additionally, the sample matrix can also affect the properties of the plasma. It has been observed that the introduction of small amounts of carbon in the plasma enhances the ionization of selenium and arsenic while large amounts of carbon decreases the ionization of those elements. So, these effects may be important when analysing food samples containing high levels of organic carbon.

Sample introduction systems should allow the rapid switchover between samples without any cross-contamination. This concept is called 'memory effect' and is present in most sample introduction systems to a certain extent. Memory effects depend also on the nature and oxidation state of the element. For example, Hg^{2+} in liquid samples shows pronounced memory in contrast to Hg^0 introduced as cold vapour. Once memory effects are evaluated, the time required for adequate washing between samples and signal stabilization can be established.

12.1.2 Extraction of the ions into the mass spectrometer

In the plasma the ions are formed at atmospheric pressure and at a temperature of about 7000 K while for mass spectrometric analysis the ions should be in vacuum and at low temperatures. The extraction interface of modern ICP-MS instruments is very similar to that developed in the early 1980s. A water-cooled nickel cone with an orifice of approximately 1 mm diameter, the 'sampler', is set in front of the plasma. A high-capacity rotary pump creates a supersonic jet which expands into the low pressure zone behind the sampler cone. A second nickel cone of steeped slope, the 'skimmer', is set within this expanding supersonic jet. Behind the skimmer the pressure drops further with the help of turbomolecular pumps. The final extracted ion beam is positively charged due mainly to the Ar^+ ions from the plasma.

12.1.3 Mass spectrometers employed in ICP-MS

The quadrupole mass filter was selected for the first commercial ICP-MS instruments because of cost and ease of coupling considerations and its use has continued until now. Commercial ICP-MS instruments incorporating a double focusing analyser appeared in the early 1990s and they are the preferred instruments for high-sensitivity work. These instruments use a reverse Nier–Johnson geometry and are single collector instruments. The use of collision/reaction cells in combination with quadrupole analysers started in the late 1990s. Nowadays, quadrupoles, hexapoles and octapoles are employed as collision/reaction cells in different commercial instruments. These instruments remove most polyatomic interferences from argon or matrix components by the judicious selection of a collision/reaction gas. Quadrupole ICP-MS instruments equipped with collision/reaction cells are incorporated in most modern instruments and are well suited for their application to elemental analysis in routine testing laboratories.

12.1.4 Ion detection

Most commercial ICP-MS instruments employ secondary electron multipliers as ion detectors. These detectors can work in both analogue and ion-counting modes. The ion-counting mode detects every single ion arriving at the detector and is linear up to about 10^6 ions/s.

For larger ion currents the analogue mode provides two additional orders of magnitude of linearity. So the combination of both modes shows abou eight orders of magnitude of linear response. Detection limits in quadrupole ICP-MS instruments depend on the ionization degree of the elements and the blank values but they are usually well below nanogram per millilitre levels for most elements. Thus in the same sample we could analyse elements at trace and ultratrace levels without changing the instrumental configuration.

12.2 Spectral interpretation in ICP-MS

The ICP ion source produces mainly monoatomic monopositive ions. Thus spectral interpretation in ICP-MS is straightforward as the isotopic composition of the elements can be considered constant in nature [2]. However, certain polyatomic ions may complicate the interpretation of the mass spectra and the selection of the right isotopes for quantification. A typical ICP-MS spectrum from the acid digestion of white beans for the mass range 0–80 u using a quadrupole analyser is shown in Figure 12.1, in logarithmic scale. The main spectral features of the ICP-MS coupling are indicated in the spectrum. Signals at several selected masses are always saturated and so those masses are not normally measured in quadrupole ICP-MS (forbidden masses). Masses 14, 15, 16, 17, 18 and 19 for nitrogen and water derived ions and masses 36, 38, 40 for argon (including mass 41 for ArH^+) are not measured in quadrupole ICP-MS. Other polyatomic ions observed are N_2^+ at mass 28, O_2^+ at mass 32, ArO^+ at mass 56 and Ar_2^+ at mass 80. Thus the main isotopes of silicon (28), iron (56) and selenium (80) are interfered in quadrupole ICP-MS. For non-interfered elements, ICP-MS provides easy identification. For example, the isotope distributions observed for copper at masses 63 and 65 and zinc at masses 64, 66, 67, 68 and 70 in Figure 12.1 agree very well with the theoretical abundances for the natural elements [2]. The data in Figure 12.1 also show that it comes from a vegetable sample as the signal for potassium at mass 39 is much larger than that from sodium at mass 23.

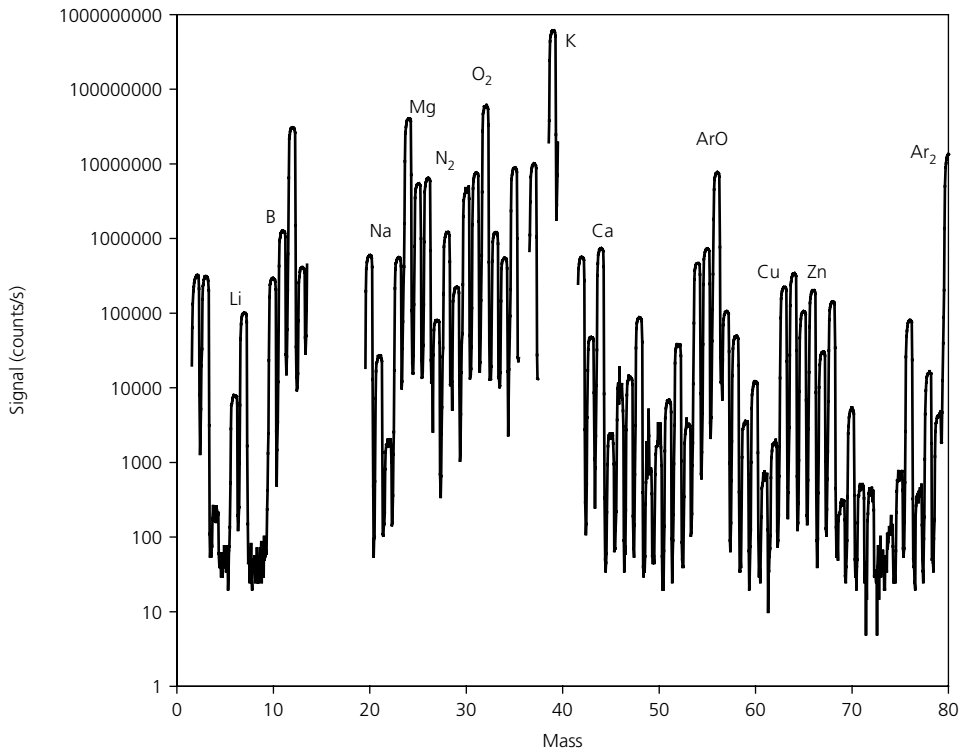


Figure 12.1 Mass spectrum obtained by ICP-MS for the acid digestion of a white bean sample in the mass range 0–80 u.

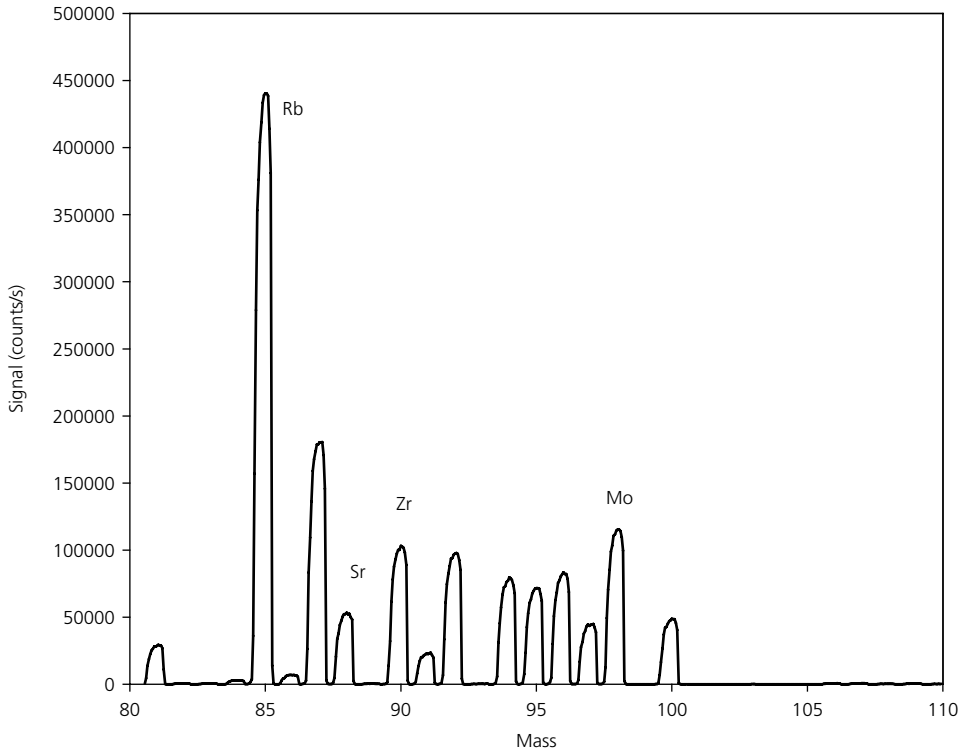


Figure 12.2 Mass spectrum obtained by ICP-MS for the acid digestion of a white bean sample in the mass range 80–110 u.

The mass spectrum of the same sample in the 80–110 u range is shown in Figure 12.2, here in linear scale. In this mass range no polyatomic interferences from argon or background gases are observed (except for Ar_2H^+ at mass 81). The main elements present in the digestion of white beans are Rb (85 and 87), Sr (84, 86, 87 and 88), Zr (90, 91, 92, 94 and 96) and Mo (92, 94, 95, 96, 97, 98 and 100). Again, the observed isotope composition of these elements follows closely that expected based on their natural abundances [2]. Isobaric overlaps between different elements (e.g. ^{87}Rb and ^{87}Sr in Figure 12.2) cannot be resolved and a single peak is always observed. It is worth noting that Rb and Sr are always present in vegetable samples and their ratio changes depending on the characteristics of the growing soil. The content of these elements can be used for provenance purposes as we discussed in later sections.

12.2.1 Removal of spectral interferences

As seen in Figure 12.1 the main spectral interferences observed in ICP-MS arise from polyatomic ions. There are two general ways to eliminate those interferences: the use of collision/reaction cells or high-resolution instruments. Collision/reaction cells are very efficient at removing polyatomic interferences from argon and other matrix components. Typical examples are the elimination of the $^{40}Ar^{35}Cl^+$ interference on $^{75}As^+$, the elimination of $^{40}Ar^{16}O^+$ on $^{56}Fe^+$ or the elimination of $^{40}Ar_2^+$ on $^{80}Se^+$. However, collision/reaction cells do not perform very efficiently for the elimination of metal oxide and hydroxide interferences. For example the interferences of $^{40}Ca^{16}O^+$ on $^{56}Fe^+$ and $^{40}Ca^{16}O^1H^+$ on $^{57}Fe^+$ cannot be eliminated easily in collision/reaction cells. In those cases, the selection of ^{54}Fe for the determination of iron in food and beverages may be justified. Additionally, double focusing magnetic sector ICP-MS instruments working at 3000–4000 resolution ($M/\Delta M$) can be the alternative. Table 12.1 shows the masses and procedures for the elimination of polyatomic and isobaric spectral interferences for a list of elements of interest in food and beverages. For some of the elements we have included two/three masses as these masses would be the usual selection when performing isotope dilution MS [3].

Table 12.1 Masses usually selected for the determination of trace metals by ICP-MS in foods and beverages, their typical interferences and the way to eliminate them.

Element	Masses	Typical spectral interferences	Alternative removal procedures
Li	6	—	—
	7	—	—
Be	9	—	—
B	10	—	—
	11	—	—
Na	23	—	—
Mg	24	—	—
Al	27	$^{12}C^{14}N^{1}H$, $^{13}C^{14}N$, $^{12}C^{15}N$	High resolution, collision/reaction cell
K	39	$^{38}Ar^{1}H$	High resolution, collision/reaction cell
Ca	43	—	—
	44	$^{12}C^{16}O_2$	High resolution, collision/reaction cell
Ti	47	$^{31}P^{16}O$	High resolution, collision/reaction cell
	48	^{48}Ca	Chemical separation
V	51	$^{35}Cl^{16}O$	High resolution, collision/reaction cell
Cr	52	$^{40}Ar^{12}C$	High resolution, collision/reaction cell
	53	$^{37}Cl^{16}O$	High resolution, collision/reaction cell
Mn	55	—	—
Fe	54	$^{40}Ar^{14}N$, ^{54}Cr	High resolution, collision/reaction cell, chemical separation
	56	$^{40}Ar^{16}O$, $^{40}Ca^{16}O$	High resolution, collision/reaction cell
	57	$^{40}Ar^{16}O^{1}H$, $^{40}Ca^{16}O^{1}H$	High resolution, collision/reaction cell
Co	59	—	—
Ni	58	^{58}Fe	Chemical separation
	60	—	—
Cu	63	$^{23}Na^{40}Ar$	High resolution, collision/reaction cell
	65	—	—
Zn	64	$^{32}S^{16}O_2$	High resolution, collision/reaction cell
	66	$^{34}S^{16}O_2$	High resolution, collision/reaction cell
	67	—	—
Ga	69	$^{138}Ba^{2+}$	High resolution
	71	—	—
Ge	72	—	—
As	75	$^{35}Cl^{40}Ar$	Collision/reaction cell
Se	77	$^{37}Cl^{40}Ar$	Collision/reaction cell
	78	$^{38}Ar^{40}Ar$	Collision/reaction cell
	80	$^{40}Ar_2$	Collision/reaction cell
Rb	85	—	—
	87	^{87}Sr	Chemical separation
Sr	86	—	—
	87	^{87}Rb	Chemical separation
	88	—	—
Zr	90	—	—
Mo	95	$^{39}K^{40}Ar^{16}O$	High resolution, collision/reaction cell
	98	—	—
Cd	111	$^{95}Mo^{16}O$	Chemical separation
	114	$^{98}Mo^{16}O$, ^{114}Sn	Chemical separation
Sn	120	—	—
Sb	121	—	—

(Continued)

Table 12.1 (Continued)

Element	Masses	Typical spectral interferences	Alternative removal procedures
I	127	—	—
Cs	133	—	—
Ba	138	—	—
Hg	202	—	—
Tl	205	—	—
Pb	208	—	—
Bi	209	—	—
Th	232	—	—
U	238	—	—

As can be observed in Table 12.1, most spectral interferences can be eliminated either by collision/reaction cells or by high-resolution instruments. However, the interferences from oxides of the heavy elements (e.g. molybdenum oxide interferences on cadmium isotopes) cannot be eliminated by either collision/reaction cells or high-resolution instruments. For these elements a chemical separation could be the only way to eliminate these spectral interferences.

Pure isobaric interferences (e.g. ^{87}Rb on ^{87}Sr) cannot be resolved even at the highest resolution setting of current magnetic sector instruments. For high precision work a chemical separation needs to be performed (e.g. for the measurement of Sr isotope ratios in the presence of Rb). However, for most applications a mathematical correction equation can be applied and the chemical separation can be avoided.

12.2.2 Qualitative analysis

The qualitative identification and detection of trace elements in food samples by ICP-MS is very simple. Based on the spectra shown in Figures 12.1 and 12.2 the presence or absence of different elements in the sample can be elucidated. However, some more information from the ICP-MS spectrum can be obtained when an internal standard element is added to samples and blanks and the relative intensities are measured for the masses shown in Table 12.1 for each element. In this way the relative importance of the signals for the different elements can be compared. For example, Figure 12.3 shows the data obtained for the bean sample shown in Figures 12.1 and 12.2 (open circles) and a digestion blank (filled circles) at one of the selected masses for each element using rhodium as internal standard.

The relative signal for each element can be compared directly with the blank. As can be observed, Mg and K are the main components of the digested samples which can be expected for a vegetable sample. Other elements clearly present in the sample include Rb, Sr and many transition elements. It is of interest to note that zirconium, observed clearly in Figure 12.2, seems to be a contamination of the digestion reagents as the signal ratio for the digestion blank is very similar to that in the sample. Note that the relative signals shown in Figure 12.3 cover about eight orders of magnitude so elements found at very low concentrations (e.g. uranium) can be detected and identified in the presence of elements at very high concentrations (e.g. Mg, K). Thus the graph shown in Figure 12.3 is ideal for identifying the presence of different elements in the sample and to have an approximate idea of their relative concentration.

12.3 Quantification modes in ICP-MS

In comparison with other traditional techniques of trace element analysis, such as atomic absorption spectrometry (AAS) or AES, where the calibration of the response for each element is the only quantification mode, ICP-MS offers different alternatives depending on the accuracy and precision required. First, a semi-quantitative analysis can be performed easily as the response of ICP-MS for each element is a simple function of its mass, its ionization potential and the isotope abundance of the monitored isotope. Second, quantitative analysis based on a linear calibration can be performed as in any other instrumental analysis technique.

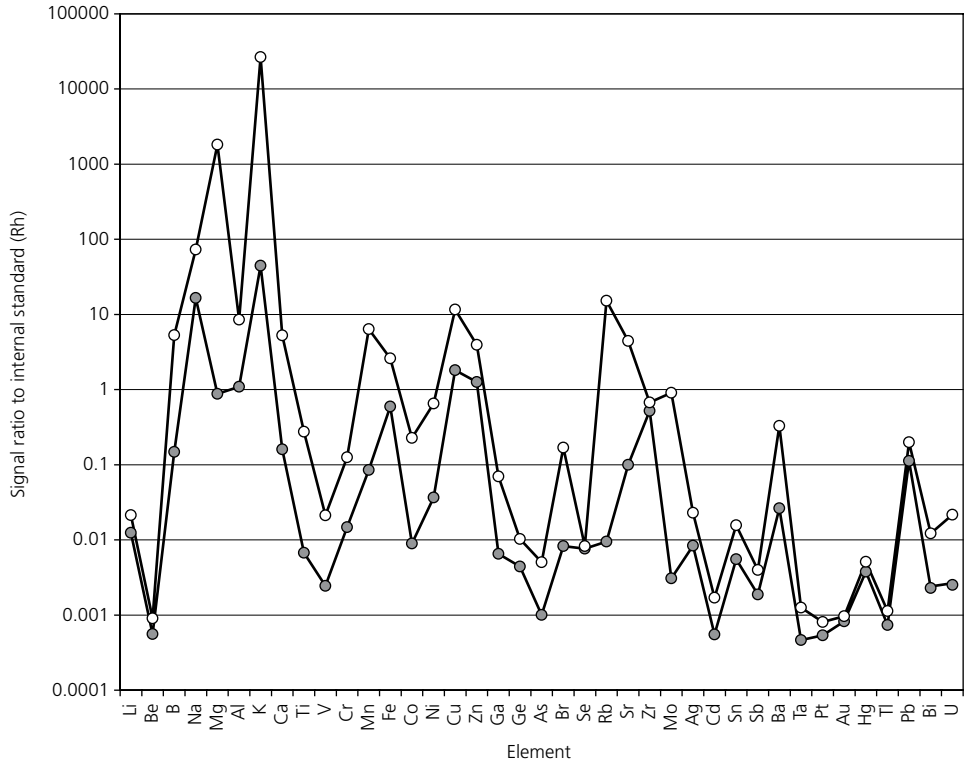


Figure 12.3 Signal ratio between the element and the internal standards for a white bean sample (open circles) and a digestion blank (filled circles).

However, in ICP-MS it is recommended that an internal standard is always used to correct for drift and some matrix interferences. Finally, there is always the possibility of using the isotopic information provided by ICP-MS to carry out isotope dilution procedures. As discussed later in this chapter, multi-element isotope dilution can be carried out in the on-line mode which is suitable for routine analysis at low cost. We will describe briefly the three different alternatives indicated above.

12.3.1 Semi-quantitative analysis using the response curve

If we prepare a multi-element solution and measure it using ICP-MS for several masses of the different elements present, we will obtain a response curve similar to that shown in Figure 12.4 for a mixture of 10 ppb of Mg, Al, Sc, V, Mn, Cu, Zn, Se, Sr, Mo, Pd, Sn, Sb, Cs, Pr, Nd, Tl, Pb, Bi, Th and U. The measured signal was divided by the concentration and the natural isotope abundance for each element and plotted against the mass of the measured isotopes (open circles).

As can be observed the response is a simple function of the mass of each element. However, there are some elements shown in the graph (Zn, Se, Sb, Au, Bi) for which the response is much lower than that corresponding to their mass. This is due to the fact that these elements are not ionized quantitatively in ICP-MS because of their high first ionization potential. In these cases it is possible to correct the response by a given ionization degree calculated based on the Saha equation [4]. When the ionization correction is applied (filled circles), the response in ICP-MS follows a simple polynomial curve. The practical application of this characteristic is that semi-quantitative analysis can be performed easily by ICP-MS once the response factors are known for each element. Unfortunately, the shape and magnitude of the response curve may change with the experimental conditions and sample matrix. Thus the response curve needs to be updated for each measured sample.

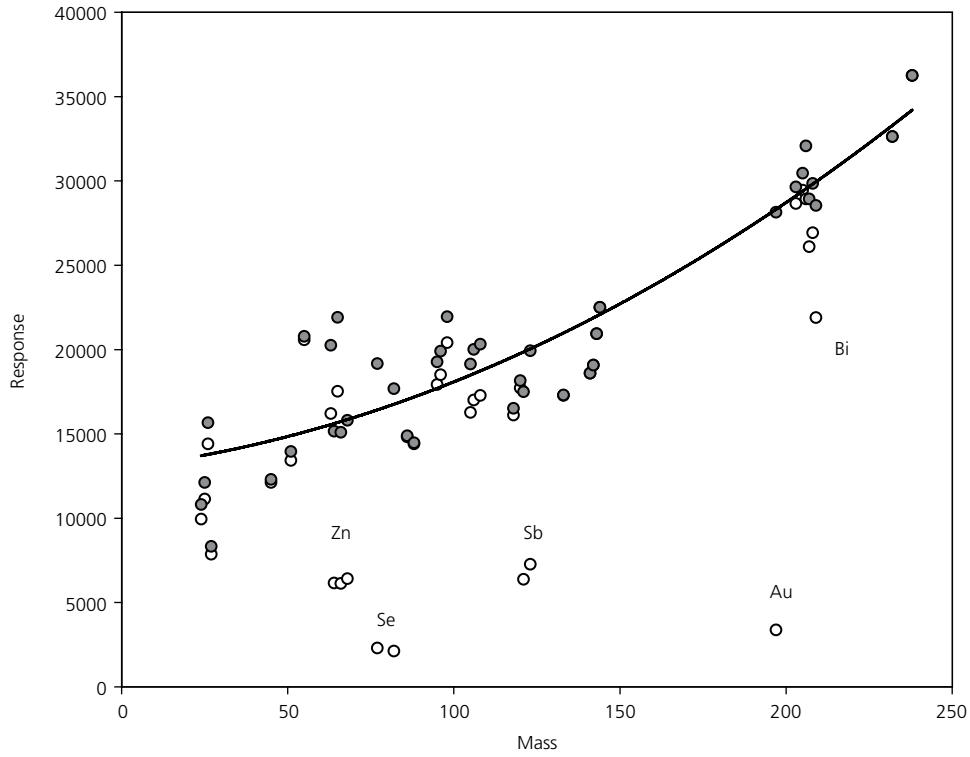


Figure 12.4 Response curve in the ICP-MS.

This is performed by adding a series of internal standards to the sample covering the mass range to be measured. Ideally, those internal standards should be monoisotopic and easily ionized in the plasma. Typical internal standards are Be, Sc, Ga, Y, Rh, Pr, Tb, Re and Th. Obviously, selection of the internal standard will depend on its absence from the samples measured. For most food and beverage samples this is usually the case for these elements.

12.3.2 Calibration with internal standards

Quantitative analysis by ICP-MS can be done as in any other instrumental technique by aqueous calibration or standard additions. The use of internal standards, such as Rh, is recommended to correct for matrix effects during nebulization, sensitivity drift with time and to improve reproducibility. When calibrations are to cover more than one order of magnitude of concentration it is recommended to use weighted regression lines as the standard deviation of the measurements changes with the signal intensity or the signal ratio to the internal standard. Also, when the concentrations in the samples are in the low part of the calibration graph, the use of weighted regression lines is recommended. This is to avoid negative concentration values due to the incorrect calculation of the intercept of the calibration graph by classic linear regression.

For the determination of some elements it is necessary to observe particular precautions. For example, inorganic mercury in solution shows pronounced memory effects so the calibration graph should be prepared at the minimum concentration which covers the expected concentrations in the sample. Excessive concentrations in the calibration standards may pose a serious contamination risk to the samples due to memory effects. Another known effect is the increase in the ionization degree of arsenic and selenium because of the presence of carbon in the samples. In these cases the addition of a small percentage of an organic solvent such as methanol or isopropanol to samples and calibration standards is common practice. In this way the

sample matrix mimics that of the calibration standards and the ionization effect is corrected. This is particularly important for the analysis of alcoholic beverages after a simple dilution of the sample.

Another important consideration when performing multi-element calibrations is the inter-element effects particularly when some elements are at high concentrations in the calibration standards and some other at very low concentration. For example, at high concentrations of calcium the formation of $^{40}Ca^{16}O$ and $^{40}Ca^{16}O^{1}H$ may interfere with masses 56 and 57 of iron. So, the calibration graph for iron at these masses will be affected by the calcium concentration. In this case we can select mass 54 for iron determination but only if there is no chromium in the samples or calibration standards. Another example is the use of gallium as internal standard. There is spectral interference from $^{138}Ba^{2+}$ which appears at mass 69, the main isotope of gallium. When a high concentration of barium is present in the calibration standards, the signal at mass 69 will be affected. This is observed in the qualitative graph shown in Figure 12.3 where there is apparently a positive confirmation for gallium in the sample. Unfortunately, gallium was measured here using mass 69 and the apparent detection is simply the effect of the double ionized barium isotopes. Thus it is always important to check for inter-element effects in calibration graphs.

12.3.3 Isotope dilution mass spectrometry

The highest precision and accuracy in quantitative analysis using ICP-MS is normally offered by isotope dilution mass spectrometry (IDMS). The principle of IDMS is shown schematically in Figure 12.5 using copper as example.

Natural abundance copper contains isotopes 63 and 65 at a ratio of about 2 : 1 and this abundance can be considered constant in nature. A certified solution of highly enriched ^{65}Cu (the tracer or spike) can be obtained commercially. When we mix a known amount of copper from the tracer, N_t, with an unknown amount of copper from the sample, N_s, the isotope distribution

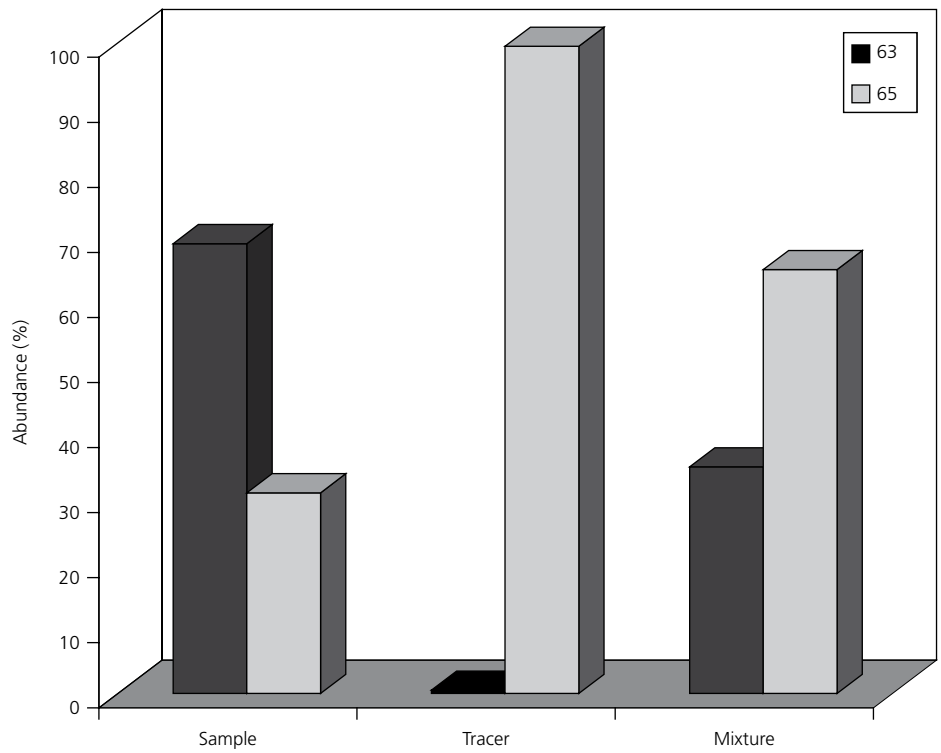


Figure 12.5 Principle of IDMS for elemental analysis.

observed in the mixture, A_m, will be a linear function of the isotope distributions from the sample, A_s, and tracer, A_t, respectively. The parameters of the linear function will be the molar fractions of copper in the mixture coming from the sample (x_s) and the tracer (x_t), respectively. This can be expressed as:

$$\begin{bmatrix} A_m^{63} \\ A_m^{65} \end{bmatrix} = \begin{bmatrix} A_t^{63} & A_s^{63} \\ A_t^{65} & A_s^{65} \end{bmatrix} \times \begin{bmatrix} x_t \\ x_s \end{bmatrix} \tag{12.1}$$

where the values of x_t and x_s can be obtained by resolving this set of two equations with two unknowns. Once the molar fractions are known the amount of natural abundance copper in the sample is obtained from:

$$N_s = N_t \times \frac{x_s}{x_t} \tag{12.2}$$

as the ratio of molar fractions is equal to the ratio of moles. When the element contains more than two isotopes, equation (12.1) provides more equations than unknowns and its solution is obtained by multiple linear regression.

From a practical point of view the actual determination of the isotope composition in the mixture is performed by isotope ratio measurements using the equation:

$$A_m^i = \frac{R_m^{i/j}}{\sum_{i=1}^{n} R_m^{i/j}} \tag{12.3}$$

where isotope j is the same reference isotope for all measured isotope ratios. In this way isotope dilution measurements are not affected by matrix effects or signal drift as isotope ratios are known to be very robust and precise measurements in ICP-MS.

For routine multi-element analysis, IDMS is better performed in the on-line configuration shown in Figure 12.6. The sample, coming from an autosampler, is pumped with the help of a peristaltic pump to a T-piece where it is mixed on-line with a multi-element solution of the spike just before entering the nebulizer of the ICP-MS. In this way several elements can be determined simultaneously and even monoisotopic elements determined using internal standards added to the spike solution. More details on the use of IDMS in elemental analysis can be obtained from Garcia Alonso and Rodríguez-González [3].

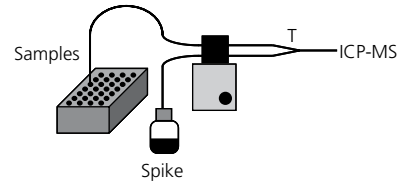


Figure 12.6 IDMS in the on-line configuration as applied for routine analysis.

12.4 Total elemental analysis

It is well known that food and beverages are the main source of elements for humans. Certain elements, such as sodium, magnesium, phosphorus, sulfur, chlorine, potassium, calcium, iron, copper and zinc, are considered essential nutrients and they are required in milligram per kilogram quantities per day. Other essential elements, such as chromium, manganese, cobalt, selenium, molybdenum and iodine, are required in microgram per kilogram quantities per day. Essential elements may become toxic at high concentrations so their content in food and beverages needs to be controlled. Other elements, such as arsenic, cadmium, mercury and lead, are considered exclusively toxic and should be excluded from the diet [5]. It must be said also that not all chemical forms of these elements possess the same toxicity. Inorganic arsenic is considered extremely toxic while arsenobetaine, the most abundant arsenic compound in fish, can be considered non-toxic. So, methods for the speciation of toxic metals need to be developed. These are discussed in section 12.5.

There is a third type of element present in food and beverages which are considered neither essential nor toxic (e.g. rubidium and strontium). These elements have no known physiological function but can be found in human serum at relatively high concentrations [6]. Their determination in food and beverages is normally not required by legislation but it has found application in certain fields such as provenance studies.

Therefore, to assure that food products are safe their elemental content must be controlled. From the essential/toxic point of view, the determination of trace elements in food and beverages presents two different goals: (i) to ensure the required intake of essential elements and (ii) to determine the levels of toxic elements and compare them with regulated maximum levels. Additionally, the content of other elements in food and

beverages, such as strontium, may serve to establish provenance, particularly for vegetable and dairy products. It is clear that a multi-element analysis technique with low detection limits is required. ICP-MS can be considered such a technique and so it is increasingly employed for the determination of the elemental content of food and beverages.

12.4.1 Legal requirements

During last few years, measurement of the levels of toxic and essential elements in foodstuffs has become a major topic of public interest. Various international bodies and food agencies have introduced legislation that specifes maximum allowable concentrations for certain metals in foodstuffs. For example, the European Commission Directive EC 1881/2006 specifies maximum levels for lead, cadmium, mercury and inorganic tin in a variety of foodstuffs [7]. Maximum allowed lead content varies from 0.02 mg/kg in milk products and infant formulae up to 1.5 mg/kg in bivalve molluscs. Maximum allowed inorganic tin content ranges from 20 mg/kg in soy up to 200 mg/kg in canned food. In a similar way, the US Food and Drug Administration (FDA) sets action levels for poisonous or deleterious substances in human food and animal feed, including cadmium, lead, mercury and others [8]. Nowadays, there is also increasing interest in the regulation of baby foods since babies and infants are a sensitive population when exposed to environmental contaminants. The European Commission has regulated the allowed levels of lead and cadmium in materials such as cereals, fruit and vegetables, raw materials that can be further employed for the production of baby food. For example, tin is regulated at 50 mg/kg specifically in canned baby food and infant formula [7]. Canada has a specific tolerance level of 0.1 mg/kg for arsenic and 0.2 mg/kg for lead in ready-to-serve fruit juices, nectars and beverages [9]. The FDA suggests that lead content above 0.1 mg/kg would provide an unacceptable risk to children who regularly consume sweets. This is extrapolated to guidance levels for certain trace elements in many food items.

With legislation covering many different food types, the number of samples that food laboratories need to analyse is very large. Sensitive, accurate, precise and robust analytical methods are required for the measurements of minor and trace elements in foodstuffs. The chosen technique must measure toxic elements, elemental species, micronutrients and macronutrients, and must be able to identify whether products have been contaminated during (i) the production process, (ii) their packaging or (iii) by the cooking tools used to prepare them. ICP-MS addresses all these requirements in the most efficient way. In combination with an adequate sample preparation procedure, ICP-MS is capable of providing total element concentrations well below the detection limits required by the legislation and it is nowadays the technique mostly employed for the certification of food reference materials in combination with IDMS [3].

12.4.2 Sample preparation procedures

Food element analysis presents a difficult analytical challenge because of the variety of food types and the wide range of element concentrations that need to be measured. In a first step, a representative sample must be obtained. Then the sample must be homogenized to obtain a representative subsample. Typical procedures for homogenization of solid food samples include lyophilization, cryogenic milling or dry ashing. When selecting the right procedure for homogenization we have to take into account possible losses of volatile elements and the risk of sample contamination. In our laboratory we normally carry out lyophilization of food samples followed by milling using polytetrafluoroethylene (PTFE) balls. Additionally, lyophilization provides a dry sample which can be stored for longer periods than fresh samples. Then, for routine ICP-MS determination of trace metals the samples should be in the form of an acidic aqueous solution so, before analysis, solid samples should be digested. This is a critical step in most analytical methods for routine determination of chemical elements in food samples. Among the most frequently applied digestion procedures for food analysis are open wet digestion in quartz tubes and closed vessel microwave digestion. Typical digestion acids include nitric acid and hydrofluoric acid. HF is normally required in small amounts and only for the digestion of vegetable samples which may contain traces of silicon. Furthermore, hydrogen peroxide needs to be added for the removal of the organic matter. It is important to check that volatile elements are not lost during digestion and that is why closed vessel digestion in microwave ovens is the most usual analytical procedure. It is also important to pay attention to the type of containers employed to avoid adsorption effects on the container walls or sample contamination from trace element

impurities in the materials employed. Quartz, PTFE or perfluoroalkoxy (PFA) are the most employed materials for sample digestion for ICP-MS analysis as they provide the lowest levels of impurities. Additionally, special attention must be paid to the purity of the water and concentrated acids in order to avoid sample contamination. Sub-boiling distillation can be employed to purify concentrated acids and reduce the levels of trace elements in the reagent blanks.

We usually carry out sample digestions and dilutions on a weight basis to avoid the use of volumetric flasks and minimize dilution errors. In this way, we can perform the addition of enriched isotopes (for IDMS) or internal standards (for aqueous calibration or semi-quantitative analysis) in a very precise way. High-density polyethylene is a good and cheap material to store digested samples and can be discarded after use. A better material is PFA but it must be reused because of cost considerations.

For liquid samples (e.g. alcoholic beverages) a simple dilution is normally required. In those cases, the content of dissolved organic carbon will be high and special precautions must be taken for the determination of certain elements such as arsenic or selenium, which show ionization enhancement or depression in the ICP depending on the carbon content in the samples. The addition of an organic modifier, such as isopropyl alcohol, to samples and calibration standards is the usual procedure for the accurate determination of these elements.

12.4.3 Examples of food and beverages analysis

Accurate and precise elemental analysis of food and beverages is a necessary prerequisite for assessing their nutritional composition, identifying food contamination sources and determining potentially toxic elements. Elemental food content has been analysed using different techniques such as flame AES, flame and graphite furnace AAS, inductively coupled plasma atomic emission spectrometry (ICP-AES) and ICP-MS. In the last few years, and owing to its analytical characteristics (low detection limits, multi-element capabilities, isotopic information and wide linear range), ICP-MS is considered the most suitable technique for elemental food analysis.

The literature on use of ICP-MS for studying the elemental content of food and beverages has increased considerably in recent years so only a few representative examples will be cited here. Beverages (wine, beer, alcoholic drinks, cider, fruit juice, coffee, tea, bottled water); fruits and vegetables (tomato, lettuce, potato, orange, clementine, peach, strawberry, onion, asparagus); meat and meat-derived products; fish and fish-derived products; cereals (wheat, rice); dairy products (cheese, milk, yoghurt); baby/infant foodstuff; and spices and herbs (vanilla, oregano, pepper, ginger, garlic) can be mentioned as examples of the different food and beverages studied.

Wine is by far the most studied beverage. Elements usually quantified include Na, Mg, K, Ca and Fe among the major elements, and Al, Cr, Mn, Cu, Zn, Se, Br, Rb and Sr for the minor elements. Some other trace elements, and especially the lanthanides, have been monitored in both red and white wine samples [10]. The number of publications dealing with beer analysis is smaller. Recently, Mahmood *et al*. [11] monitored the content of V, Cr, Co, Ni, As, Se, Mo, Cd, In, Sb, Cs, Pb, Bi and U in 40 commercial beer samples in order to establish their elemental profile for fingerprinting purposes. A fast method for the determination of Ca, Cd, Co, Cu, Fe, Mn, Mo, Na, Ni, Pb, Rb, Sr, V and Zn in fruit juices by ICP-MS after only a simple dilution was proposed by Tormen *et al*. [12]. A dilution factor of 1 : 20 allowed direct sample introduction into the plasma without clogging the cross-flow nebulizer or causing deposits on the cones and ion lens system. In addition, external calibration can be carried out using aqueous standard solutions and Rh as internal standard. The detection limits obtained were much lower than the maximum values allowed by regulatory agencies for these elements.

When considering baby/infant foodstuff, it is necessary to examine both the nutritive value of the foods, in order to establish whether the correct levels of nutrients are present, and the risk of exposure to toxic elements. Carbonell-Barrachina *et al*. [13] have analysed baby foods from Spain, the UK, the USA and China for essential macroelements (Ca and Na), essential trace elements (Fe, Cu, Zn, Mn, Se, Cr, Co and Ni) and toxic trace elements (As, Pb, Cd and Hg). Their results indicate that, for instance, pure baby rice samples contained relevant amounts of Pb in Spain, As in the UK, As, Cr and Ni in the USA, and Cr and Cd in China. In another study Zand *et al*. [14] determined the concentration of 20 essential and non-essential elements in a representative range of ready-to-feed infant foods (poultry and fish based) currently on sale in the UK. They concluded

that the concentrations of toxic elements found in this study were not of concern.

Milk and dairy products, especially yoghurt, are a good source of many valuable nutrients and minerals in the human diet. Llorent-Martínez *et al*. [15] determined the content of 20 trace and minor elements in soy and dairy yoghurts consumed in Spain by ICP-MS after microwave digestion. The results indicate that Cu, Mn and Ni were higher in soybean products while slight differences were observed in the content of Fe and Zn. Additionally, elemental profiles can be a powerful tool for distinguishing between cow and buffalo milk, and therefore between the dairy products obtained from them as described by Benincasa *et al*. [16].

When considering food analysis, total diet studies (TDS) are very relevant since they estimate a population's dietary intakes. The foods most frequently consumed by a population group are identified, purchased and prepared as consumed. This approach takes into account the impact of home cooking. The combination of contaminant, mineral and nutrient concentrations and dietary consumption information provides the estimated dietary exposure for population groups. For example, Sr, Ag, Sn, Ba, Fe, Te, Ga, Ge and V were quantified by ICP-MS in 1319 food samples typically consumed by the French population under a TDS [17]. The food groups with the highest levels of trace elements were (i) sweeteners, honey and confectionery, (ii) fish and fish products, (iii) tofu, (iv) ice-cream and (v) fat and oil. Fruits and vegetables presented the highest amount of Sn.

12.4.4 Validation of methodologies: food reference materials

Food analysis is one of those fields where accreditation is required for testing laboratories. Demonstration of competence is usually carried out by participation in proficiency testing exercises but validation of the methodologies developed in each laboratory requires the use of certified reference materials (CRMs). A CRM is a 'reference material accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence' [18]. It is clear that well-characterized reference materials are needed by laboratories in the food testing, dietary supplement and nutritional fields.

Reference materials can be used not only for the validation of methodologies but also to optimize the sample preparation step including the digestion of solid samples. When CRMs are as close as possible to the nature of the sample to be analysed, they are called matrix CRMs. Typical examples are pork meat, mussel tissue, rye flour and milk powder certified for their elemental content. Nowadays there are more than 100 food-related reference materials certified for their elemental content, highlighting the importance of quality assurance in food analysis. However, this number is still small due to the wide range of food and beverages matrices, the large geographical and cultural variety in food and beverages habits, and the different concentration levels in which metals can be present in foodstuffs.

Reference material producers are usually national metrology institutes (NMIs). They normally apply primary methods of measurement, such as IDMS, for the certification of the elemental content in their matrix CRMs and assure the traceability of their measurements by regular participation in key comparisons organized by the Comité Consultatif pour la Quantité de Matière (CCQM). The CCQM was created in 1993 by the Comité International des Poids et Mesures (CIPM) and coordinates the activities of NMIs to help them demonstrate their analytical competence in this field. Information on key comparisons organized by the CCQM can be found on their database (http://kcdb.bipm.org/). A list of the key comparisons organized in the field of elemental and speciation analysis in food and beverages is shown in Table 12.2.

The National Institute for Standards and Technology (NIST), the Institute for Reference Materials and Measurements (IRMM), the Federal Institute for Materials Research and Testing (BAM), the Laboratory of the Government Chemists (LGC), the National Metrology Institute of Japan (NMIJ) and the National Research Council of Canada (NRCC) can be mentioned as reference material producers with a special focus on food and beverages. Table 12.3 shows a few selected examples of food-related reference materials taken form the web-based catalogues of these NMIs.

As can be observed, the nature of the existing reference materials is very varied and covers a wide range of trace elements both essential and toxic. In the last few years we have seen an increase in the reference materials certified for elemental species (e.g. arsenobetaine, methylmercury, selenomethionine). This modern trend will be covered in the next section.

Table 12.2 Key comparisons organized by the CCQM in the field of elemental and speciation analysis in foods and beverages.

Key comparison reference	Elements and materials	Years
CCQM-K24	Cadmium in rice	2001
CCQM-K30	Lead in wine	2006–2007
CCQM-K30.1	Lead in wine	2011–2012
CCQM-K31	Arsenic in shellfish	2002–2003
CCQM-K43	Organo-mercury in salmon fish	2005
CCQM-K43.1	Arsenic, mercury, selenium and methylmercury in marine fish	2007
CCQM-K45	Tin in tomato paste	2005
CCQM-K49	Toxic and essential elements (Fe, Zn, Se, Cd, Pb, Cr, As) in bovine liver	2006
CCQM-K56	Trace elements (Ca, Fe, Zn and Cu) in whole fat soybean powder	2007
CCQM-K60	Total selenium and selenomethionine in selenized wheat flour	2008
CCQM-K75	Toxic metals (Pt and Ni) in algae	2009
CCQM-K89	Trace and essential elements (As, Ca, Cd, Pb and Zn) in *Herba ecliptae*	2011
CCQM-K97	Arsenobetaine in standard solution and in fish tissue (tuna fish)	2011
CCQM-K108	Determination of arsenic species, total arsenic and cadmium in brown rice flour	2013
CCQM-K124	Trace elements in tap water	2014, planned
CCQM-K126	Iodine, aluminium and other essential or toxic elements in infant formula	2014, planned

Source: http://kcdb.bipm.org/ with search criteria 'Amount of Substance and Inorganics'.

Table 12.3 Examples of certified reference materials in the field of elemental and speciation analysis in food and beverages.

Reference	Matrix/material	Certified elements and elemental species
NIST SRM 1548a	Typical diet	Al, As, Cd, Cs, Cu, Fe, I, Mn, Ni, Pb, Se, Sn, Zn
NIST SRM 1549a	Whole milk powder	Ba, Ca, Mg, Mn, P, K, Se, Na, Sr, Zn
NIST SRM 1566b	Oyster tissue	Ca, Cl, Mg, K, Na, S, Al, As, Cd, Co, Cu, Fe, Pb, Mn, Hg, methylmercury, Ni, Rb, Se, Ag, Th, V, Zn
NIST SRM 1568b	Rice flour	Al, As, Br, Cd, Ca, Cl, Cu, Fe, Mg, Mn, Hg, Mo, P, K, Rb, Se, Na, S, Zn, dimethylarsinic acid, monomethylarsonic acid, inorganic arsenic
NIST SRM 1577c	Bovine liver	Ag, As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, S, Se, Sr, V, Zn
NIST SRM 1849a	Infant/adult nutritional formula	Ca, Cu, Cr, I, Fe, Mg, Mn, Mo, P, K, Se, Na, Zn
NIST SRM 2384	Baking chocolate	Cu, Mg, Mn, P, K, Na, Zn
NIST SRM 2385	Slurried spinach	Ca, Fe, Mg, Mn, P, K, Zn
NIST SRM 3233	Fortified breakfast cereal	Ba, Cd, Ca, Cu, Fe, Mg, Mn, P, K, Na, Sr, Zn
NIST SRM 3234	Soy flour	Ca, Cu, Fe, Mg, Mn, P, K, Zn
ERM-BB184	Bovine muscle	As, Cd, Cu, Fe, Mn, Se, Zn
ERM-BB186	Pig kidney	Cd, Cu, Fe, Mn, Pb, Se, Zn
ERM-BB384	Pork muscle	Ca, Mg, Na, P
ERM-BB422	Fish muscle	As, Cd, Cu, Fe, Hg, I, Mn, Se, Zn
ERM-BC084a	Tomato paste	Cd, Pb, Sn
ERM-BC211	Rice	Total As, dimethylarsinic acid, sum of arsenite and arsenate
ERM-BD150	Skimmed milk powder	Ca, Cd, Cl, Cu, Fe, Hg, I, K, Mg, Mn, Na, P, Pb, Se, Zn
ERM-CA011b	Hard drinking water	Al, Sb, As, Ba, Be, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Mg, Mn, Mo, Ni, K, Se, Na, Sr, V, Zn
ERM-CA022a	Soft drinking water	Al, As, B, Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Se, Zn
ERM-CE464	Tuna fish	Total Hg, methylmercury
NMIJ-CRM-7402–a	Cod fish tissue	Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Hg, Na, Mg, K, Ca, arsenobetaine, methylmercury
NMIJ-CRM-7403–a	Swordfish tissue	Mn, Fe, Cu, Zn, As, Se, Sr, Cd, Hg, Na, Mg, P, K, Ca, arsenobetaine, methylmercury
NMIJ-CRM-7503–a	White rice flour	Mn, Fe, Cu, Zn, As, Cd, arsenite, arsenate, dimethylarsinic acid
NRCC-SELM-1	Yeast	Total Se, selenomethionine, methionine
NRCC-LUTS-1	Lopster hepatopancreas	As, Cd, Ca, Cr, Co, Cu, Fe, Pb, Mg, Mn, Ni, K, Se, Ag, Sr, Zn

12.5 Speciation analysis

The nutritional and toxicological properties of the elements in food and beverages depend not only on their concentration but also on the chemical form in which elements are present in the samples. The study of such chemical forms is known as speciation analysis and its relevance for foodstuffs analysis is now recognized worldwide. Typical examples are methylmercury in fish and inorganic arsenic in rice. Legislation concerning these specific forms of the elements is coming slowly and food testing laboratories need to be prepared for this demanding task. Fortunately, food CRMs for speciation analysis have existed for a few years (see Table 12.3) and NMIs are investing a lot of effort to cope with the increasing demand for these materials.

Speciation analysis is not an easy task. First, the elemental species should be extracted quantitatively from the solid material without transformation (e.g. interconversion reactions) and then should be detected specifically. Among the different approaches used to undertake elemental speciation in food and beverages, hyphenated techniques, where an efficient separation technique (chromatography or electrophoresis) is coupled on-line to an element-specific atomic detector, are preferred. Nowadays ICP-MS has become the main atomic technique used for the specific on-line elemental detection in speciation analysis due to its low detection limits, easy coupling to analytical separation techniques and the ability to perform IDMS calculations. Additionally, the use of IDMS with multiple tracers has allowed the detection and correction of interconversion reactions between the different species of the same element [3], which was the Achilles heel of speciation analysis until very recently.

12.5.1 Separation techniques employed in speciation analysis using ICP-MS

A literature search based on the Atomic Spectrometry Updates (ASU) published in the *Journal of Analytical Atomic Spectrometry* between 2009 and 2013 [19–21] showed that liquid chromatography is the preferred technique for the coupling of a separation technique to ICP-MS when applied to trace element speciation in food and beverages, covering more than 75% of the published applications. The coupling of high-performance liquid chromatography (HPLC) to ICP-MS is very simple as the flow rates used in HPLC are compatible with those used by most of ICP-MS nebulizers. Consequently, the exit of the column can be connected directly to a suitable nebulizer as shown in Figure 12.7.

Unfortunately, plasmas are not very compatible with high amounts of organic solvents such as those required as mobile phases in some types of liquid chromatography. Thus new sample introduction systems, such as those employing low-flow nebulizers or desolvating systems, have been developed to avoid such problems. The most important characteristics of a low-flow nebulizer are near quantitative nebulization efficiency and its ability to introduce in the plasma tolerable amounts of

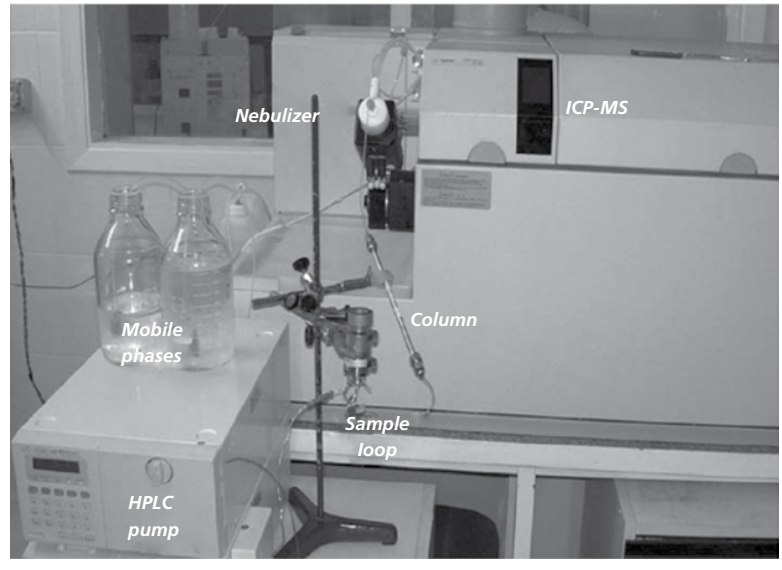


Figure 12.7 Typical HPLC/ICP-MS coupling. Note that the HPLC column is connected directly to the nebulizer to minimize band broadening.

organic solvents. Furthermore, memory effects with this type of nebulizer are much less severe than with conventional pneumatic nebulizers and they are compatible with micro-HPLC columns. HPLC coupled to ICP-MS has been mainly employed for the speciation of arsenic, selenium and chromium and for elements present as metalloproteins in human and formula milk (e.g. Fe, Cu, Zn) [22, 23]. Applications for the speciation of mercury and tin have also been developed using HPLC/ICP-MS but for these two elements gas chromatography (GC) seems to be the preferred technique [22–24].

The coupling of GC with ICP-MS shows better resolution and sensitivity than HPLC/ICP-MS coupling and the gases used in GC are fully compatible with the ICP ion source. However, a heated interface between the exit of the column and the torch is necessary to transport the compounds separated in the GC column to the ICP avoiding their condensation in the transfer line. The coupling of a gas chromatograph to ICP-MS is shown in Figure 12.8.

For speciation analysis using GC/ICP-MS the analytes should be converted to volatile thermally stable compounds and that requires a derivatization reaction. For organotin compounds or organomercury compounds the most used derivatization reaction is ethylation or propylation using aqueous tetraethyl- or tetrapropyl-borate [24].

12.5.2 Elements of interest in food and beverages

Figure 12.9 shows the percentage of papers published in recent years on elemental speciation in food and beverages using ICP-MS as elemental detector [19–21]. As it can be seen, only seven elements (As, Br, Cr, Hg, I, Se and V) were of interest in the last few years. It is worth noting that the publications related to the speciation of Se, As and Hg comprised more than three-quarters of the total, indicating the large interest in these elements in food and beverages. We will discuss briefly the reasons for this interest and the analytical techniques employed.

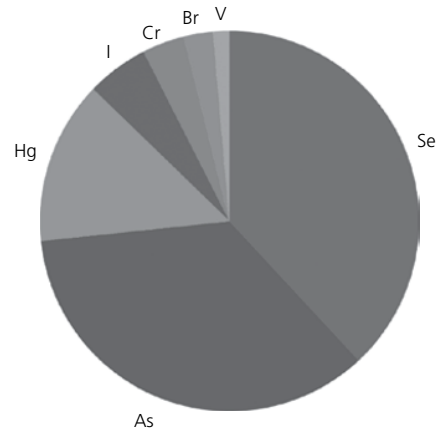


Figure 12.9 Percentage of published articles on metal speciation in food and beverages using ICP-MS in terms of the element monitored. Data taken from the ASU reviews "Clinical and biological materials, foods and beverages" in *Journal of Analytical and Atomic Spectrometry* published between 2009 and 2013.

Arsenic

The speciation of arsenic in food and beverages is mandatory as inorganic forms of arsenic [As(III) and As(V)] and methylated forms such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are highly toxic, with As(III) being more toxic than As(V) and inorganic forms more toxic than methylated forms. The organic forms, such as arsenobetaine, arsenocholine, trimethylarsine oxide (TMAO) and arsenosugars, are not considered to be toxic. The content of total As in seafood products is significantly higher than in any other food but, due to the elevated content (~95%) of non-toxic cationic compounds, mainly arsenobetaine [26], fish consumption does not pose a risk of arsenic poisoning. For many years contaminated drinking water has been designated the main source for human exposure to inorganic As. However, rice, rice-based products and other cereals have been identified as significant dietary sources of inorganic As [27].



Figure 12.8 GC/ICP-MS coupling using a home-made interface [25].

Anion-exchange chromatography coupled to ICP-MS is usually the technique used for arsenic speciation in food and beverages [28]. Gradient reverse-phase chromatography coupled to ICP-MS has been used for analysis of arsenic-containing lipid-soluble compounds in fish oils [29]. The gradient in reverse-phase chromatography usually involves high amounts of organic solvents (such as methanol) which produce changes in response due to the carbon effect. This effect can be avoided by adding an organic solvent via a capillary in the end cap directly to the spray chamber (so that the spray chamber is saturated with vapours of the organic solvent) or adding the organic solvent through a T-piece after the column and before the nebulizer [29].

Mercury

Mercury is one of the most hazardous pollutants in the environment. Mercury released into the air as elemental mercury is deposited on land and water. It is then transformed into the more toxic methylmercury (MeHg) by bacteria. MeHg thus generated can be accumulated through the aquatic food chain and concentrated hundreds or thousands of times, posing a potential risk to humans and wildlife that consume fish. It is well known that MeHg is more toxic than inorganic mercury (iHg). More than 75% of the total mercury content in the edible portion of fish is in the form of MeHg so most of the papers published during the last few years in this field are focused on the determination of MeHg in fish or fish-related products [30]. Apart from fish, mercury can also be accumulated from water and soil into different types of crops that produce grains and vegetables. As cereals, vegetables and fish are consumed by humans, periodic monitoring of these samples for toxic mercury species is of great concern.

For mercury speciation analysis in food and beverages, GC or HPLC coupled to ICP-MS has been extensively used. GC/ICP-MS requires a derivatization step with sodium tetraethylborate [31], sodium tetrapropylborate [32] or sodium tetraphenylborate [33] followed by extraction with an appropriate organic solvent. HPLC/ICP-MS has been performed mainly by reverse-phase chromatography [34]. Although normally not necessary, cold vapour has been sometimes used as an introduction system between HPLC and ICP-MS with the aim of increasing the sensitivity of the method [35].

Selenium

Selenium is an essential micronutrient for humans in a very narrow concentration range. Outside this range deficiency or toxicity occurs. Symptoms of selenium deficiency occur at intakes below 20 μg/day and symptoms of toxicity occur at more than 800 μg/day. The recommended dietary allowance of selenium is 55 mg/day. The toxicity of Se again depends to a large extent on its chemical forms, with inorganic Se compounds being more toxic than organoselenium compounds. The predominant forms of Se in food are selenomethionine (SeMet), the main selenium-containing compound in selenized yeast and cereals, and selenocysteine (SeCys). Both amino acids are found mainly bound in protein chains. Other non-protein organoselenium compounds can be found in certain samples (e.g. selenoneine in fish tissues or selenosugars in urine). Inorganic selenium occurs less frequently and in very low amounts in food. Speciation of selenium in food and beverages has focused mainly on dietary supplements as selenized yeast, vegetable and animal food enriched with this element.

Following indications that selenium supplementation could reduce the incidence of some cancers, selenium-enriched yeast and other materials have been commercialized as dietary supplements. Since dietary supplements are not regulated as pharmaceuticals, there are concerns about the quality, storage conditions, stability and selenium content in selenium supplements. On the other hand, selenium enters the food chain through plants. Accumulation of Se by plants is due to their ability to transform inorganic Se into a variety of organoselenium species, which has important implications for human nutrition and health. Selenium has no known physiological function in plants and is metabolized via biosynthesis of SeCys and SeMet, which are non-specifically incorporated into proteins in place of cysteine and methionine, respectively. Selenium-accumulator plants, which can contain from hundreds to several thousands of micrograms of Se per gram in their tissues, differ from non-accumulator plants in that they biosynthesize primarily non-protein selenoamino acids, such as Se-methyl-selenocysteine (MeSeCys) and γ-glutamyl-Se-methyl-selenocysteine (γ-Glu-MeSeCys), thereby preventing the damaging effects on plant functions resulting from incorporation of SeCys and SeMet in proteins. Wheat is one of the most important dietary sources of Se. The fertilization of food crops with selenium in some countries has increased Se in food chains, with consequent increases in human Se status. Other selenium-enriched vegetables (garlic, broccoli, mushroom, potatoes, radish, etc.) have also been investigated. The amount of selenium in animal tissues, chicken eggs or milk can be increased by using a source of selenium (e.g. Se-rich yeast) as a feed supplement.

Thus, the biofortification of Se in animal foods may be an effective strategy to improve nutritional requirements. However, an understanding of Se species present in foods is essential in understanding their metabolic pathways and potential health benefits. The difficulties in identifying the individual Se-containing proteins stimulate the interest in methods allowing at least the distinction between the fraction of selenium present in the form of SeMet (unspecific replacement of S by Se during supplementation), SeCys (genetically encoded incorporation of selenium into the true selenoproteins) and non-covalently bound inorganic selenium.

Most of the analytical methods developed started with an extraction procedure (frequently including an enzymatic digestion of the proteins to obtain the amino acids), separation of the extracted species using one or more separation techniques, detection of the selenium species by on-line coupling of a separation technique with an ICP-MS instrument and identification, in a parallel experiment, of these seleniun compounds by organic MS [36].

Separation of the selenium compounds has been performed by reverse-phase [37], reverse-phase ion pairing [38], anion-exchange [39], cation-exchange [40] or size-exclusion [41] chromatography coupled to ICP-MS, although the latter is usually used for purification of the extracts before applying a second chromatographic technique with analytical purposes [42]. Frequently, speciation analysis has been performed by combining the results obtained using two or more of these types of chromatography [43]. Sometimes, separation of the selenium compounds involves the sequential combination of more than one type of separation method. Thus, capillary or nano-HPLC/ICP-MS has been used as a technique for the mapping of selenopeptides extracted with trypsin from one-dimensional gel bands or two-dimensional gel spots [44]. Another example is the collection of fractions by size-exclusion chromatography and the subsequent individual injection of these fractions in a second chromatographic step (e.g. reverse-phase or anion-exchange chromatography) [45], monitoring in both cases the element by ICP-MS.

The determination of selenium by ICP-MS has been hampered by serious spectroscopic interferences. Major isotopes of selenium (^{80}Se, ^{78}Se and ^{76}Se) are all subject to severe Ar_2^+ interferences as shown in Table 12.1. Other Se isotopes of lower abundance (^{82}Se, ^{77}Se and ^{74}Se) are also subject to molecular interferences from halogens (BrH^+, ClO^+) which can be present in the analysed samples. The most employed solution to eliminate such interferences is the use of collision/reaction cells with H_2 [46] or methane [47].

12.5.3 Extraction procedures

In general, the extraction procedures used for speciation analysis with ICP-MS as elemental detector does not differ significantly from those using other detectors. Extraction of the compounds can be done by heating in the presence of different reagents such as HNO_3, HNO_3/methanol, methanol/water, water, trifluoroacetic acid, tetramethylammonium hydroxide and methanesulfonic acid. These extractions used to be microwave or ultrasound assisted. Accelerated solvent extraction has also been used. Sometimes, the cooking process is simulated to study the release of metallic compounds from the food [48].

On the other hand, enzymatic hydrolysis is an appealing methodology because enzymes break down specific bonds of the substrate (biomolecule hydrolysis, mainly of proteins) and allow selective analyte release from the sample matrix without chemical species changes. Most of the enzymes used (Pronase E, pepsin, pancreatin or trypsin) are mainly proteolytic (proteases), and they attack the peptide bonds of proteins and peptides. Other enzymes, such as lipases, are able to hydrolyse fats into long-chain fatty acids and glycerol, while amylases hydrolyse starch and glycogen to maltose and to residual polysaccharides. The enzyme must be carefully selected to suit the nature of the sample (high protein, fat or carbohydrate content). In some cases, a mixture of different enzymes can simultaneously be used but this approach is not always possible because the optimum pH for a certain enzyme cannot fix the optimum working pH of the other enzyme. Although the enzyme extraction may be done in a shaking water bath at 37 ± 2°C overnight [49], several attempts based on the use of ultrasound energy [50], and more recently microwave energy and pressurization [51], have been carried out to speed up (from hours to minutes) the enzymatic hydrolysis process.

The integrity of the species must be maintained during sample extraction, preparation and storage. Mercury species transformation might occur during sample preparation. In order to avoid this problem, alternative extraction procedures have been suggested with reagents containing thiol ligands, such as mercaptoethanol or L-cysteine, EDTA/mercaptoethanol/methanol, KOH/methanol or HCl/methanol. In a recent publication

[52], an ultrasound-assisted homogenization/extraction was carried out in the presence of perchloric acid, L-cysteine and toluene/methanol (1 : 1). Perchloric acid was used for protein denaturation and precipitation, toluene helped to destroy lipid structures potentially sequestering MeHg and L-cysteine was used to form water-soluble complexes with iHg and MeHg.

In the case of selenium, the determination of SeCys is problematic due to the poor stability of this amino acid [53]. Water containing dithiothreitol (antioxidant) and phenylmethylsulfonylfluoride (inhibitor of protease activity) [44] has been used for selenium compound extraction in yeast. Proteins of edible animal tissues, chicken eggs or cows' milk have been extracted with urea under reducing conditions (dithiothreitol) to break the Se–Se, S–S and Se–S bonds, with subsequent derivatization of SeCys and SeMet by carbamidomethylation with iodoacetamide to alkylate cysteine and SeCys residues followed by quantitative proteolysis with protease XIV and lipase [54].

12.5.4 Bioavalability studies

Bioavailability is a term used to describe the proportion of a nutrient in food that can be utilized for normal body function. Additionally, the term 'bioaccessibility' indicates the maximum fraction of a trace element, or other substances, in food that is theoretically released from the matrix in the gastrointestinal tract and thus available for intestinal absorption (i.e. enters the bloodstream). In general, bioavailability and bioaccessibility are affected by the type and/or composition of food, the different cooking procedures of the foodstuff and also the gastrointestinal conditions. Although the use of proteolytic digestion provides information about speciation in the original sample, more detailed information on the identity and content of the different species released after gastrointestinal digestion is needed in an attempt to evaluate bioaccessibility of an element in the different foods and beverages.

Determination of the bioavailability of trace elements in foodstuffs is mainly carried out by *in vitro* methods, although some *in vivo* methods can be found in the literature [46]. Most *in vitro* methods involve two different steps: a gastric digestion stage simulating the stomach, and a subsequent intestinal digestion stage simulating the digestion in the intestine and absorption of nutrients. *In vitro* methods use conditions similar to those found in the human body during digestion. These procedures can measure the bioavailable fraction through two different approaches. First, the *in vitro* method can assess the maximum concentration of soluble substances in the simulated gastrointestinal medium (simulated extract isolated from the treated foodstuff after a filtration/centrifugation step). In this case, the *in vitro* procedures determine the bioaccessible fraction [55]. Second, some *in vitro* methods use a semi-permeable membrane with a specified pore size (molecular weight cut-off) during the intestinal digestion step to simulate the nutrient absorption mechanism. In this case, the nutrient is assessed in the liquid medium inside the membrane (dialysate fraction), and the *in vitro* methods commonly determine the dialysable fraction of the nutrient [56]. In both cases, the combination of a separation technique with ICP-MS allows monitoring of the bioavailable/bioaccessible species in foodstuffs [55, 56].

12.5.5 Isotope dilution mass spectrometry

IDMS can be performed in speciation analysis by ICP-MS using two different strategies: the species-unspecific spiking mode and the species-specific spiking mode [22]. In the species-unspecific spiking mode, addition of the isotope tracer or spike is carried out post-column after separation of the species in the sample has taken place. Figure 12.10 shows a schematic diagram of the instrumental set-up. The continuous addition of the spike solution containing the enriched isotopes is performed by a peristaltic pump in such a way that it is completely and continuously mixed through a T-piece with the effluent of the column containing the species to be determined. In brief, the basis of this on-line isotope dilution technique relies on the plotting of the mass flow versus retention time. The mass flow is calculated by measuring the corresponding isotope ratio throughout the whole chromatographic run. Then, in order to obtain the concentration of the different species in the sample, the various peaks detected have to be integrated and normalized to the injection volume.

The spike here may exist in a chemical form different from that of the species to be determined, since the only preconditions to be fulfilled are the absence of substance loss before spiking and the complete mixing between the analyte separated species and the spike prior to the ionization process, in order to accurately measure the corresponding isotope ratio. This mode is especially useful either when the structure and composition of the sought species are not exactly known or

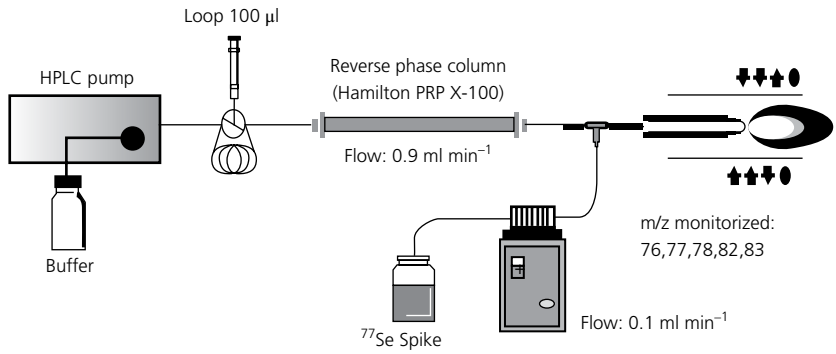


Figure 12.10 Scheme of HPLC/ICP-MS coupling for post-column isotope dilution analysis of selenocompounds in wheat and yeast [57].

when the corresponding isotopically labelled compounds are not commercially available or cannot be synthesized. This mode of spiking does not offer the full range of advantages associated with IDMS [3]. Any loss of sample prior to the chromatographic separation will not be corrected for. However, it is the only alternative for quantification when a standard for the analyte species is not available. Also, this mode of spiking can only be applied when the ionization efficiency of the element is independent of the chemical form in which the element is presented to the ion source. Hence, only ICP-MS has been used until now for post-column isotope dilution as the ionization efficiencies in this ion source can be considered independent of the chemical form of the element. This strategy has been used in the speciation of selenium in wheat and yeast [46, 57] using ^{76}Se or ^{77}Se as enriched spike.

Conversely, the species-specific spiking mode requires the use of a spike solution containing the species to be analysed in an isotopically labelled form. Therefore, in contrast to the species-unspecific mode, the composition and structure of the species of interest must be exactly known in order to either synthesize the corresponding enriched species or acquire the proper spike solution if it is commercially available. In this mode, the spike solution is added to the sample at the beginning of the analytical procedure, and once complete mixing between the added enriched species and the naturally occurring ones in the sample is achieved, all traditional advantages of isotope dilution analysis can be fully exploited. Using this strategy MeHg has been determined in fish using a commercial available ^{201}Hg or ^{202}Hg-enriched monomethylmercury [31, 49] and SeMet has been determined in wheat and yeast [58, 59] using ^{77}SeMet obtained from yeast cultivated in a medium enriched with ^{77}Se. Furthermore, when several species of the same element need to be analysed, each compound can be enriched in a different isotope of the element opening the way to new forms of quantitation and correction of species interconversion reactions as indicated previously. Thus, ^{199}iHg and ^{201}MeHg have been used to correct for species interconversion in the speciation of mercury in fish [32]. For more information on the use of IDMS in speciation analysis, please consult the corresponding references [3, 22, 23].

12.6 Provenance studies

Nowadays, there is increasing concern worldwide about food quality and authenticity. Hence, the aim of international legislation is to ensure the safety and quality of products and to protect them against fraud and imitation. Food authenticity, which includes geographic and production origins, can influence the purchasing decision of consumers in developed countries. Indeed, increasing numbers of consumers are willing to pay more for food with a guaranteed geographic origin. The development of analytical methods to accurately determine the geographic origin of food is thus essential not only for research purposes, but also for the protection of national health. At the European level, food products could have three possible geographical indications:

1 PDO (protected designation of origin) denoting agricultural products that are produced, processed and prepared in a given geographical area using recognized know-how;

2 PGI (protected geographical indication), denoting agricultural products and foodstuffs closely linked to a geographical area since at least one production, processing or preparation was carried out in the area;
3 TSG (traditional specialities guaranteed), which highlights traditional character in composition or means of production [60].

During the last few years, a large number of instrumental techniques have been recommended to assess the authenticity of food and beverages including HPLC, GC, infrared spectroscopy, nuclear magnetic resonance and DNA technologies. All these techniques are based on the determination of different organic components of food and/or beverages. However, as organic components are influenced by environmental, agricultural and meteorological conditions, their use to determine a product origin is not always reliable. As an alternative, determination of element content has been postulated [60]. Elements are more stable and mineral contents are less variable than organic compounds in food and beverages. It is well known that elemental soil composition could be transferred to growing vegetation, and multi-element composition of animal tissues could reflect the vegetation that the animals eat. Therefore, multi-element analysis in combination with isotopic fingerprints (e.g. strontium isotope ratios) and further statistical analysis of the data has been shown to be a suitable approach for determining the geographical origin and authenticity of various foods and beverages [60].

For example, we have evaluated both multi-element analysis and Sr isotope abundance ratios in combination with multivariate statistical analysis to study the geographical origin of ciders [61]. The content of Na, Mg, Al, K, Ca, Ti, V, Mn, Zn, As, Rb, Sr, Mo, Ba together with the $^{87}Sr/^{86}Sr$ isotope abundance ratio were found to be the best variables for discriminating the origin of the ciders. Linear discriminant analysis was able to classify correctly 100% of cider samples coming from Spain, France, England and Switzerland [61].

Abbreviations

AAS: Atomic absorption spectrometry
AES: Atomic emission spectrometry
ASU: Atomic Spectrometry Updates
BAM: Federal Institute for Materials Research and Testing
CCQM: Comité Consultatif pour la Quantité de Matière
CIPM: Comité International des Poids et Mesures
CRM: Certified Reference Material
DMA: Dimethylarsinic acid
FDA: Food and Drug Administration
GC: Gas chromatography
γ-Glu-MeSeCys: γ-Glutamyl-Se-methyl-selenocysteine
HPLC: High performance liquid chromatography
ICP: Inductively coupled plasma
IDMS: Isotope dilution mass spectrometry
iHg: Inorganic mercury
IRMM: Institute for Reference Materials and Measurements
LGC: Laboratory of the Government Chemists
MeHg: Methylmercury
MeSeCys: Se-methyl-selenocysteine
MMA: Monomethylarsonic acid
MS: Mass spectrometry
NIST: National Institute for Standards and Technology
NMI: National Metrology Institute
NMIJ: National Metrology Institute of Japan
NRCC: National Research Council of Canada
PDO: Protected designation of origin
PFA: Perfluoroalkoxy
PGI: Protected geographical indication
PTFE: Polytetrafluoroethylene
RF: Radiofrequency
SeCys: Selenocysteine
SeMet: Selenomethionine
TDS: Total diet studies
TMAO: Trimethylarsine oxide
TSG: Traditional specialities guaranteed

References

1 Houk RS, Fassel VA, Flesch GD, Svec HJ, Gray AL, Taylor CE. Inductively coupled argon plasma as an ion source for mass spectrometric determination of trace elements. *Anal Chem* 1980;**52**:2283–2289.
2 Berglund M, Wieser ME. Isotopic composition of the elements 2009. *Pure Appl Chem* 2011;**83**:397–410.
3 Garcia Alonso JI, Rodríguez-González P. *Isotope Dilution Mass Spectrometry*. Royal Society of Chemistry, Cambridge, 2013.
4 Montes Bayón M, Garcia Alonso JI, Sanz-Medel A. Semiquantitative elemental analysis of water samples using double focusing ICP-MS. *J Anal Atom Spectrom* 1998;**13**:1027–1032.
5 Mann J, Truswell AS. *Essentials of Human Nutrition*, 4th edn. Oxford University Press, New York, 2012.

6 Sariego Muñiz C, Fernández-Martin JL, Marchante-Gayón JM, García Alonso JA, Cannata-Andía JB, Sanz-Medel A. Reference values for trace and ultratrace elements in human serum determined by double-focusing ICP-MS. *Biol Trace Elem Res* 2001;**82**:259–272.

7 European Commission. Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Available at https://www.fsai.ie/uploadedFiles/Consol_Reg1881_2006.pdf

8 US Food and Drug Administration. Supporting document for recommended maximum level for lead in candy likely to be consumed frequently by small children. Available at http://www.fda.gov/Food/FoodborneIllnessContaminants/Metals/ucm172050.htm

9 Government of Canada. Food and Drug Regulations. CRC, c. 870 (Section B.16.100). Available at http://laws-lois.justice.gc.ca/eng/regulations/C.R.C.,_c._870/FullText.html

10 Mejia J, Mounicou, S, Caruso JA. Plasma spectrometry for elemental speciation and characterization of beverages. *J AOAC Int* 2004;**87**:205–224.

11 Mahmood N, Petraco N, He Y. Elemental fingerprint profile of beer samples constructed using 14 elements determined by inductively coupled plasma–mass spectrometry (ICP-MS): multivariation analysis and potential application to forensic sample comparison. *Anal Bioanal Chem* 2012;**402**:861–869.

12 Tormen L, Torres DP, Dittert IM, Araújo RGO, Frescura VLA, Curtius AJ. Rapid assessment of metal contamination in commercial fruit juices by inductively coupled mass spectrometry after a simple dilution. *J Food Compos Anal* 2011;**24**:95–102.

13 Carbonell-Barrachina AA, Ramírez-Gandolfo A, Wu X *et al*. Essential and toxic elements in infant foods from Spain, UK, China and USA. *J Environ Monit* 2012;**14**:2447–2455.

14 Zand N, Chowdhry BZ, Wray DS, Pullen FS, Snowden MJ. Elemental content of commercial 'ready to-feed' poultry and fish based infant foods in the UK. *Food Chem* 2012;**135**:2796–2801.

15 Llorent-Martínez EJ, Fernández de Córdova ML, Ruiz-Medina A, Ortega-Barrales P. Analysis of 20 trace and minor elements in soy and dairy yogurts by ICP-MS. *Microchem J* 2012;**102**:23–27.

16 Benincasa C, Lewis J, Sindona G, Tagarelli A. The use of multi element profiling to differentiate between cowand buffalo milk. *Food Chem* 2008;**110**:257–262.

17 Millour S, Noël L, Chekri R *et al*. Strontium, silver, tin, iron, tellurium, gallium, germanium, barium and vanadium levels in foodstuffs from the Second French Total Diet Study. *J Food Compos Anal* 2012;**25**:108–129.

18 Emons H, Held A, Ulberth F. Reference materials as crucial tools for quality assurance and control in food analysis. *Pure Appl Chem* 2006;**78**:135–143.

19 Taylor A, Branch S, Day MP, Patriarca M, Whited M. Atomic Spectrometry Update. Clinical and biological materials, foods and beverages. *J Anal Atom Spectrom* 2009;**24**:535–579.

20 Taylor A, Branch S, Day MP, Patriarca M, Whited M. Atomic Spectrometry Update. Clinical and biological materials, foods and beverages. *J Anal Atom Spectrom* 2011;**26**:653–692.

21 Taylor A, Day MP, Hill S, Marshall J, Patriarca M, Whited M. Atomic Spectrometry Update. Clinical and biological materials, foods and beverages. *J Anal Atom Spectrom* 2013;**28**:425–459.

22 Rodríguez-González P, Marchante Gayón JM, García Alonso JI, Sanz-Medel A. Isotope dilution analysis for elemental speciation: a tutorial review. *Spectrochim Acta B* 2005;**60**:151–207.

23 Rodríguez-González P, García Alonso JI. Recent advances in isotope dilution analysis for elemental speciation. *J Anal Atom Spectrom* 2010;**25**:239–259.

24 García Alonso JI, Ruiz Encinar J. Separation techniques: gas chromatography and other gas based methods. In: Cornelis R (ed.) *Handbook of Elemental Speciation*. John Willey & Sons Ltd, Chichester, 2003, pp. 163–200.

25 Montes Bayón M, Gutiérrez Camblor M, García Alonso JI, Sanz Medel A. An alternative GC-ICP-MS interface design for trace element speciation. *J Anal Atom Spectrom* 1999;**14**:1317–1322.

26 Nam SH, Oh HJ, Min HS, Lee JH. A study on the extraction and quantitation of total arsenic and arsenic species in seafood by HPLC–ICP-MS. *Microchem J* 2010;**95**:20–24.

27 Carbonell-Barrachina AA, Wu CC, Ramirez-Gandolfo A *et al*. Inorganic arsenic contents in rice-based infant foods from Spain, UK, China and USA. *Environ Pollut* 2012;**163**:77–83.

28 Raber G, Stock N, Hanel P, Murko M, Navratilova J, Francesconi KA. An improved HPLC–ICPMS method for determining inorganic arsenic in food: application to rice, wheat and tuna fish. *Food Chem* 2012;**134**:524–532.

29 Ruiz-Chancho MJ, Taleshi MS, Goessler W, Francesconi KA. A method for screening arsenolipids in fish oils by HPLC-ICPMS. *J Anal Atom Spectrom* 2012;**27**:501–504.

30 Batista BL, Rodrigues JL, de Souza SS, Souza VCO, Barbosa F. Mercury speciation in seafood samples by LC–ICP-MS with a rapid ultrasound-assisted extraction procedure: application to the determination of mercury in Brazilian seafood samples. *Food Chem* 2010;**126**:2000–2004.

31 Bravo AG, Loizeau JL, Bouchet S *et al*. Mercury human exposure through fish consumption in a reservoir contaminated by a chlor-alkali plant: Babeni reservoir (Romania). *Environ Sci Pollut Res* 2010;**17**:1422–1432.

32 Castillo A, Rodríguez-González P, Centineo G, Roig-Navarro AF, García Alonso JI. Multiple spiking species-specific isotope dilution analysis by molecular mass spectrometry: simultaneous determination of inorganic mercury and methylmercury in fish tissues. *Anal Chem* 2010;**82**:2773–2783.

33 Dressler VL, Santos CMM, Antes FG, Bentlin FRS, Pozebon D, Flores EMM. Total mercury, inorganic mercury and methyl mercury determination in red wine. *Food Anal Method* 2012;**5**:505–511.

34 Chang LF, Jiang SJ, Sahayam AC. Speciation analysis of mercury and lead in fish samples using liquid chromatography–inductively coupled plasma mass spectrometry. *J Chromatogr A* 2007;**1176**:143–148.

35 Lin LY, Chang LF, Jiang SJ. Speciation analysis of mercury in cereals by liquid chromatography chemical vapor generation inductively coupled plasma-mass spectrometry. *J Agric Food Chem* 2008;**56**:6868–6872.

36 Lipiec E, Siara G, Bierla K, Ouerdane L, Szpunar J. Determination of selenomethionine, selenocysteine, and inorganic selenium in eggs by HPLC–inductively coupled plasma mass spectrometry. *Anal Bioanal Chem* 2010;**397**: 731–741.

37 Infante HG, Borrego AA, Peachey E *et al*. Study of the effect of sample preparation and cooking on the selenium speciation of selenized potatoes by HPLC with ICP-MS and electrospray ionization MS/MS. *J Agric Food Chem* 2009;**57**:38–45.

38 Bierla K, Szpunar J, Lobinski R. Specific determination of selenoaminoacids in whole milk by 2D size-exclusion-ionparing reversed phase high-performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC–ICP MS). *Anal Chim Acta* 2008;**624**:195–202.

39 Cuderman P, Kreft I, Germ M, Kovacevic M, Stibilj V. Selenium species in selenium-enriched and drought-exposed potatoes. *J Agric Food Chem* 2008;**56**:9114–9120.

40 Cubadda F, Aureli F, Ciardullo S *et al*. Changes in selenium speciation associated with increasing tissue concentrations of selenium in wheat grain. *J Agric Food Chem* 2010;**58**: 2295–2301.

41 Yamashita Y, Amlund H, Suzuki T *et al*. Selenoneine, total selenium, and total mercury content in the muscle of fishes. *Fish Sci* 2011;**77**:679–686.

42 Bierla K, Dernovics M, Vacchina V, Szpunar J, Bertin G, Lobinski R. Determination of selenocysteine and selenomethionine in edible animal tissues by 2D size-exclusion reversed-phase HPLC-ICP MS following carbamidomethylation and proteolytic extraction. *Anal Bioanal Chem* 2008;**390**:1789–1798.

43 Alzate A, Perez-Conde MC, Gutierrez AM, Camara C. Selenium-enriched fermented milk: a suitable dairy product to improve selenium intake in humans. *Int Dairy J* 2010;**20**: 761–769.

44 Tastet L, Schaumloffel D, Bouyssiere B, Lobinski R. Identification of selenium-containing proteins in selenium-rich yeast aqueous extract by 2D gel electrophoresis, nanoHPLC–ICP MS and nanoHPLC–ESI MS/MS. *Talanta* 2008;**75**:1140–1145.

45 Chan QL, Caruso JA. A metallomics approach discovers selenium-containing proteins in selenium-enriched soybean. *Anal Bioanal Chem* 2012;**403**:1311–1321.

46 Kirby JK, Lyons GH, Karkkainen MP. Selenium speciation and bioavailability in biofortified products using species-unspecific isotope dilution and reverse phase ion pairing–inductively coupled plasma–mass spectrometry. *J Agric Food Chem* 2008;**56**:1772–1779.

47 Amoako PO, Uden PC, Tyson JF. Speciation of selenium dietary supplements: formation of S-(methylseleno)cysteine and other selenium compounds. *Anal Chim Acta* 2009;**652**: 315–323.

48 Mihucz VG, Tatar E, Virag I, Zang C, Jao Y, Zaray G. Arsenic removal from rice by washing and cooking with water. *Food Chem* 2007;**105**:1718–1725.

49 Chung SWC, Chan BTP. A reliable method to determine methylmercury and ethylmercury simultaneously in foods by gas chromatography with inductively coupled plasma mass spectrometry after enzymatic and acid digestion. *J Chromatogr A* 2010;**1218**:1260–1265.

50 Dufailly V, Nicolas M, Richoz-Payot J, Poitevin E. Validation of a method for arsenic speciation in food by ion chromatography-inductively coupled plasma/mass spectrometry after ultrasonic-assisted enzymatic extraction. *J AOAC Int* 2011;**94**:947–958.

51 Moreda-Piñeiro J, Alonso-Rodríguez E, Moreda-Piñeiro A *et al*. Simultaneous pressurized enzymatic hydrolysis extraction and clean up for arsenic speciation in seafood samples before high performance liquid chromatography–inductively coupled plasma-mass spectrometry determination. *Anal Chim Acta* 2010;**679**:63–73.

52 Santoyo MM, Figueroa JAL, Wrobel K, Wrobel K. Analytical speciation of mercury in fish tissues by reversed phase liquid chromatography–inductively coupled plasma mass spectrometry with Bi3+ as internal standard. *Talanta* 2009;**79**: 706–711.

53 Dernovics M, Lobinski R. Characterization of the selenocysteine-containing metabolome in selenium-rich yeast. Part II. On the reliability of the quantitative determination of selenocysteine. *J Anal Atom Spectrom* 2008;**23**:744–751.

54 Juniper DT, Phipps RH, Bertin G. Effect of dietary supplementation with selenium-enriched yeast or sodium selenite on selenium tissue distribution and meat quality in commercial-line turkeys. *Animal* 2011;**5**:1751–1760.

55 Hinojosa Reyes L, Ruiz Encinar J, Marchante-Gayón JM, García Alonso JI, Sanz-Medel A. Selenium bioaccessibility assessment in selenized yeast after '*in vitro*' gastrointestinal digestion using two-dimensional chromatography and mass spectrometry. *J Chromatogr A* 2006;**1110**:108–116.

56 Moreda-Piñeiro J, Alonso-Rodríguez E, Romaris-Hortas V *et al*. Assessment of the bioavailability of toxic and non-toxic arsenic species in seafood samples. *Food Chem* 2012;**130**:552–560.

57 Díaz Huerta V, Hinojosa Reyes L, Marchante-Gayón JM, Fernández Sánchez ML, Sanz-Medel A. Total determination and quantitative speciation analysis of selenium in yeast and wheat flour by isotope dilution analysis ICP-MS. *J Anal Atom Spectrom* 2003;**18**:1243–1247.

58 Hinojosa Reyes L, Moreno Sanz F, Herrero Espílez P, Marchante-Gayón JM, García Alonso JI, Sanz-Medel A. Biosynthesis of isotopically enriched selenomethionine: application to its accurate determination in selenium-enriched yeast by isotope dilution analysis-HPLC-ICP-MS. *J Anal Atom Spectrom* 2004;**19**:1230–1235.

59 Hinojosa Reyes L, Marchante-Gayón JM, García Alonso JI, Sanz-Medel A. Application of isotope dilution analysis for the evaluation of extraction conditions in the determination of total selenium and selenomethionine in yeast-based nutritional supplements. *J Agric Food Chem* 2006;**54**: 1557–1563.
60 Drivelos SA, Georgiou CA. Multi-element and multi-isotope-ratio analysis to determine the geographical origin of foods in the European Union. *Trends Anal Chem* 2012;**40**:38–51.
61 García-Ruiz S, Moldovan M, Fortunato G, Wunderli S, García Alonso JI. Evaluation of strontium isotope abundance ratios in combination with multi-elemental analysis as a possible tool to study the geographical origin of ciders. *Anal Chim Acta* 2007;**590**:55–66.

CHAPTER 13

Electroanalytical methods: application of electrochemical techniques for mineral elements analysis in food

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Abstract: The role of modern electrochemical methods as efficient analytical tools for the determination of mineral elements in food is addressed in this chapter. Ion-selective electrodes using new materials for the analysis of alkaline earth and alkaline ions, divalent metal ions other than alkaline earth ions, trivalent metal ions and anions can be used for this purpose. Moreover, sensitive voltammetric techniques involving various types of electrodes and methods based on stripping analysis, as well as amperometric sensors for continuous detection, are also more and more frequently applied for these types of analyses. Some selected examples illustrate the latest electroanalytical trends for the determination of mineral elements in food, with particular emphasis on the strategies developed to improve the analytical characteristics of the corresponding methodologies. The future trends in this area are also addressed at the end of the chapter.

Keywords: electroanalysis, mineral elements, food, ion-selective electrodes, stripping analysis, continuous monitoring

13.1 Introduction

Electrochemical techniques are used to a great extent in food analysis because of their important advantages, such as (i) the electrical nature of the electrochemical signal allowing direct transduction of the reaction rate in the readout; (ii) the small volumes, even of some few nanolitres, required to perform the electrochemical measurements due to the interfacial nature of such measurements; (iii) the detection limits that can be achieved which are usually adequate for the detection of numerous analytes of interest; and (iv) the relative simplicity and low cost of the electrochemical instrumentation that allows easy availability of these devices. The application of electrochemical techniques to the determination of mineral elements in food dates from early potentiometric and polarographic analysis [1, 2]. Currently, ion-selective electrodes based on new materials, more sensitive voltammetric techniques using various types of electrodes and methods based on stripping analysis, as well as amperometric sensors for continuous detection are more and more frequently applied for these determinations. The exciting role of electrochemical methods as efficient analytical tools in this field is addressed in this chapter. Some examples showing the latest trends are presented and commented, and the efforts made to improve the analytical characteristics are especially addressed.

13.2 Ion-selective electrodes

Potentiometric methods employing ion-selective electrodes (ISEs) are widely applied to the determination of mineral elements in food because of the ease of preparation and the simplicity of the involved procedures, relatively fast responses, reasonable selectivity, wide linear dynamic range, long lifetimes and acceptable mechanical stability, as well as the simple instrumentation, low cost and suitability for continuous monitoring. They provide readouts in the form of potential measurements

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associated with the ion of interest, also allowing discrimination between free (ionized) and bonded (complexed) ions in the sample, as well as between the activities of the different oxidation states of a specific ion. In general terms, the development of ISEs is nowadays a mature discipline that is mainly focused on reaching detection limits an order of magnitude lower than previously thought possible and making these devices more robust. A great variety of ISEs exists which can be used for the determination of both high concentrations or trace contents of all mineral elements of interest in food. The following examples summarize some applications of interest.

13.2.1 Alkaline earth and alkaline ions

The determination of calcium in milk is of great significance. Calcium ions play crucial roles in the physicochemical properties of casein micelles [3], as free Ca^{2+} can interact with the negatively charged casein, leading to variations in the repelling forces among them. Furthermore, calcium forms linkages between the protein molecules either as colloidal calcium phosphate or by direct binding to casein. Free calcium activity in milk can be measured by using a Ca^{2+} ISE [4]. This was used, for instance, to evaluate the effect of microwave energy on the mineral balance and on changes in ionic calcium content after microwave treatment [5]. However, the main problem is the existence of matrix effects that can affect the determinations. Consequently, proper standards for calibration are necessary in order to equilibrate the ionic compositional differences between milk samples and conventional calcium solutions [6]. Various strategies were followed to overcome this inconvenience. For example, calcium solutions containing imidazole have been used to calibrate the Ca-ISE [7] and, more recently, significant quantities of sodium, magnesium, phosphate and citrate have been added to stock calcium ion solutions for the preparation of calibration standards [6].

Different Ca^{2+}-ISE configurations have been used. For example, poly(vinyl chloride) (PVC) membrane sensors using bilirubin (1,3,6,7-tetramethyl-4,5-dicarboxyethyl-2,8-divinyl-[b-13]-dihydrobilenone) as a neutral carrier in the presence of various plasticizers employed as solvent mediators to incorporate the carrier into the membranes have been reported [8]. A calibration graph with a linear Nernstian slope of 29.5 ± 0.8 mV between 5×10^{-7} and 10^{-2} M and a detection limit of 3×10^{-7} M (12 ppb) were achieved. The sensor was applied to the analysis of baby foods, among other samples. More recently, a solid-state electrode of reduced size using carbon nanotubes as the transducing layer was reported for the direct determination of Ca^{2+} in sap of plants. This configuration overcomes problems encountered with commercial ISEs in the analysis of such complex samples and shows a range of linearity from 10^{-5} to $10^{-2.5}$ M with a limit of detection of $10^{-6.2}$ M, thus making it an excellent tool for determining Ca^{2+} in a wide range of plant species [9]. Ca^{2+} and Mg^{2+} together with alkaline ions were also determined in water samples using a potentiometric sensor array consisting of a series of ISEs prepared by combination of different ionophores in the same PVC membrane. Multivariate calibration methods were used to provide reliable models for the sensor array data in the analysis of water samples [10].

The specific determination of magnesium ion in food using ISEs constitutes a major challenge. Mg(II) is one of the most abundant elements in the human body. A variety of enzyme activities, cell functions and electrolyte levels are directly or indirectly dependent on magnesium. Moreover, magnesium ion is present in a number of dietary products such as meat, fish, seafood and dairy products. Mineral water also contains magnesium in fairly high quantities. Although ISEs are ideally suitable for the determination of Mg(II), some problems such as a narrow working concentration range, long response time and interference from other alkaline earth metal ions, especially calcium, arise [11]. In a recent publication, a PVC membrane containing 5,10,15,20-tetrakis(2-furyl)-21,23-dithiaporphyrin as the electroactive material was proposed to fabricate Mg(II)-selective sensor. Porphyrins have intense and selective binding affinities towards different metal ions. This is because such materials possess electron-rich interior cavities and the ability to complex through dipole–dipole interactions with metal ions of compatible dimensions. The developed device exhibited a wide working concentration range of 9.2×10^{-6} to 1.0×10^{-1} M, with a Nernstian slope of 30.0 ± 1.0 mV/decade and a response time of 15 s. The limit of detection was of 8.0×10^{-6} M. The sensor showed a lifetime of about 8 weeks and exhibited excellent selectivity over a number of monovalent, bivalent and trivalent cations including alkaline, alkaline earth metal, heavy and transition metal ions. It was successfully applied to the analysis of soft drinks and baby foods [11].

Regarding alkaline ions, although a variety of selective electrodes have been described in the literature based mainly on the use of crown ethers and related macrocyclic hosts for the determination of Na^+ [12] or valinomcyn in the case of potassium, recent applications to the analysis of these ions in food are very scarce. Some examples in which these sensors are combined with flow techniques are discussed in section 13.5.1.

13.2.2 Divalent metal ions other than alkaline earth ions

ISEs have been widely applied to the determination of divalent metal ions such as copper, cobalt, zinc and manganese, which are present in foods at low concentrations. Copper is an essential element in the human body and plays an important role in various biological processes. Copper deficiency results in anaemia while its accumulation results in Wilson's disease [13]. A variety of ISEs for Cu^{2+} with application in food analysis has been reported in the literature. For example, a very recent paper describes a copper ISE based on the simple incorporation of a synthesized ionophore in a carbon paste matrix. The incorporation of nanomaterials such as carbon nanotubes or ZnS or gold nanoparticles (AuNPs) loaded on activated carbon (AC) provided a significant enhancement of the response properties. The highest improvements were obtained in the presence of AuNPs–AC. A linear range from 6×10^{-8} to 10^{-1} M Cu^{2+} was achieved. This remarkably wide interval is probably due to the strong coordination of the metal ion with the ionophore in the presence of the nanomaterial. As an interesting application, the electrode was used to determine Cu^{2+} in cinnamon and ginger species [14].

The preparation of miniaturized ISEs is a more and more relevant line of research. An interesting strategy for reducing the size of conventional ISEs is the construction of coated wire ion-selective electrodes, which imply the incorporation of electroactive species into a thin polymeric film coated directly on a metallic or graphite conductor. For example, a coated platinum Cu^{2+} ISE using 2-((2-(2-(2-(2-hydroxy-5-methoxybenzylidene amino)phenyl)disufanyl)phenylimino)methyl)-4-methoxyphenol Schiff base was developed. Schiff bases are characterized by their ability to form metal chelate complexes with great stability and biological activity. The developed electrode showed a wide linear range of concentrations (1.2×10^{-7} to 1.0×10^{-1} M) and a low detection limit of 9.8×10^{-8} M. Furthermore, a short response time of 9 s and long stability of 3.5 months were found. Good results were also obtained by application of the electrode to the analysis of coffee, black tea and drinking waters [15].

Cobalt is an essential element in animal nutrition. It is present in vitamin B_{12}, and its deficiency may cause anaemia, retarded growth and loss of appetite. However, Co(II) may be toxic at high doses and cause irritation of gastrointestinal tract and vomiting, among other disorders [16]. Cobalt ISEs for the determination of this ion in food samples were developed making use of macrocycles 2,3,4:9,10,11-dipyridine-1,3,5,8,10,12-hexaazacyclotetradeca-2,9-diene and 2,3,4:9,10,11-dipyridine-1,5,8,12-tetra-methylacrylate-1,3,5,8,10,12-hexaaza-cyclotetradeca-2,9-diene, in the form of PVC membrane and coated graphite electrode (CGE). These configurations provided limits of detection of 4.7×10^{-8} M and 6.8×10^{-9} M, respectively. The CGE showed good behaviour in partially non-aqueous media in the presence of methanol, ethanol or acetonitrile. Among other applications, this electrode was used for the determination of Co(II) in water and beer samples [17].

In a similar approach, a comparative study on the fabrication of Mn(II)-selective polymeric membrane electrode (PME) and CGE based on Schiff bases was described. Manganese ion is involved in lipid, carbohydrate and protein metabolism, and thus trace amounts of manganese in the diet are considered nutritionally necessary [18]. However, as for other metals, the chronic intake of manganese produces toxic effects, including injury to the central nervous system and Parkinson's disease [19]. CGE showed a working concentration range between 4.1×10^{-7} and 1.0×10^{-1} M with a limit of detection of 6.7×10^{-8} M, a response time of 12 s and a shelf-life of 105 days. The proposed CGE was successfully used for the analysis of water, soil, vegetables and medicinal plants [20]. Also, two other macrocyclic ligands were synthesized and employed to construct ISEs for the quantification of Zn^{2+} ion, which is involved in immune system function and neurological disorders such as epilepsy and Alzheimer's diseases [21]. The electrode was applied to the analysis of water, milk, tea and biological samples [22].

13.2.3 Trivalent metal ions

Iron is a very important element in biological systems as it is involved in the transport of oxygen. Abnormally high levels of iron in the body lead to haemochromatosis

while iron deficiency leads to anaemia. The determination of Fe(III) ion is of great interest in food and water analysis. **Table** 13.1 summarizes the analytical characteristics of various ISEs developed for the determination of Fe(III) [23–30]. Different species have been used as ionophores for the preparation of these ISEs, Schiff bases being the preferred option [23, 24, 27, 29, 30] due to their ability to form strong complexes with transition metals. Most of the reported ISEs are PVC-based membrane electrodes showing high sensitivity, it also being possible to optimize the composition to yield high selectivity [24]. Moreover, a CGE exhibiting similar analytical characteristics to those of the PVC-based membrane electrode was prepared [25]. The methods developed with these ISEs showed in general a wide dynamic range, Nernstian behaviour and a low detection limit. A novel interesting application (not included in **Table** 13.1) involves the use of various ISEs using a carbon paste electrode (CPE) modified with different ionophores for the determination of Fe(III) in water samples and fish tissue [31]. The ease to construct CPEs were prepared with carbon powder mixed with the selective agent and a binder as the main components and providing a stable response are important benefits of this approach, with the additional advantage of the possibility of surface renewing by polishing.

As is well known, chromium toxicity in humans depends critically on its oxidation state. Cr(III) has important roles in glucose, lipid and protein metabolism, but Cr(VI) is toxic [32]. Also in this case, the application of ISEs using different ionophores has demonstrated high utility when applied to the analysis of water and food samples. An illustrative example is the development of a CPE containing 1-[(2-hydroxyethyl)amino]-4-methyl-9H-thioxanthen-9-one for the selective determination of Cr(III). The response of electrode was linear in the range 3.2×10^{-7} to 1.0×10^{-1} M, and satisfactory results were obtained in the analysis of coffee and tea leaves [34]. Schiff bases such as *N*-(acetoacetanilide)-1,2-diaminoethane and *N*,*N*′-bis(acetoacetanilide)-triethylenetetraamine were also used as ionophores for the preparation of PVC-based membrane Cr(III) ISEs, showing good analytical behaviour that allowed the determination of this ion in turmeric species among other samples [33]. Moreover, a Cr(III)-selective electrode based on the use of glyoxal bis(2-hydroxyanil) as ionophore was also reported and applied to the analysis of cacao powder among other samples [34].

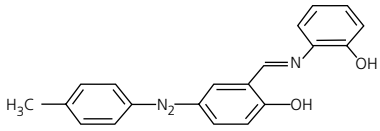
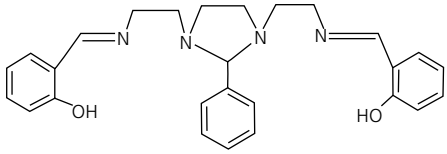
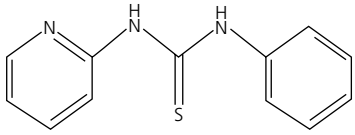
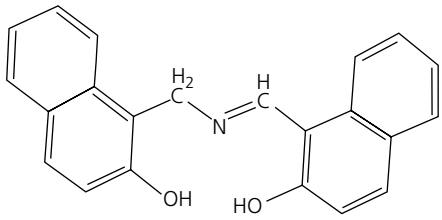
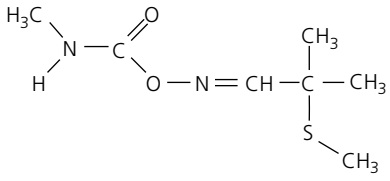
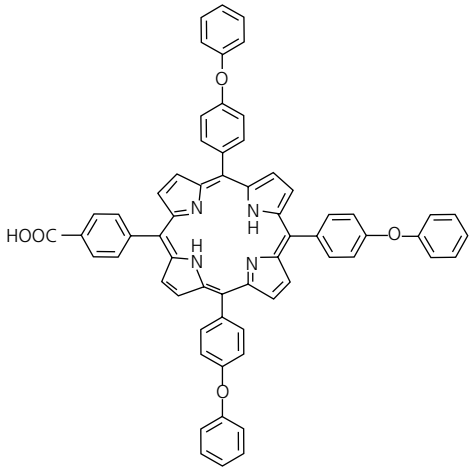
13.2.4 Anions

Fluoride is an important element for all mineralized tissues in the body [35]. It is present in various foodstuffs such as tea or seafood. An analytical method was reported for the determination of fluoride in oysters, fish, shrimps, prawns and tea in which the samples were treated by alkaline fusion and quantification was accomplished using a fluoride ISE [36]. The zwitterionic morpholinoethanesulfonic acid (MES) was used as a total ionic strength adjustor buffer system in order to improve the analytical characteristics of the ISE for application to the analysis of tea and herbs. The operation of the sensor under flow injection conditions provided a sensitivity of 60 mV per decade and a detection limit of 2.1×10^{-7} M [37].

Iodine is also an essential micronutrient utilized by the thyroid gland for biosynthesis of the thyroid hormones thyroxine and triiodothyronine. An excess of iodine can produce goitre and hypothyroidism as well as hyperthyroidism [38]. The results obtained in the determination of iodide ion in milk using a commercial ISE were compared with those from a silver electrode used as amperometric detector after ionic chromatographic separation. The low cost and simplicity of the ISE-based method together with a high selectivity were the highlighted characteristics [39]. Another potentiometric sensor for the determination of iodide was reported based on the electrodeposition of poly(3-aminophenylboronic acid) (PAPBA) onto a platinum electrode. The interaction of the polymer with the anion was the basis of the approach, since the boronic acid groups are able to form a stable covalent bond in a similar way to that observed for fluoride [40]. A good analytical performance was observed for this electrode, with a linear calibration plot in the 10^{-6} to 10^{-1} M iodide ion concentration range. The limit of detection was found to be 8×10^{-7} M. The PAPBA electrode was employed as a sensing platform for the determination of iodide ions in commercial table salt [41].

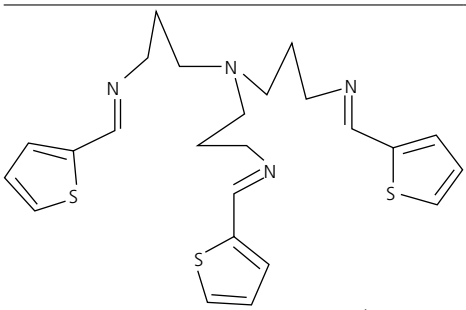
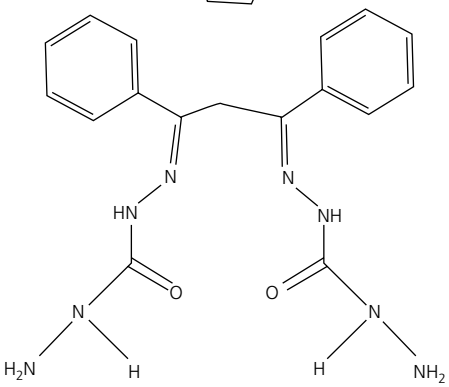
Phosphorus is involved in the utilization of carbohydrates and fats and its deficiency can cause retarded growth or poor mineralization of bones among other malfunctions. This element exists in living organisms mostly in the form of phosphate ions [42]. ISEs using diverse ionophores such as macrocyclic compounds have been developed for phosphate ion determination. For example, an all-solid-state ISE for monohydrogen phosphate ions was reported and applied to the

Table 13.1 Ionophores and analytical characteristics of some ISEs for the determination of Fe(III) in drinking water.

Ionophore		Analytical characteristics	Reference
	2-[(2-Hydroxy-1-propenyl-buta-1,3-dienyl imino)-methyl]-4-p-tolylazo phenol	Slope: 28.5 ± 0.5 mV/decade Concentration: 3.5×10^{-6} to 4×10^{-2} M LOD: $(2.5 \pm 0.1) \times 10^{-6}$ M Response time: <15 s Sample: water	[23]
	2-Phenyl-1,3-bis[3′-aza-4′-(2′-hydroxyphenyl)-prop-4-en-1′-yl]-1,3-imidazolidine	Slope: 20.0 mV/decade Concentration: 6.3×10^{-6} to 1.0×10^{-1} M LOD: 5.0×10^{-6} M Response time: 15 s. Lifetime: 60 days Sample: water	[24]
	1-Phenyl-3-pyridin-2-yl-thiourea	Slope: 20.2 ± 0.8 (CGE); 19.9 ± 0.4 mV/decade (PME) Concentration: 3.0×10^{-7} to 1.0×10^{-2} M (CGE); 6.9×10^{-7} to 1.0×10^{-2} M (PME) LOD: 2.0×10^{-7} M (GCE); 3.9×10^{-7} M(PME) Response time: <10 s. Lifetime: 60 days Sample: water	[25]
	1,1′-(Iminobis(methan-1-yl-1-ylidene)) dinaphtalen-2-ol	Slope: 19.9 mV/decade Concentration: 1.0×10^{-7} to 1.0×10^{-1} M LOD: 5.0×10^{-8} M Response time: <12 s. Lifetime: 60 days Sample: water	[26]
	*S*-Methyl-*N*-(methylcarbamoyloxy) thioacetimidate	Slope: 21.2 mV/decade Concentration: 9.1×10^{-6} to 1.0×10^{-1} M Response time: 20s. Lifetime: 60 days Sample: water	[27]
	5-(4-Carboxyphenyl)-10,15, 20-tris(4-phenoxyphenyl)-porphyrin	Slope: 21.6 mV/decade Concentration: 1.0×10^{-7} to 1.0×10^{-1} M LOD: $(8.6 \pm 0.4) \times 10^{-8}$ M Response time: 20 s. Lifetime: 42 days Sample: water	[28]

(Continued)

Table 13.1 (Continued)

Ionophore		Analytical characteristics	Reference
	Tris(3-ophenal) propylamine	Slope: 19.8 ± 0.3 mV/decade Concentration: 1.0×10^{-8} to 1.0×10^{-2} M LOD: 8.3×10^{-9} M Response time <10 s. Lifetime: 70 days Sample: water	[29]
	Dibenzoylmethane bis(carbohydrazone)	Slope: 19.7 mV/decade Concentration: 1.0×10^{-9} to 1.0×10^{-3} M Response time: ~9 s. Lifetime: 95 days Sample: water	[30]

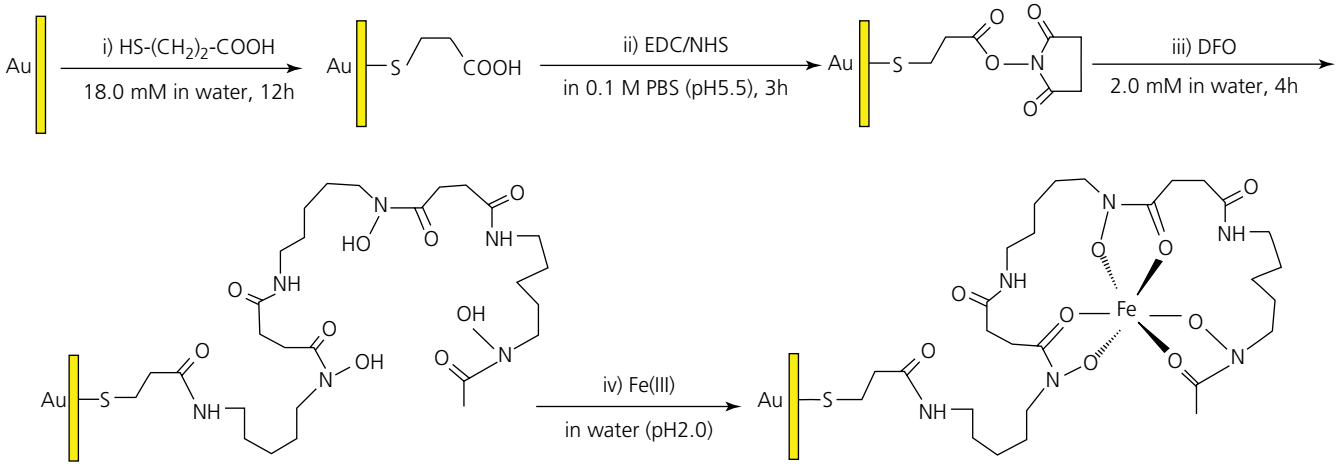
PME, poly(vinyl chloride) membrane electrode; CGE, coated graphite electrode.

analysis of a standard reference material consisting of a complex mixture of different foods representing the daily intake [43].

13.3 Electrochemical sensors and biosensors

Sensors are small devices that can be used for the direct measurement of an analyte in the sample matrix. Ideally, these devices should respond to the analyte in a continuous and reversible manner without sample disturbance, thus avoiding the need for sample treatment or even sample uptake. Unfortunately, this ideal situation is very rarely achieved with electrochemical techniques and sample treatment is often necessary when complex real samples are analysed. However, the limited selectivity associated with these techniques is strongly minimized when a recognition system with high selectivity for certain analytes is immobilized or retained at the surface of the electrochemical transducer. In this section, non-potentiometric electrochemical sensors involving the chemical or biochemical modification of the electrode surface and the measurement of current at fixed or variable potential, which have been described and applied to the determination of mineral elements in food, are considered.

Organic compounds containing different functional groups have been used as recognition systems for the development of Fe(III) electrochemical sensors. A selective and sensitive electrode using desferrioxamine (DFO), a bacterial hydroxamic siderophore that shows high affinity for Fe(III) ions, has been recently reported. The sensor was constructed by immobilizing DFO onto a 3-mercaptopropionic acid (MPA) self-assembled monolayer deposited on a gold electrode (**Figure** 13.1). Once Fe(III) was accumulated at the modified electrode, the electrochemical detection could be performed by measuring the reduction current of the metal ion by square wave voltammetry (SWV) or by the impedimetric effect



Schematic representation of the proposed mechanism for the Au–MPA–DFO SAM formation and its interaction with Fe(III).

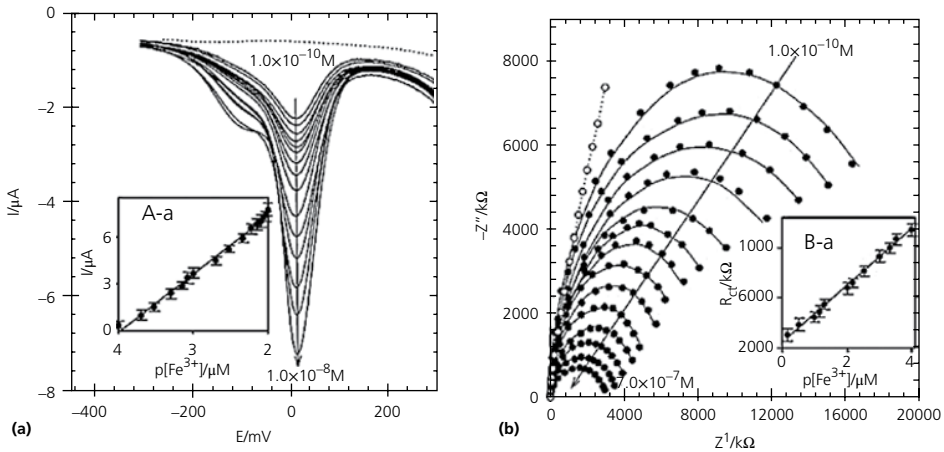


Figure 13.1 Schematic display for the construction of the Au-MPA-DFO modified electrode and its interaction with Fe(III). (A) SWVs and (B) electrochemical impedance spectroscopy (EIS) recordings, and the respective calibration plots. From Shervedani and Akrami [44]. Reproduced with permission of Elsevier Science

of the accumulated ion against a redox probe. A linear response from 1.0×10^{-10} to 1.0×10^{-7} M and a limit of detection of 2.0×10^{-11} M were achieved. A particularly relevant characteristic is the high selectivity of the sensor towards the most common metal ions and anions as well as organic compounds such as dopamine or uric acid. The method was applied to the analysis of corn leaves among other samples [44].

The concept of ion-channel mimetic sensor (ICS) was applied to the construction of a Mg(II) voltammetric sensor using the calcium ionophore ETH 5234, an oxapentanediamide-type ionophore, taking advantage of the different stoichiometry of the complexes formed with both ions. The mechanism of ICS is based on the charged analyte-gated permeability changes when redox markers cross self-assembled monolayers (SAMs) (**Figure** 13.2) [45]. The response was suggested to be an electrostatic ion–ion interaction induced signal because Mg(II) complexation with ETH 5234 provides a positive charge that is accumulated on the surface of the modified electrode, thus favouring the approximation of the redox probe and consequently facilitating the redox reaction. Accordingly, the measured current from the electroactive probe at the modified electrode can be used to determine the ion of interest (**Figure** 13.2). The sensor exhibited high selectivity for Mg^{2+} by controlling the ionophore concentration on the electrode surface, allowing the measurement of this ion in a 1 mM Ca^{2+} background solution [46].

An electrochemical sensor for Cu(II) was prepared based on the Cu(I) catalysed alkyne–azide cycloaddition reaction (CuAAC). This reaction is one of the most studied click reactions introduced by Sharpless and colleagues in 2001 [46] and is characterized by its high efficiency and good selectivity [47]. **Figure** 13.3 shows the fundamentals of the sensor. Firstly, 1-azidoundecan-

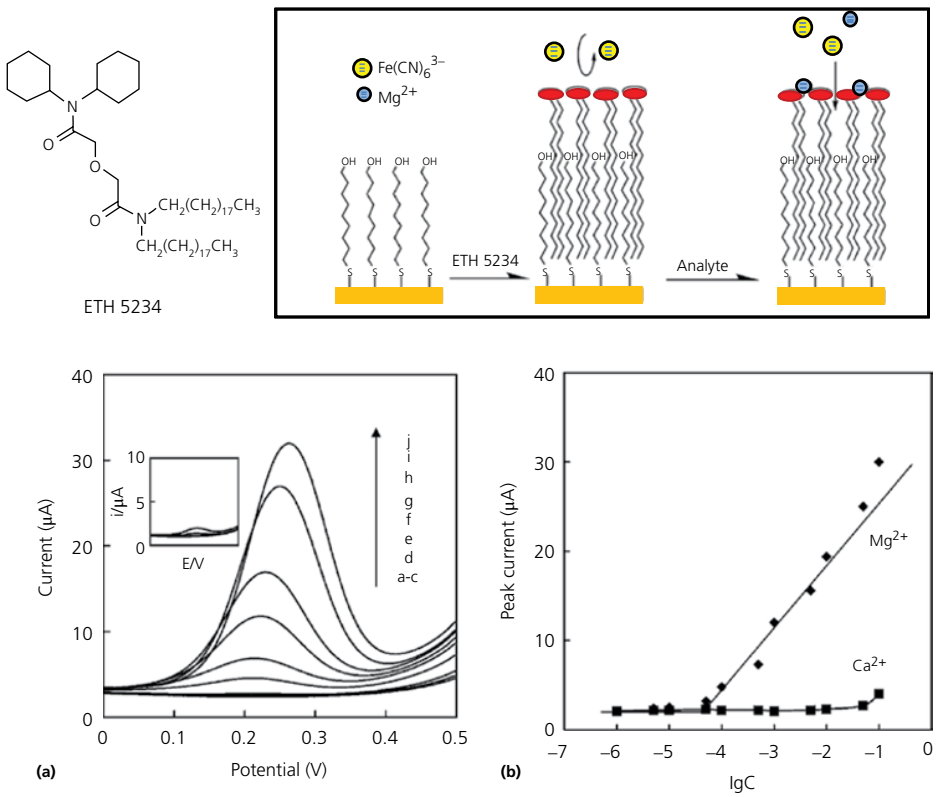


Figure 13.2 (*Top*) Chemical structure of ETH 5234 and scheme of the ion-channel mimetic sensor prepared by embedding of the ionophore in a SAM. (*Bottom*) SWV traces **(a)** and calibration plots **(b)** obtained with different concentrations of Mg^{2+} or Ca^{2+} at ETH 5234/SAM-Au electrodes in buffered 1 mM $Fe(CN)_6^{3-}$ 0.1 M KNO_3 solutions. Adapted with permission from Zhu *et al.* [45]. Copyright 2010 American Chemical Society

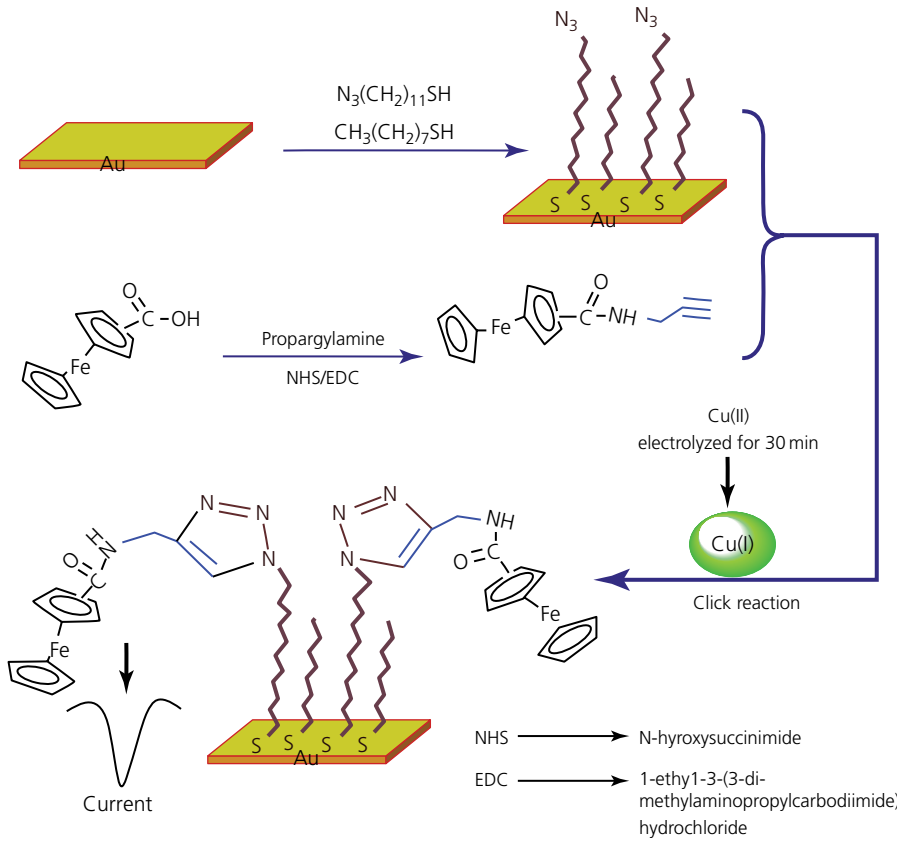


Figure 13.3 Fundamentals of Cu(II) detection via Cu(I) catalysed alkyne–azide cycloaddition reaction. From Qiu *et al.* [47]. Reproduced with permission of Elsevier.

11-thiol and octylthiol were assembled on the gold electrode surface to form the azide terminal modified electrode. Then, propargyl-functionalized ferrocene was covalently coupled on the electrode surface via CuAAC reaction, where Cu(I) was obtained from the analytical Cu(II) solution via electrochemical reduction. The differential pulse voltammetric current measured with the propargyl-functionalized Fc modified electrode could be related to the Cu(II) concentration in the sample. A linear response to the logarithm of Cu(II) concentration in the 1.0×10^{-14} to 10^{-9} M range was found as well as good selectivity in the presence of other common metal ions. As an application, the method was used to determine Cu(II) in yoghurt.

Functionalized graphene has found relevant applications in the field of electrochemical sensors due to the unique physicochemical properties of this material. In a recent article, the simple incorporation of starch to graphene nanosheets has led to the preparation of a sensitive and highly selective platform for the determination of iodide in seafood samples [48]. The recognition mechanism was based on the plugging of the ion in the grooves of the double helix of starch to form the starch–iodide complex. Peak currents measured by cyclic voltammetry were linear with the logarithm of iodide concentrations in the 10–1000 μM range.

Regarding electrochemical biosensors, few have been designed for the determination of metallic ions or anions in food. The reported configurations are mainly based on the inhibition that these species produce on the activity of some enzymes. Almost all the biosensors of this type have focused on the determination of heavy metal residues, with few examples of application to mineral elements, of which a representative example is vanadium.Vanadium ion is considered essential for humans [49]. Although its function in the organism is

not very clear, it seems to provide some insulin-like effects. Conversely, high doses can induce DNA breakdown among other disturbances. Very recently, a method for the determination of vanadium ions in tap water was proposed in which the enzyme activity of alkaline phosphatase on the conversion of riboflavin-5-monophosphate to riboflavin was affected by the presence of low concentrations of these ions. The enzyme was immobilized on a screen printed carbon electrode modified with gold nanoparticles. In the presence of metavanadate, the production of riboflavin decreases with subsequent decrease in the oxidation current, this decrease being proportional to the concentration of inhibitor. The detection limit using amperometric detection at +1.45 V (vs. Ag/AgCl) was 3.9 μM [50].

Based on the activator role that calcium plays on the activity of catalase enzyme in the degradation of hydrogen peroxide, a biosensor for such ion was developed. The biosensor had a linear relation to calcium concentrations between 1 and 10 mM with 1-min response time, and was used for the determination of calcium in milk, spring and mineral water [51]. Another example relies on the use of pyruvate oxidase for the determination of phosphate. The enzyme was immobilized on a gold substrate functionalized with copper nanoparticles onto a *p*-mercaptobenzoic acid SAM and the bioelectrode was applied to the determination of phosphate by measuring the electrochemical responses of hydrogen peroxide. A linear dynamic range between 40 and 350 μM was obtained [52].

13.4 Stripping analysis

Electrochemical stripping analysis can boast the merits of high sensitivity, selectivity and versatility. Stripping techniques are fairly inexpensive and easy to run. Furthermore, they provide both high accuracy and precision, with the possibility of portable analysis. Additionally, an insignificant effect of the matrix is possible when using medium exchange protocols. All these advantages explain the widespread use of these techniques for the determination of low concentrations of analytes in complex samples such as food [53]. The determination of metals is one of the principal uses of these techniques and so they are frequently used for mineral elements present in foods at trace levels.

13.4.1 Voltammetric stripping

Applications of stripping voltammetry in the field of food analysis are numerous. With the aim of providing a wide review of the most relevant strategies, Table 13.2 summarizes some recent examples of the use of stripping methodologies for the determination of important elements in a variety of samples [54–61, 63, 65–72, 74]. A relevant element for which stripping analysis has demonstrated great usefulness is molybdenum. This is one of the biologically essential microelements and has indispensable functions, stimulating the synthesis of proteins and nucleic acids and activating some enzymatic processes such those catalysed by xanthine oxidase [54]. However, excess molybdenum may disturb calcium and phosphorus metabolism, causing osteoporosis among other diseases. The electrochemical determination of Mo(VI) is usually performed by adsorptive stripping voltammetry after complexation with an organic ligand. Various methods have been described using CPEs, with the advantages over mercury of non-toxicity, easy modification, wide accessible potential window, low cost and renewability. Table 13.2 shows details of the oxidation of the adsorbed Mo(VI)–morin complex at a sodium dodecyl sulfate modified CPE (SDS/CPE) [54] as well as those related to the adsorption of Mo(VI)–alizarin violet complex at an acetylene black paste electrode [55]. Both methods were applied to the analysis of a variety of food samples with limits of detection in the range 10^{-9} to 10^{-10} M. Complexation of Cu(II) was also used to develop methods for the determination of cupric ion traces by accumulation of the respective complexes onto diverse electrode materials. For example, in a recent method, 1,10-phenantroline was used as the ligand and oxidation responses from the adsorbed species by SWV at a glassy carbon electrode were employed for the analysis of different food samples [56]. Another interesting strategy involved modification of a glassy carbon electrode with poly(2-amino-4-thiazoleacetic acid) and multiwalled carbon nanotubes [57].

As is well known, much effort has been made in the last few years to substitute the widely used mercury electrodes in stripping voltammetry by other greener electrodes. However, it is interesting to note that, as **Table** 13.2 shows, some recent applications involving mercury electrodes still appear in the literature. This is undoubtedly because of the excellent electrochemical behaviour of this material and its fitness for these kinds of measurements. Among all the reported methods, it is

Table 13.2 Stripping voltammetric methods for trace analysis of mineral elements in foods.

Species	Electrode	Method	Analytical characteristics	Sample	Reference
Mo(VI)	SDS/CPE	Adsorptive ASV; E_{ac} = –0.1 V (vs. SCE); accumulation of Mo(VI)–morin	Linear range: 8.0×10^{-10} to 10×10^{-8} M (3×10^{-6} M morin; t_{ac} = 120 s) LOD: 4.0×10^{-10} M (S/N = 3)	Water, tomato, cucumber, wheat	[54]
Mo(VI)	ABPE	Adsorptive ASV; E_{ac} = –200 mV (vs. SCE); accumulation of Mo(VI)–alizarin violet complex	Linear range: 6.0×10^{-10} to 1.0×10^{-6} M (4.0×10^{-6} M alizarin violet; t_{ac} = 120 s) LOD: 2.0×10^{-9} M	Plant foodstuffs	[55]
Cu(II)	GCE	Adsorptive ASWV; E_{ac} = –0.5 V (vs. Ag/AgCl); accumulation of Cu(II)–1,10-phenantroline complex	Linear range: 0.1–50 ng/mL (t_{ac} = 120 s) LOD: 18.5 pg/mL	Honey, orange juice, tea, vegetables, juice	[56]
Cu(II)	Poly(2-amino-4-thiazole acetic/ MWCNTs/GCE	DPASV; accumulation at open circuit	Linear range: 7.0×10^{-7} to 5.0×10^{-5} M (t_{ac} = 300 s) LOD: 5.0×10^{-10} M (S/N = 3)	Water	[57]
Cu(II)	HMDE	Adsorptive ASV; accumulation of Cu(II)–thiosemicarbazide complex	Linear range: 0.01–90.0 ng/mL LOD: 7 pg/mL	Rice, tea, tomato, water	[58]
Cu(II)	HMDE	AdsSV; accumulation of Cu(II)–TZAMB complex	Linear range: 9.5×10^{-9} to 4.7×10^{-7} M LOD: 9.5×10^{-9} M	Rice, vegetables	[59]
Cu(II) Zn(II)	HMDE	ASV; E_{ac} = –1.5 V	LOD: 0.990 µg/L (Cu); 0.738 µg/L (Fe) t_{ac} = 120 s	Edible mushrooms	[60]
Zn(II)	HMDE	Adsorptive ASWV; E_{ac} = –0.1 V; accumulation of alizarin complex	Linear range: 5×10^{-8} to 4×10^{-7} M (1×10^{-6} M alizarin; t_{ac} = 60 s)	Instant coffee	[61]
Zn(II)	BiF/GCE	ASV E_{ac} = –1.4 V (vs. Ag/AgCl)	Linear range: 5×10^{-7} to 3×10^{-6} M (t_{ac} = 120 s) LOD: 9.6×10^{-9} M (S/N = 3)	Milkvetch	[63]
Se(IV)	HMDE	DPCSV; E_{ac} = –300 mV (vs. Ag/AgCl); accumulation of Cu_2Se	Linear range: 0.01–0.1 µg/L (t_{ac} = 600 s) LOD: 0.4 ng/L	Potatoes	[65]
Se(IV)	HMDE	DPCSV; E_{ac} = –0.35 V (vs. SCE); accumulation of Cu_2Se	Linear range: 0.15–8 µg/L LOD: 0.07 µg/L	Rice	[66]
Fe(II)	HMDE	Ads-SSWV; accumulation of Fe(II)–DCDT complex	Linear range: 0–30 ng/mL LOD: 0.55 ng/mL	Olive oil	[67]
Fe(II)	Dithiodianiline/ AuNPs/CPE	DPASV; adsorption of Fe(II); E_{ac} = –1.0 V (vs. SCE)	Linear range: 0.1–100 nM LOD: 0.05 nM ($3s_b/m$); t_{ac} = 40 s	Wheat seeds, barley seeds, lentils	[68]
Fe(II) Fe(III)	Pt-RDE	DPSAV; E_{ac} = –1.2 V (vs. Ag/AgCl)	Linear range: 0.026–15 mM (total iron) LOD: 13 µM	Fermented and fresh carrot juice	[69]
Mn(II)	MBPCE	ASV; E_{ac} = –0.200 V (vs. Ag/AgCl)	Linear range: 6.0×10^{-7} to 5.0×10^{-4} M LOD: 1.07×10^{-7} M; t_{ac} = 4 min	Wheat flour, rice, vegetables	[70]
Mn(II)	Hg(Ag)FE	DPASV E_{ac} = –1.75 V (vs. Ag/AgCl)	Linear range: 10–260 nM LOD: 0.3 nM; t_{ac} = 120 s	Water	[71]
Mn(II) Cu(II) Fe(III)	CPE	SW-AdsCSV; accumulation of 5-Br-PADAP-complexes; E_{ac} = +1.0 V (vs. Ag/AgCl)	LOD: 0.066 (Mn), 0.108 (Cu), 0.093 (Fe) µg/L	Water	[72]

(Continued)

Table 13.2 (Continued)

Species	Electrode	Method	Analytical characteristics	Sample	Reference
Cr(VI)	HMDE	Ads catalytic SV in the presence of nitrate; accumulation of Cr(III)–rubeanic acid complex	Linear range: 0.01–6 ng/mL LOD: 0.002 ng/mL; t_{ac} = 50 s	Vinegar	[74]

SDS, sodium dodecyl sulfate; CPE, carbon paste electrode; ASV, anodic stripping voltammetry; SCE, saturated calomel electrode; morin, 20,3,40,5,7-pentahydroxyflavone; alizarin violet, 4-hydroxy-3-[(2-hydroxy-1-naphthalenyl)azo]-benzenesulfonic acid monosodium; CSDPV, cathodic stripping differential pulse voltammetry; DCDT, 5,5-dimethylcyclohexane-1,2,3-trione 1,2-dioxime 3-thiosemicarbazone; TZAMB, 2-[2,3,5-triazolylazo]-5-dimethyl-aninobenzonic; ABPE, acetylene black paste electrode; CCSA, constant current stripping voltammetry; MBPCE, bentonite–porphyrin carbon paste electrode; Hg(Ag)FE, mercury film silver based electrode; 5-Br-PADAP, (5-bromo-2-pyridylazo)-5-diethylaminophenol.

worth mentioning various applications for the determination of copper and zinc with the hanging mercury drop electrode (HMDE) [58–61]. However, the toxicity of mercury has also led to the use of the environmentally friendly bismuth film electrode (BiFE), which is characterized by a simple preparation process, sufficiently accessible potential window, and high sensitivity, among other advantages [62]. An interesting example of the use of stripping methodologies with BiFE in food analysis is the determination of zinc in milkvetch, a dietary supplement [63]. In this method, a glassy carbon electrode was coated by *in situ* deposition of bismuth film and the target metal at –1.4 V after removal of dissolved oxygen. A good sensitivity with a limit of detection in the nanomolar range and a slope two times higher than that obtained using the mercury film electrode was achieved using an accumulation time of 120 s.

Stripping voltammetry has also been used for the speciation of selenium in foodstuffs. Selenium is considered an essential trace element, as it is a key component of selenoproteins [64]. However, this element is toxic at relatively high doses. Both selenium toxicity and bioavailability are dependent on the chemical form present in food. In an interesting method, the determination and speciation of selenium in potatoes was performed using cathodic stripping voltammetry at an HMDE. Hydrolysis of the starch with amyloglucosidase enzyme followed by acidic digestion in a microwave oven was carried out. Nitric acid or a mixture of nitric and sulfuric acids were respectively used to produce Se(IV), which was directly analysed, or Se(VI), which was further reduced to Se(IV) by heating. The results of the analysis provided information about the contents of Se(IV) and total selenium in starch. Furthermore, in order to determine total selenium in proteins, the remaining residue from the enzyme hydrolysis was also treated with sulfonitric mixture. In both cases, the electrochemical measurements were carried out by differential pulse cathodic stripping voltammetry in the presence of 1 mg/L Cu(II), giving rise to the accumulation of the analyte in the form of Cu_2Se on the mercury drop [65]. A similar method was also used for the analysis of rice [66].

Various methodologies based on the adsorption of metal complexes on electrode surfaces have also been applied for the determination of iron. For example, CPEs modified with selective ligands were used for metal accumulation on the basis of the complex formation between the ion Fe(II) and the modifier. Following this scheme, a method for the determination of iron in olive oil by adsorptive stripping SWV was developed using 5,5-dimethylcyclohexane-1,2,3-trione 1,2-dioxime 3-thiosemicarbazone (DCDT) as the ligand. This compound reacted with Fe(II) and it was accumulated by application of a potential of –0.02 V versus Ag/AgCl. Measurement of the peak current from square wave cathodic voltammograms allowed a linear calibration plot up to 30 ng/mL iron to be obtained with a limit of detection of 13.75 ng/g in the olive oil [67]. Another methodology involved the use of dithiodianilide and gold nanoparticles for the preparation of a modified CPE for the determination of Fe(II) in the presence of Fe(III) [68]. Stripping voltammetry was also used for the determination of Fe(II) and Fe(III) in aqueous solutions containing complexing ligands of both ions which are typically present in foods. The method was applied to the analysis of fermented and fresh carrot juice using differential pulse anodic stripping voltammetry (ASV) at a platinum rotating disc electrode. The obtained results were related with the interactions of the more relevant organic acids with these ions and their bioavailability [69].

Various alternatives have been proposed for the stripping determination of manganese. Here, three relevant examples can be highlighted. Firstly, preparation of an original platform using a chemically modified bentonite–porphyrin carbon paste electrode (MBPCE) solved problems found when mercury electrodes were used, such as the low solubility of this ion in mercury, the low potential value (–1.7 V vs. SCE) needed for deposition of manganese, or the formation of inter-metallic compounds [70]. Use of the MBPCE was advantageous due to the catalytic properties of the layered silicates as bentonite and the complexing capacity of porphyrin. All these properties gave rise to the development of a sensitive and selective method where the ASV peak currents measured by cyclic voltammetry were linear in a wide concentration range (see **Table** 13.2).

Mercury electrodes have also been replaced, at least partially, by other less toxic materials for manganese trace determination. This is the case of the cyclic renewable mercury film silver-based electrode (Hg(Ag)FE) employed for the analysis of water samples. This electrode was characterized by the easy construction and renovation of the surface as well as by long-term stability. The method provided a higher sensitivity than that achieved using an HMDE, which was attributable, among other factors, to the larger Hg(Ag)FE active surface area. A linear calibration plot ranging from 10 nM to over 260 nM for an accumulation time of 15 s was obtained. Furthermore, the detection limit for an accumulation time of 120 s was as low as 0.3 nM [71]. On the other hand, the simultaneous determination of Mn(II), Cu(II) and Fe(III) by square-wave adsorptive cathodic stripping voltammetry (SW-AdCSV) was also reported. A CPE was used to accumulate the ions in the form of metal complexes, from a solution containing 2-(5′-bromo-2′-pyridylazo)-5-diethylaminophenol (5-Br-PADAP) using an accumulation potential of +1.0 V (vs. Ag/AgCl/3MKCl). The method was successfully applied to the analysis of bottled natural water samples [72].

Under the heading of catalytic adsorptive stripping voltammetry are grouped various highly sensitive and selective methods widely applied in trace analysis [73]. One of these methods has been used recently for the determination of Cr(VI) in the presence of rubeanic acid and nitrate as the catalyst. The rationale of this method implies the electrochemical reduction of chromate to Cr(III) followed by the adsorptive accumulation of the Cr(III)–rubeanic acid complex at an HMDE. Subsequently, the application of a cathodic scan produces reduction to Cr(II), which is then reoxidized by means of nitrate. The catalytic redox cycle produces an increase in the response. A detection limit as low as 0.002 ng/mL for an accumulation time of 50 s was obtained with no interference from other common ions [74].

13.4.2 Potentiometric stripping

Potentiometric stripping (PSA) is similar to stripping voltammetry in the accumulation step, but once accumulated the species of interest undergoes chemical oxidation or reduction by a suitable reagent in solution or by application of a constant current, while the potential of the electrode is monitored as a function of time [75]. In both cases, the potential versus time response is recorded and the analyte concentration is related to the time required for complete stripping from the electrode surface. One advantage of this technique is the high resistance to electrode fouling. Moreover, the spontaneous stripping makes possible a high insensitivity to interferences from other electroactive substances.

Various PSA methods have been applied to the determination of traces of selenium. Cathodic stripping potentiometry at a glassy carbon mercury film electrode was used for the determination of selenium in seed oils. Selenium was extracted from the matrix by treatment with hydrochloric acid at 90 °C. A potential of –150 mV was applied for 60 s to pre-concentrate the element in the form of amalgam and the deposited Se(Hg) was further reduced to H_2Se by application of a –30 μA constant current showing a peak at –530 mV. Under these experimental conditions, limits of detection lower than 0.5 ng/g were obtained [76]. Another method for selenium was based on the use of sulfide as internal standard for quantification of this element by chronopotentiometric stripping at a mercury film glassy carbon electrode. This strategy enabled simpler and faster calculation of the analyte concentration by comparing its behaviour with that of the standard, thus also avoiding multiple steps and electrode damage, as well as increasing the accuracy of the response measurement for selenium. The method was applied to the analysis of biscuits and pasta samples [77].

Ion-pairing and extraction of iodide at a CPE modified with tricresyl phosphate were the bases for a PSA method for the determination of iodide in table salt. Deposition was performed at a potential of +0.8 V for 30 s and a cathodic stripping step was run by applying a

constant current of –5 μA. In this case, the linear range was between 2 and 100 mg/kg and the limit of detection was 1 mg/kg [78].

13.5 Continuous monitoring

As already discussed, one of the most important advantages of electroanalytical techniques is the possibility of miniaturization without loss of sensitivity. This enabled a large expansion in the construction of sensors and biosensors very suitable for integration as detectors for electrochemical responses in continuous monitoring systems, both automatic analysers and instruments for high-resolution separations. Nowadays, it can be said that all electrochemical techniques based on the use of a variety of electrode materials have been used for these applications. As discussed in the following examples in the field of food analysis, there is relatively intense activity with regard to the design of methods for continuous electrochemical detection, with the determination of mineral elements occupying a prominent position.

13.5.1 Flow-injection approaches

Electrochemical detection has been used in flow analysis since the early years of its development, applications being particularly numerous in injection techniques and currently including microfluidic systems [79]. Some examples relying on the use of relevant electrode and/or manifold configurations are considered in this section.

An interesting design is the flow injection analysis (FIA)–potentiometric system for the assay of chloride in food samples using a solid-state Ag/AgCl chloride detector and a dialysis unit that enables direct analysis of liquid food samples without any other previous treatment (**Figure** 13.4). Clogging of the dialysis membrane by lipids present in milk or coconut water samples was prevented by addition of Triton X-100, allowing the injection of hundreds of samples without decrease in sensitivity. **Figure** 13.4b shows an example of the potentiometric responses for repetitive injections of 2.5-times diluted milk containing 80 mg/L chloride, yielding a relative standard deviation as low as 1.2% for potentiometric measurements [80].

A PVC-membrane potentiometric sensor array and multivariate calibration were used for the determination of Ca(II) and Mg (II) in mineral waters. By coupling the array to a sequential injection analysis (SIA) system, an automated electronic tongue was developed, and the information involved was processed using a multilayer artificial neural network (ANN) [81].

Calcium and magnesium ions were also determined using a pH-sensitive detector for flow-through potentiometry consisting of a graphite/quinhydrone/silicone composite electrode. Automated analysis of mineral and drinking waters was performed using EDTA (Na_2H_2Y) as the carrier solution. The principle of this method is based on the detection of the protons released when metallic ions are complexed by Y^{4-}, while the sample zone passes through the electrode. The peak areas were proportional to the calcium and/or magnesium concentration in the range 1×10^{-5} to 5×10^{-2} M. The detection limit for both ions was about 1×10^{-5} M, the standard deviation was less than 2%, and the sampling frequency was 60 determinations per hour [82]. Furthermore, the combination of FIA with an all-solid-state potassium ISE gave very good results in the determination of potassium ion in soy sauce. The electrode was prepared by coating an electroactive polypyrrole:poly(4-styrenesulfonate) film electrode with a plasticized PVC membrane containing valinomycin and showed a highly stable response, being easily miniaturized. As an additional advantage the FIA method allows fast and simple sample treatment. The continuous assay of potassium ions was possible in the range 10^{-5} to 10^{-1} mol/L, with a high frequency of analysis of 150 samples per hour and good precision [83].

Voltammetric and amperometric measurements have been widely used in flow analysis. An improvement in the detection limit under hydrodynamic conditions was usually achieved for voltammetric signals in comparison with stationary measurements, which can be attributed to the increased transport rate of the analyte to the working electrode surface [79]. Regarding amperometric detection, both the electrode material and the geometrical design of the flow cell determine the sensitivity and performance of detection.

Stripping voltammetry was used in the flow electrochemical analysis of mineral ions. For example, a method for the determination of zinc ion making use of a graphite felt electrode has been described. In general, porous electrodes are appropriate for use in flow systems because of their high surface area and good hydrodynamic properties [84]. In particular, graphite felt,

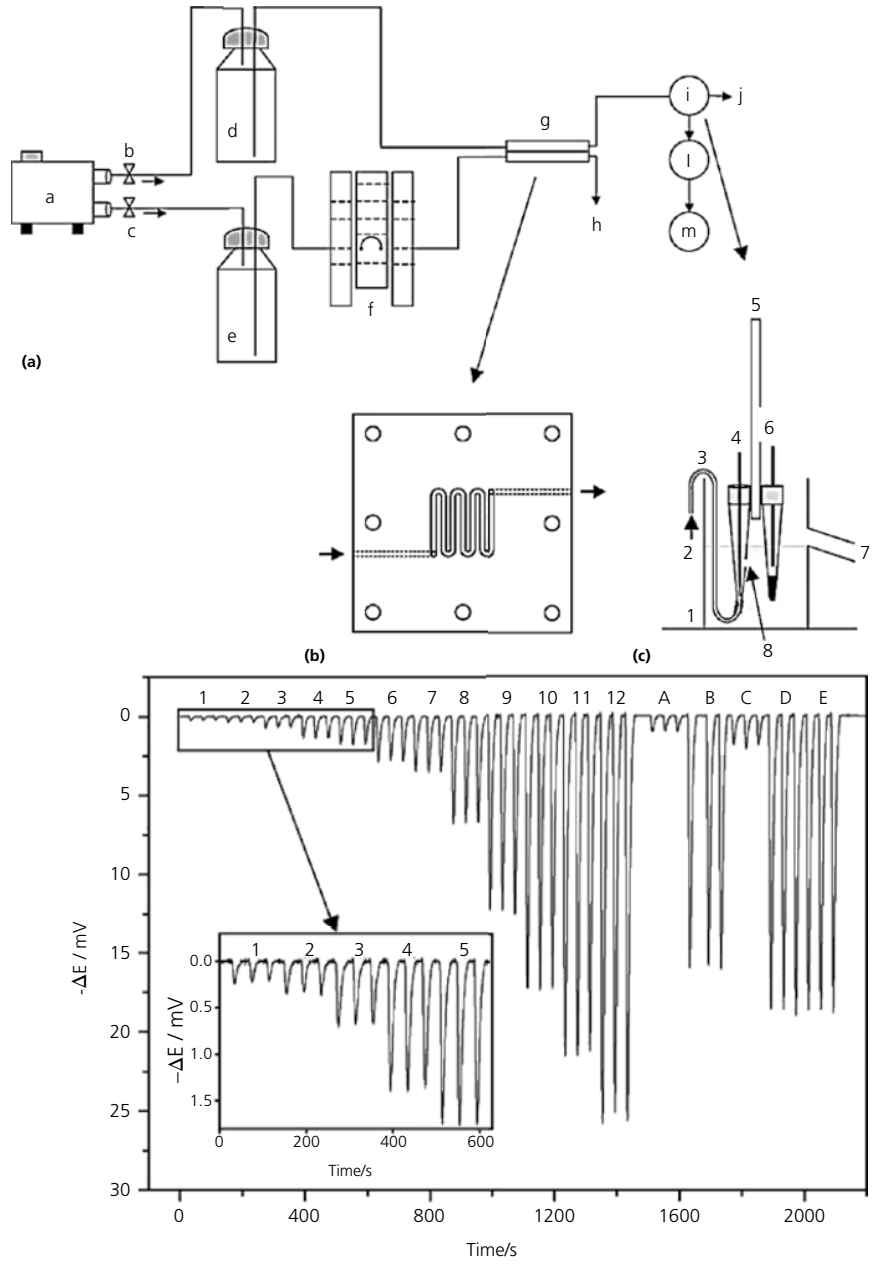


Figure 13.4 (*Top*) Flow injection manifold used for FIA-potentiometric chloride determination: **(a)** air pump (a); flow regulator valves (b,c); solution carrier flask of the detector (d) and sample (e); injector (f); dialysis unit (g); waste (h,j); flow cell (i); multimeter (l); computer (m). **(b)** Superior view of the dialysis unit. **(c)** Schematic flow cell. (*Bottom*) 1–12: potentiogram for consecutive injections with in-line dialysis of 4–1000 mg/L chloride standard solutions; **(a–c)** milk samples; **(d, e)** coconut water samples. From Da Silva *et al.* [80]. Reproduced with permission of Elsevier

consisting of a high number of graphite fibres 10 μm in diameter, possesses a high area/volume ratio (616/cm) and a large void volume (around 90%), providing great electrochemical efficiency and low detection limits. Electrochemical flow detection using this graphite felt electrode was used for the determination of zinc ion in tap water and a food supplement. The method allowed determination of zinc ion in a linear range between 10^{-6} and 10^{-4} M, using an accumulation potential of –1.4 V for 5 min [84].

A vanadium oxide-modified glassy carbon electrode was prepared by casting vanadium tri(isoproxide) oxide ($VO(OC_3H_7)_3$) and poly(propylene carbonate) (PPC) onto a glassy carbon electrode surface. Based on the catalytic oxidation of iodide on the coated electrode, an FIA method with amperometric detection for the determination of iodide with a linear range of 5×10^{-7} to 1×10^{-3} M was developed and successfully applied to the analysis of iodide in dry edible seaweed [85]. A similar configuration based on the activation of a barrel plated nickel electrode (Ni-BPE) in alkaline media to form a $Ni(OH)_2$/NiO(OH) film was prepared and used for the selective adsorption of phosphate. This adsorption process suppressed the current corresponding to the electrocatalytic oxidation of glucose at the activated Ni-BPE, which made possible a scheme for FIA determination of the anion (**Figure** 13.5). Using a detection potential of 0.55 V (vs. Ag/AgCl), with 25 μM glucose in 0.1 M NaOH as the carrier solution, a linear range up to 1 mM phosphate was achieved. Furthermore, determination of this anion was possible in the presence of various anionic species such as chloride, acetate or carbonate, among others [86].

13.5.2 Chromatographic analysis with electrochemical detection

The methods developed for liquid chromatography are undoubtedly the most popular of all the continuous monitoring methods that use electrochemical detection. Choice of adequate electrode material and flow through the cell are crucial parameters for obtaining satisfactory results after chromatographic separation [87]. Applications for the determination of mineral elements make use mainly of ion chromatography (IC). For example, the determination of iodide was accomplished by electrochemical detection at a silver-based solid CPE after ion chromatography using an anion-exchange column. The silver composite electrode was polarized at +0.08 V versus Ag/AgCl. Remarkably, the presence of iodide ions in solution facilitates the oxidation of metallic silver, whose response currents are directly related with iodide concentration. The calibration plot for iodide was linear in the concentration range between 0.635 and 63.5 μg/L. The IC-amperometric detection method was successfully applied to the determination of iodide in skim milk and table salt among other samples [88]. On the other hand, a platinum electrode modified with an iodine-based film was also used for the amperometric determination of iodide after ion-exchange chromatography. The electrode exhibited high analytical responses for iodide electro-oxidation with good stability and long life. The detection limit was 0.5 μg/L. The method was applied to quantify the iodide content of milk and common vegetables, among other samples [89].

The common anions and cations in mineral waters were determined by amperometric detection after chromatographic separation using a gold electrode modified by electropolymerization of diphenylamine in the presence of SDS. The rationale of the method was the electrochemical transformation of the electroactive film that involves incorporation of the electro-inactive anions or cations to counterbalance the electric charge generated in the oxidation or reduction. Therefore, by applying a selected potential to the electrode, current will only flow if charge-compensating ions pass through the electrode. In this case, a set of well-defined peaks was obtained by ion-exclusion/cation-exchange chromatography when applying a potential of +1.35 V (vs. SCE) using a citric acid solution as eluent [90].

13.6 Future trends

The determination of mineral elements cannot be considered as a priority research objective in modern electroanalytical chemistry because there are many well-established competitive analytical methodologies in this field. However, the inherent advantages of electroanalysis in terms of high sensitivity, simplicity of approaches, friendly use, portability and low cost can be exploited to develop efficient strategies devoted to the analysis of mineral elements in foodstuffs. The area in which more work needs to be done is the automation of methodologies, and this is where electrochemical approaches are particularly appropriate when considering the inherent characteristics of

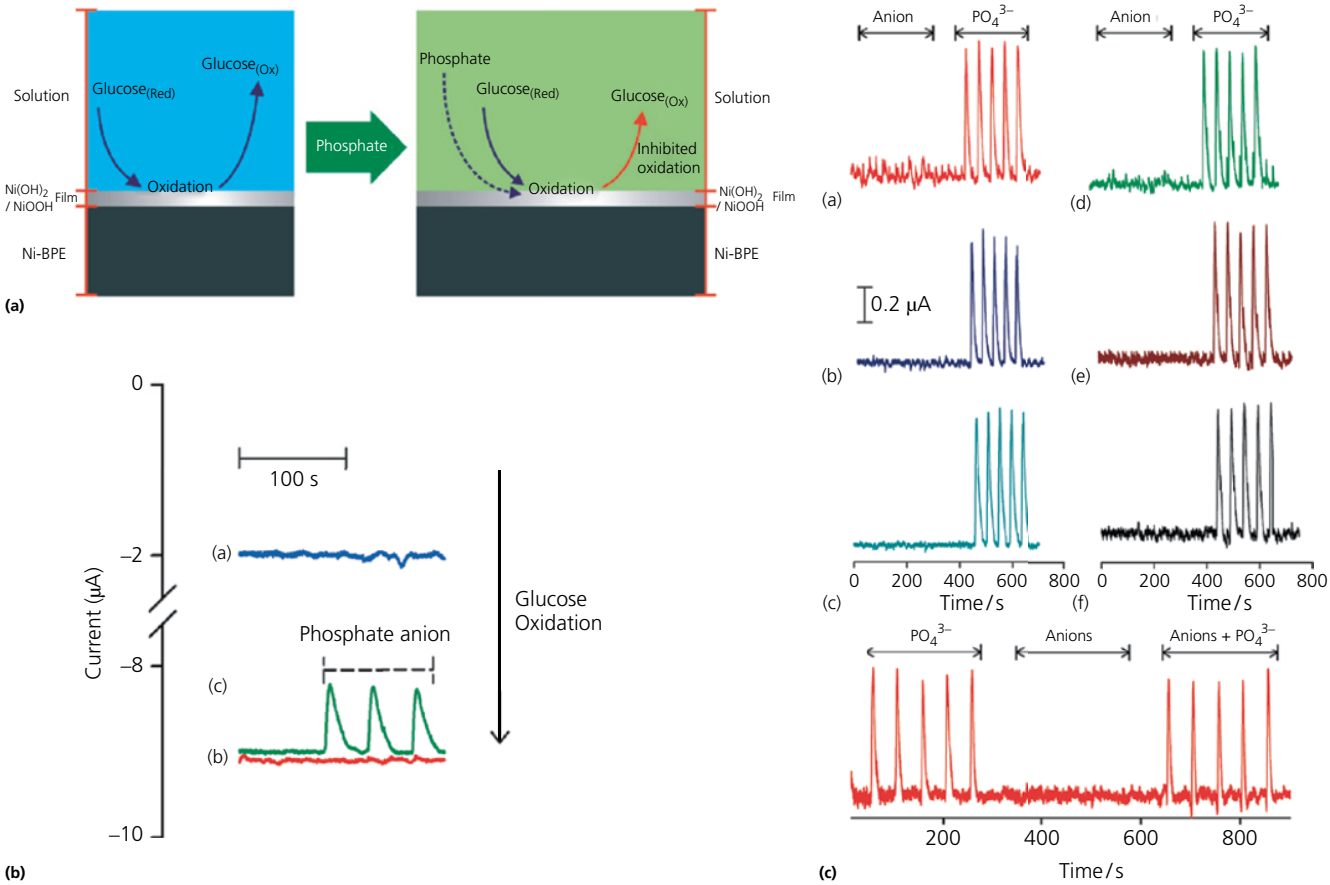


Figure 13.5 **(a)** Detection scheme of the proposed system. **(b)** FIA responses of the activated Ni-BPE in 0.1 M NaOH (a), in 0.1 M NaOH with 25 μM glucose (b), and sequential injection of 500 μM phosphate in 0.1 M NaOH with 25 μM glucose as carrier solution (c). **(c)** FIA responses of 500 μM phosphate and (a) nitrate, (b) sulfate, (c) oxalate, (d) chloride, (e) acetate, (f) carbonate and (g) all anions (including 5 μM perchlorate, chromate and arseniate) wherein the interfering anion and phosphate are present simultaneously. *E*app = +0.55 V vs. Ag/AgCl; 300 μL/min. Adapted with permission from Cheng *et al.* [86]. Copyright 2010 American Chemical Society

electrochemical measurements. Automated, reliable, electroanalytical methodologies will be extremely useful both in food quality assessment and control of the industrial processes in the agricultural and food industry, opening new avenues for the integration of electrochemical driven devices in routine industrial operation.

Abbreviations

ABPE: Acetylene black paste electrode
AC: Activated carbon
ANN: Artificial neural network
AuNP: Gold nanoparticle
ASV: Anodic stripping voltammetry
BiFE: Bismuth film electrode
5-Br-PADAP: 2-(5′-Bromo-2′-pyridylazo)-5-diethylaminophenol
CCP: Colloidal calcium phosphate
CCSA: Constant current stripping voltammetry
CGE: Coated graphite electrode
CPE: Carbon paste electrode
CSDPV: Cathodic stripping differential pulse voltammetry
CuAAC: Cu(I) catalysed alkyne–azide cycloaddition reaction
CWE: Coated wire ion-selective electrode
DCDT: 5,5-Dimethylcyclohexane-1,2,3-trione 1,2-dioxime 3-thiosemicarbazone
DFO: Desferrioxamine
EDTA: Ethylenediaminetetraacetic acid
FIA: Flow injection analysis
Hg(Ag)FE: Mercury film silver based electrode
HMDE: Hanging mercury drop electrode
IC: Ion chromatography
ICS: Ion-channel mimetic sensor
ISE: Ion-selective electrode
LOD: Limit of detection
MBPCE: Modified bentonite–porphyrin carbon paste electrode
MES: Morpholinoethanesulfonic (acid)
MPA: 3-Mercaptopropionic acid
Ni-BPE: Barrel plated nickel electrode
PAPBA: Poly(3-aminophenylboronic acid)
PME: Polymeric membrane electrode
PPC: Poly(propylene carbonate)
PSA: Potentiometric stripping
PVC: Poly(vinyl chloride)
SAM: Self-assembled monolayer
SCE: Saturated calomel electrode
SDS: Sodium dodecyl sulfate
SDS/CPE: Sodium dodecyl sulfate modified carbon paste electrode
SIA: Sequential injection analysis
SW-AdCSV: Square-wave adsorptive cathodic stripping voltammetry
SWV: Square wave voltammetry
TZAMB: 2-[2,3,5-triazolylazo]-5-Dimethyl-aninobenzonic

References

1 Zuman P. Half a century of research using polarography. *Microchem J* 1997;**57**:4–51.
2 Mannino S, Wang J. Electrochemical methods for food and drink analysis. *Electroanalysis* 1992;**4**:835–840.
3 Gao R, van Leeuwen HP, van Valenberg HJF, van Boekel MAJS. Accurate determination of the Ca^{2+} activity in milk-based systems by Ca-ISE: effects of ionic composition on the single Ca^{2+} activity coefficient and liquid junction potentials. *Food Chem* 2011;**129**:619–623.
4 Silanikove N, Shapiro F, Shamay A. Use of an ion-selective electrode to determine free Ca ion concentration in the milk of various mammals. *J Dairy Res* 2003;**70**:241–243.
5 De la Fuente MA, Olano A, Juárez M. Mineral balance in milk heated using microwave energy. *J Agric Food Chem* 2002;**50**:2274–2277.
6 Gao R, Temminghoff EJM, van Leeuwen HP, van Valenberg HJF, Eisner MD, van Boekel MAJS. Simultaneous determination of free calcium, magnesium, sodium and potassium ion concentrations in simulated milk ultrafiltrate and reconstituted skim milk using the Donnan Membrane Technique. *Int Dairy J* 2009;**19**:431–436.
7 Lin MJ, Lewis MJ, Grandison AS. Measurement of ionic calcium in milk. *Int J Dairy Technol* 2006;**59**:192–199.
8 Mahmoud WH. Novel bilirubin-based PVC calcium sensors. *Anal Sci* 2003;**19**:361–365.
9 Hernández R, Riu J, Rius FX. Determination of calcium ion in sap using carbon nanotube-based ion-selective electrodes *Analyst* 2010;**135**:1979–1985.
10 Saurina J, López-Avilés E, Le Moal A, Hernández-Cassou S. Determination of calcium and total hardness in natural waters using a potentiometric sensor array. *Anal Chim Acta* 2002;**464**:89–98.
11 Kumar P, Shim YB. A novel Mg(II)-selective sensor based on 5,10,15,20-tetrakis(2-furyl)-21,23-dithiaporphyrin as an electroactive material. *J Electroanal Chem* 2011;**661**:25–30.
12 Chandra S, Lang H. A new sodium ion selective electrode based on a novel silacrown ether. *Sensors Actuators B* 2006;**114**:849–854.
13 Somani BL, Ambade V. A kinetic method amenable to automation for ceruloplasmin estimation with inexpensive and stable reagents. *Clin Biochem* 2007;**40**:571–574.

14 Ghaedi M, Montazerozohori M, Sahraei R. Comparison of the influence of nanomaterials on response properties of copper selective electrodes. *J Ind Eng Chem* 2013;**19**: 1356–1364.

15 Shokrollahi A, Abbaspour A, Ghaedi M, Naghashian Haghighi A, Kianfar AH, Ranjbar M. Construction of a new Cu^{2+} coated wire ion selective electrode based on 2-((2-(2-(2-(2-hydroxy-5-methoxybenzylideneamino)phenyl)disufanyl)phenylimino)methyl)-4-methoxyphenol Schiff base. *Talanta* 2011;**84**:34–41.

16 Kumar P, Shim YB. A novel cobalt(II)-selective potentiometric sensor based on *p*-(4-*n*-butylphenylazo)calix[4] arene. *Talanta* 2009;**77**:1057–1062.

17 Singh AK, Singh P, Bhattacharjee G. Determination of cobalt ions at nano-level based on newly synthesized pendant armed macrocycle by polymeric membrane and coated graphite electrode. *Talanta* 2009;**80**:685–693.

18 Belane AK, Dakora FD. Levels of nutritionally-important trace elements and macronutrients in edible leaves and grain of 27 nodulated cowpea (Vigna unguiculata L. Walp.) genotypes grown in the Upper West Region of Ghana. *Food Chem* 2011;**125**:99–105.

19 Peneder TM, Scholze P, Berger ML *et al*. Chronic exposure to manganese decreases striatal dopamine in human alpha-synnuclein transgenic mice. *Neuroscience* 2011;**180**:280–292.

20 Singh AK, Bandi KR, Upadhyay A, Jain AK. A comparative study on fabrication of Mn^{2+} selective polymeric membrane electrode and coated graphite electrode. *Mater Sci Eng C* 2013;**33**:626–633.

21 Ibupoto ZH, Usman Ali SM, Chey CO, Khun CK, Nur O, Willander M. Selective zinc ion detection by functionalised ZnO nanorods with ionophore. *J Appl Phys* 2011;**110**: 104702-1–104702-6.

22 Singh P, Singh AK, Jain AK. Electrochemical sensors for the determination of Zn^{2+} ions based on pendant armed macrocyclic ligand. *Electrochim Acta* 2011;**56**:5386–5395.

23 Mashhadizadeh MH, Shoaei IS, Monadi N. A novel ion-selective membrane potentiometric sensor for direct determination of Fe(III) in the presence of Fe(II). *Talanta* 2004;**64**:1048–1052.

24 Gupta VK, Jain AK, Agarwal S, Maheshwari G. An iron(III) ion-selective sensor based on a μ-bis(tridentate) ligand. *Talanta* 2007;**71**:1964–1968.

25 Motlagh MG, Taher MA, Ahmadi A. PVC membrane and coated graphite potentiometric sensors based on 1-phenyl-3-pyridin-2-yl-thiourea for selective determination of iron(III). *Electrochim Acta* 2010;**55**:6724–6730.

26 Babakhanian A, Gholivand MB, Mohammadi M *et al*. Fabrication of a novel iron(III)-PVC membrane sensor based on a new 1,1′-(iminobis(methan-1-yl-1-ylidene))dinaphthalen-2-ol synthetic ionophore for direct and indirect determination of free iron species in some biological and non-biological samples. *J Hazard Mater* 2010;**177**:159–166.

27 Gupta VK, Sethi B, Upadhyay N, Kumar S, Singh R, Singh LP. Iron (III) selective electrode based on *S*-methyl *N*-(methylcarbamoyloxy) thioacetimidate as a sensing material. *Int J Electrochem Sci* 2011;**6**:650–663.

28 Vlascici D, Fagadar-Cosma E, Popa I, Chiriac V, Gil-Agustí M. A novel sensor for monitoring of iron(III) ions based on porphirins. *Sensors* 2012;**12**:8193–8203.

29 Zamani HA, Ganjali MR, Faridbod F, Salavati-Niasari M. Heptadentate Schiff-base based PVC membrane sensor for Fe(III) ion determination in water samples. *Mater Sci Eng C* 2012;**32**:564–568.

30 Chandra S, Deepshikha, Sarkar A. Spectral, thermal and electrochemical investigation of carbohydrazone derived ionophore as Fe(III) ion selective electrode. *Spectrochim Acta A* 2013;**107**:271–279.

31 Awad Ali T, Mohamed GG, El-Dessouky MMI, Abou El Ella SM, Mohamed RTF. Modified carbon paste ion selective electrodes for the determination of iron (III) in water, soil and fish tissue samples. *Int J Electrochem Sci* 2013;**8**:1469–1486.

32 Ghaedi M, Shokrollahi A, Salimibeni AR, Noshadi S, Joybar S. Preparation of a new chromium(III) selective electrode based on 1-[(2-hydroxyethyl) amino]-4-methyl-9H-thioxanthen-9-one as a neutral carrier. *J Hazard Mater* 2010;**178**:157–163.

33 Singh KA, Gupta VK, Gupta B. Chromium(III) selective membrane sensors based on Schiff bases as chelating ionophores. *Anal Chim Acta* 2007;**585**:171–178.

34 Gholivand MB, Sharifpour F. Chromium(III) ion selective electrode based on glyoxal bis(2-hydroxyanil). *Talanta* 2003;**60**:707–713.

35 Palmer C, Wolfe SH. The impact of fluoride on health. *J Am Diet Assoc* 2005;**105**:1620–1628.

36 Malde MK, Bjorvatn K, Julshhamn K. Determination of fluoride in food by use of alkali fusion and fluoride ion-selective electrode. *Food Chem* 2001;**73**:373–379.

37 Fouskaki M, Sotiropoulou S, Kocí M, Chaniotakis NA. Morpholino-ethanesulfonic acid-based buffer system for improved detection limit and stability of the fluoride ion selective electrode. *Anal Chim Acta* 2003;**478**:77–84.

38 Azizi F, Hedayati M, Rahmani M, Sheikloleslam R, Allaverdian S, Salarkia N. Reappraisal of the risk of iodine-induced hyperthyroidism: an epidemiological population survey. *J Endocrinol Invest* 2005;**28**:23–29.

39 Melichercik J, Szijarto L, Hill AR. Comparison of ion-specific electrode and high performance liquid chromatography methods for the determination of iodide in milk. *J Dairy Sci* 2006;**89**:934–937.

40 Nicolas M, Fabre B, Simonet J. Electrochemical sensing of fluoride and sugars with a boronic acid-substituted bipyridine Fe(II) complex in solution and attached onto an electrode surface. *Electrochim Acta* 2001;**46**:1179–1190.

41 Ciftci H, Tamer U. Electrochemical determination of iodide by poly(3-aminophenylboronic acid) film electrode at moderately low pH ranges. *Anal Chim Acta* 2011;**687**:137–140.

42 Galloway SD, Tremblay MS, Sexsmith JR, Roberts CJ. The effects of acute phosphate supplementation in subjects of different aerobic fitness levels. *Eur J Appl Physiol Occup Physiol* 1996;**72**:224–230.

43 Kumar P, Kim DM, Hyun MH, Shim YB. An all-solid-state monohydrogen phosphate sensor based on a macrocyclic ionophore. *Talanta* 2010;**82**:1107–1112.

44 Shervedani RK, Akrami Z. Gold–deferrioxamine nanometric interface for selective recognition of Fe(III) using square wave voltammetry and electrochemical impedance spectroscopy methods. *Biosensors Bioelectron* 2013;**39**: 31–36.

45 Zhu J, Qin Y, Zhang Y. Magnesium-selective ion-channel mimetic sensor with a traditional calcium ionophore. *Anal Chem* 2010;**82**:436–440.

46 Kolb HC, Finn MG, Sharpless KB. Click chemistry: diverse chemical function from a few good reactions. *Angew Chem Int Ed Engl* 2001;**40**:2004–2021.

47 Qiu S, Xie L, Gao S *et al*. Determination of copper(II) in the dairy product by an electrochemical sensor based on click chemistry. *Anal Chim Acta* 2011;**707**:57–61.

48 Liu SQ, Hu FT, Liu CB *et al*. Graphene sheet-starch platform based on the groove recognition for the sensitive and highly selective determination of iodide in seafood samples. *Biosensors Bioelectron* 2013;**47**:396–401.

49 Erdem A, Shahwan T, Cagir A, Eroglu AE. Synthesis of aminopropyl triethoxysilane-functionalized silica and its application in speciation studies of vanadium(IV) and vanadium(V). *Chem Eng J* 2011;**174**:76–85.

50 Alvarado-Gámez AL, Alonso-Lomillo MA, Domínguez-Renedo O, Arcos-Martínez MJ. Vanadium determination in water using alkaline phosphatase based screen-printed carbon electrodes modified with gold nanoparticles. *J Electroanal Chem* 2013;**693**:51–55.

51 Akyilmaz E, Kozgus O. Determination of calcium in milk and water samples by using catalase enzyme electrode. *Food Chem* 2009;**115**:347–351.

52 Karthikeyan R, Berchmans S. Inorganic–organic composite matrix for the enzymatic detection of phosphate in food samples. *J Electrochem Soc* 2013;**160**:B73–B77.

53 Brainina KhZ, Malakhova NA, Stojko NY. Stripping voltammetry in environmental and food analysis. *Fresenius J Anal Chem* 2000;**368**:307–325.

54 Deng PH, Feng YI, Fei JJ. A new electrochemical method for the determination of trace molybdenum(VI) using carbon paste electrode modified with sodium dodecyl sulfate. *J Electroanal Chem* 2011;**661**:367–373.

55 Deng P, Fei J, Zhang J, Feng Y. Determination of molybdenum by adsorptive anodic stripping voltammetry of molybdenum–alizarin violet complex at an acetylene black paste electrode. *Food Chem* 2011;**124**:1231–1237.

56 Chaiyo S, Chailapakul O, Sakai T, Teshima N, Siangproh W. Highly sensitive determination of trace copper in food by adsorptive stripping voltammetry in the presence of 1,10-phenantroline. *Talanta* 2013;**108**:1–6.

57 Zhao H, Jiang Y, Ma Y *et al*. Poly(2-amino-4-thiazoleacetic acid)/multiwalled carbon nanotubes modified glassy carbon electrodes for the electrochemical detection of copper(II). *Electrochim Acta* 2010;**55**:2518–2521.

58 Abbasi S, Reza Tabaraki HK. Determination of ultra trace levels of copper in food samples by a highly sensitive adsorptive stripping voltammetric method. *Food Chem* 2010;**123**: 507–512.

59 Lu G, Long D, Li D, Zhan T, Zhao H, Liu C. Determination of copper in food by stripping voltammetry using Cu(II)–[2-(2,3,5-triazolylazo)-5-dimethyl amino benzonic] complex. *Food Chem* 2004;**84**:319–322.

60 Alonso J, García MA, Pérez-López M, Melgar MJ. The concentrations and bioconcentration factors of copper and zinc in edible mushrooms. *Arch Environ Contam Toxicol* 2003;**44**: 180–188.

61 Alghamdi AH. Determination of zinc by square-wave adsorptive stripping voltammetry using alizarin as a chelating agent. *J Saudi Chem Soc* 2010;**14**:1–7.

62 Wang J. Stripping analysis at bismuth electrodes: a review. *Electroanalysis* 2005;**17**:1341–1346.

63 Guo Z, Feng F, Hou Y, Jaffrezic-Renault N. Quantitative dtermination of zinc in milkvetch by anodic stripping voltammetry with bismuth electrodes. *Talanta* 2005;**65**:1052–1055.

64 Whanger PD. Selenocompounds in plants and animals and their biological significance. *J Am Coll Nutr* 2002;**21**:223–232.

65 Ferri T, Favero G, Frasconi M. Selenium speciation in foods: preliminary results on potatoes. *Microchem J* 2007;**85**: 222–227.

66 Panigati M, Falciola L, Mussini P, Beretta G, Facino RM. Determination of selenium in Italian rices by differential pulse cathodic stripping voltammetry. *Food Chem* 2007; **105**:1091–1098.

67 Galeano Díaz T, Guiberteau A, López Soto MD, Ortiz JM. Spectrophotometric and adsorptive stripping square wave voltammetric determination of iron in olive oils, as complex with 5,5-dimethylcyclohexane-1,2,3-trione 1,2-dioxime 3-thiosemicarbazone (DCDT). *J Agric Food Chem* 2003;**51**: 3743–3747.

68 Gholivand MB, Geravandi B, Parvin MH. Anodic stripping voltammetric determination of iron(II) at a carbon paste electrode modified with dithiodianiline (DTDA) and gold nanoparticles (GNP). *Electroanalysis* 2011;**23**:1345–1351.

69 Scheers N, Andlid T, Alminger M, Sandberg AS. Determination of Fe^{2+} and Fe^{3+} in aqueous solutions containing food chelators by differential pulse anodic stripping voltammetry. *Electroanalysis* 2010;**22**:1090–1096.

70 Rezaei B, Ghiaci M, Sedaghat ME. A selective modified bentonite–porphyrin carbon paste electrode for determination of Mn(II) by using anodic stripping voltammetry. *Sensors Actuators B* 2008;**131**:439–447.

71 Piech R, Bas B, Kubiak WW. The cyclic renewable mercury film silver based electrode for determination of manganese(II) traces using anodic stripping voltammetry. *J Electroanal Chem* 2008;**621**:43–48.

72 Ghoneim EM. Simultaneous determination of Mn(II), Cu(II) and Fe(III) as 2-(5′-bromo-2′-pyridylazo)-5-diethylaminophenol complexes by adsorptive cathodic stripping voltammetry at a carbon paste electrode. *Talanta* 2010;**82**:646–652.

73 Bobrowski A, Krolicka A, Zarebski J. Characteristics of voltammetric determination and speciation of chromium: a review. *Electroanalysis* 2009;**21**:1449–1458.
74 Abbasi S, Bahiraei A. Ultra trace quantification of chromium(VI) in food and water samples by highly sensitive catalytic adsorptive stripping voltammetry with rubeanic acid. *Food Chem* 2012;**133**:1075–1080.
75 Economou A. Recent developments in on-line electrochemical stripping analysis. An overview on the last 12 years. *Anal Chim Acta* 2010;**683**:38–51.
76 Dugo G, La Pera L, Pollicino D, Saitta M. Determination of selenium content in different types of seed oils by cathodic stripping potentiometry (CSP). *J Agric Food Chem* 2003;**51**: 5598–5601.
77 Svarc-Gajic J, Stojanovic Z. Selenium determination in biscuits and pasta: development of chronopotentiometric stripping determination by using a sulphide as an internal standard. *Talanta* 2013;**115**:474–481.
78 Švancara I, Ogorevc B, Nović M, Vytřas K. Simple and rapid determination of iodide in table salt by stripping potentiometry at a carbon-paste electrode. *Anal Bioanal Chem* 2002;**372**: 795–800.
79 Trojanowicz M. Recent developments in electrochemical flow detections. A review Part I. Flow analysis and capillary electrophoresis. *Anal Chim Acta* 2009;**653**:36–58.
80 Da Silva IS, Richter EM, do Lago CL, Gutz IGR, Tanaka AA, Angnes L. FIA-potentiometry in the sub-Nernstian response region for rapid and direct chloride assays in milk and coconut water. *Talanta* 2005;**67**:651–657.
81 Calvo D, Größl M, Cortina M, del Valle M. Automated SIA system using an array of potentiometric sensors for determining alkaline-earth ions in water. *Electroanalysis* 2007; **19**:644–651.
82 Vahl K, Kahlert H, Scholz F. Rapid automatic determination of calcium and magnesium in aqueous solutions by FIA using potentiometric detection. *Electroanalysis* 2010;**22**:2172–2178.
83 Komaba S, Arakawa J, Seyama M, Osaka T, Satoh I, Nakamura S. Flow injection analysis of potassium using an all-solid-state potassium selective electrode as a detector. *Talanta* 1998;**46**:1293–1297.
84 Feier B, Floner D, Cristea C, Bodoki E, Sandulescu R, Geneste F. Flow electrochemical analyses of zinc by stripping voltammetry on graphite felt electrode. *Talanta* 2012;**98**:152–156.
85 Tian L, Liu L, Chen L, Lu N, Xu H. Electrochemical determination of iodide on a vanadium oxide–polypropylene carbonate coated glassy carbon electrode. *Talanta* 2005;**66**:130–135.
86 Cheng WL, Sue JW, Chen WC, Chang JL, Zen JM. Activated nickel platform for electrochemical sensing of phosphate. *Anal Chem* 2010;**82**:1157–1161.
87 Trojanowicz M. Recent developments in electrochemical flow detections. A review Part II. Liquid chromatography. *Anal Chim Acta* 2011;**688**:8–35.
88 Malongo TK, Patris S, Macours P, Cotton F, Nsangu J, Kauffmann J-M. Highly sensitive determination of iodide by ion chromatography with amperometric detection at a silver-based carbon paste electrode. *Talanta* 2008;**76**:540–547.
89 Cataldi TRI, Rubino A, Ciriello R. Sensitive quantification of iodide by ion-exchange chromatography with electrochemical detection at a modified platinum electrode. *Anal Bioanal Chem* 2005;**382**:134–141.
90 Xu Q, Xu C, Wang QJ *et al*. Application of a single electrode, modified with polydiphenylamine and dodecyl sulfate, for the simultaneous amperometric determination of electroinactive anions and cations in ion chromatography. *J Chromatogr A* 2003;**997**:65–67.

CHAPTER 14

X-ray

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Abstract: The application of X-ray fluorescence techniques to the determination of the mineral profile of foods has been reviewed with regard to the tremendous possibilities of these methodologies to provide fast and environmentally friendly alternative methods for evaluating the presence of essential and toxic elements or, at least, to provide screening of the mineral composition of foods and their safety for human nutrition. Special attention has been paid to modern methods of analysis that permit improvements in the analytical sensitivity of measurements or favour the use of low-cost portable instrumentation.

Keywords: X-ray, energy-dispersive XRF, wavelength-dispersive XRF, total reflection XRF, synchrotron radiation XRF, particle-induced X-ray emission

14.1 Introduction

X-ray fluorescence (XRF) spectroscopy is an analytical atomic spectrometry technique that is highly recommended for evaluating the elemental composition of solid samples and is also useful for the direct determination of mineral elements in liquids [1]. The energy involved in the X-ray radiation occupies the range between 0.1 and 25 keV, with wavelengths from 100 to 0.5 Å. It is clear that the interaction between primary X-ray radiation and matter involves, other than photon scattering, its absorption at characteristic wavelengths due to changes in the excitation state of electrons relating to the deep internal shell of atoms. This is the basis of XRF as a way to evaluate the presence of atoms in samples regardless of their specific chemical structure and composition. However, for quantitative purposes it must be taken into account that matrix effects are not negligible and, because of this, problems related to adequate calibration together with the relatively low sensitivity (generally at the percentage or parts per thousand level of concentration) justifies the infrequent use of XRF in food analysis.

14.2 X-ray spectra of foods

The absorption of X-ray radiation by an element in a sample is very simple and consists of few peaks at wavelengths characteristic of the element. Thus, K and L edges can be obtained that correspond to the energy of the photons which exactly matches the energy required to eject a K or an L electron of the target element, corresponding to those present in the two electron shells closest to the atomic nucleus.

Samples irradiated with strong primary X-ray radiation produce an emission fluorescence spectra. Excitation can be made using a selected X-ray tube with the cathode exposed to a high voltage and a suitable target anode to generate the X-ray radiation. Alternatively, a radioisotope source such as ^{55}Fe, ^{109}Cd, ^{153}Gd, ^{57}Co or ^{241}Am, which provides Mn K radiation to γ-rays together with ultra-soft X-rays, could be used. In short, after sample excitation, a series of elements present in the sample produce a typical spectrum in which the target elements can be identified by controlling the wavelength or the energy of the photons, thus providing a mineral fingerprint of the sample.

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In fact, the energy of the emitted radiation (in keV) has a direct relationship with its wavelength as $E = 1.24\,\lambda^{-1}$, and XRF spectra of foods can principally be obtained in the wavelength domain (wavelength dispersion X-ray fluorescence, WD-XRF) or the energy domain (energy dispersion X-ray fluorescence, ED-XRF). Traditionally, large and costly WD-XRF instruments were used in the fields of geological and alloy analysis but today simple, relatively low cost and portable ED-XRF instruments are available and offer excellent evaluation of the mineral profile of samples, from foods to medicine to heritage, archaeological and industrial applications.

The absorption of X-rays by a pellet prepared from a lyophilized menu sample containing 8500 mg/kg K, 1460 mg/kg Ca, 710 mg/kg Mg, 24.3 mg/kg Fe and 16.5 mg/kg Zn provided the spectrum shown in Figure 14.1, which shows the range from 0 to 40 keV. The enlargement of the spectrum from 0 to 15 keV (top right in Figure 14.1) shows the typical Kα bands of the major, minor and trace elements. Table 14.1 summarizes some of the published papers in which XRF was used to determine some minerals in foods [2–13], from pure products like wheat grains [2] or tea leaves [5] to selected foods [7] or diets [4]. It is important to note that in some of the aforementioned papers XRF data were compared with the results from inductively coupled plasma mass spectrometry (ICP-MS) or atomic absorption spectroscopy (AAS). However, for a better understanding of the real applicability of XRF in food analysis, it is interesting to study those reports where the mineral profile of foods was obtained by energy dispersion compared with those based on wavelength dispersion. Tables 14.2 and 14.3 summarize some of the papers published on ED-XRF [14–38] and WD-XRF [39–44]. It can be concluded that ED-XRF has been employed to determine a single element like As [30] or a number of elements, in some cases providing information on the authentication of foods, like soybean origin [14] or pumpkins [18].

In contrast, the expensive WD-XRF has been less frequently employed in food analysis, although in the last few years the ability of the technique to check many elements simultaneously or sequentially in the same sample has been of great value in discriminating curcuma spices produced from different countries [39] and for establishing differences between pistachios or beans produced by organic and conventional farming [40, 41].

In general, it is well accepted that WD-XRF has superior resolution and sensitivity compared with ED-XRF. However, the use of different crystals and the expensive instrumentation required dramatically affects more

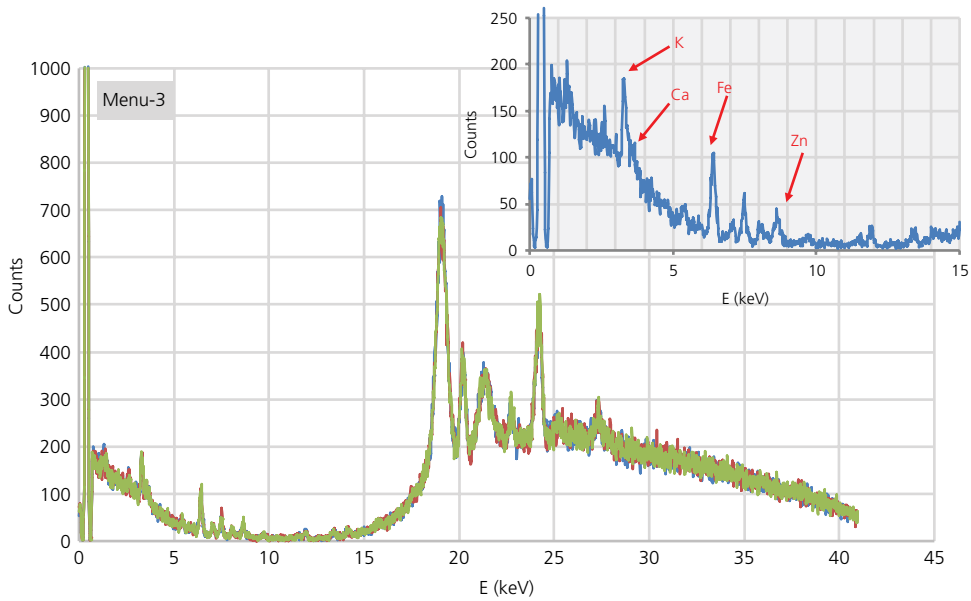


Figure 14.1 ED-XRF spectrum of a sample menu comprising a mixture of lentils, meatballs and yogurt.

Table 14.1 General papers on XRF analysis of foods.

Technique	Sample	Elements	Application	Reference
XRF, ICP-MS	Wheat grains	Be, Ca, Cd, Cr, Co, Cs, Cu, Fe, Ga, Mg, Mn, Na, Pb, Rb, Se, Sc, Sr, Ti, V, Y, Zn, Zr	Evaluation of provenance soil and their potential as fingerprints of geographical origin (PCA–LDA)	[2]
XRF, AAS, FAS	Herbs and shrubs	Al, Ca, K, Fe, Mn, Na, P, S, Sr, Zn	Use of atomic absorption spectrophotometer, flame photometer and X-ray fluorescence	[3]
XRF	Diet samples	Ca, Cu, Fe, Mn, Mo, Ni, Sr, Zn		[4]
XRF	Fresh green tea, black tea, and tea residue	Ca, K	138 samples	[5]
XRF	Fast food	Br, Ca, Cl, Fe, K, Mn, P, Rb, S, Sr, Zn	239 samples Evaluation of repeatability and reproducibility	[6]
XRF, AAS	Selected foods	Cu, Fe, Mn, Zn		[7]
XRF	Biological matrices	S	Critical comparison of X-ray-fluorescence and combustion–infrared methods	[8]
XRF	Foods and feedstuffs of vegetable and animal origin	Trace heavy metals	Evaluation also of soil extracts, sewage sludges and floor coatings	[9]
XRF	Feedstuff	Heavy metals	Preparation of samples	[10]
XRF	Food	As, Cd, Hg, Pb, Te		[11]
XRF	Feedstuff	Ca, Cl, K, P, S		[12]
XRF	Biological samples	Sr	Determination of stable strontium	[13]

PCA, principal component analysis; LDA, linear discriminant analysis.

Table 14.2 Some examples of ED-XRF studies on the mineral profile of foods.

Technique	Sample	Elements	Application	Reference
ED-XRF	Soybeans	Ba, Br, Cl, Cu, K, Mg, Mn and P	Constructing a discriminant function for geographical origin	[14]
ED-XRF	Defatted kenaf seed meal (DKSM)	K, Mg and P	Prospective edible flour from agricultural waste with high antioxidant activity	[15]
ED-XRF	Rice and pearl millet	Fe and Zn	ED-XRF results were highly correlated with ICP-OES values for both Zn and Fe. ED-XRF predicted Zn and Fe in rice to within 1.9 and 1.6 mg/kg of ICP-OES values, and Zn and Fe in pearl millet to within 7.6 and 12.5 mg/kg of ICP-OES values, at a 95% confidence level	[16]
ED-XRF	Whole grain wheat	Fe, Se and Zn	ED-XRF limits of quantification were estimated as 7, 3 and 2 mg/kg for Zn, Fe, and Se, respectively. ED-XRF results were highly correlated with ICP-OES or ICP-MS values. Standard errors of ED-XRF predictions were ±2.2 mg/kg Zn, ±2.6 mg/kg Fe, and ±1.5 mg/kg Se	[17]
ED-XRF	Pumpkin seeds from four geographical origins	Ba, Br, Ca, Cu, Fe, Mn, Mo, Ni, P, Rb, Sr and Zn	Identifying the geographic origin of individual pumpkins, 118 pumpkin samples, LDA models	[18]

(Continued)

Table 14.2 (Continued)

Technique	Sample	Elements	Application	Reference
ED-XRF	Indian major carps: rohu, katla and mrigel	Cr, Cu, Fe, Mn, Pb and Zn	Major carp species from wastewater-fed fishponds of East Calcutta Wetlands	[19]
ED-XRF	Infant foods	Ca, Cl and K		[20]
ED-XRF	Dried, powdered kelp food supplements	As, Hg and Pb	Detectable levels of arsenic that exceeded both the EPA and AHPA suggested reference doses were present in every sample. Of the 17 samples studied none were found to contain any significant amounts of Pb and Hg	[21]
ED-XRF	Predator fish	Cr, Cu, Fe, Mn, Pb and Zn	Determined values are lower than the maximally allowed concentrations of heavy metals in fish muscle	[22]
ED-XRF	Food additive specifications	SiO_2	Good agreement with the ICP results	[23]
ED-XRF	Milk-and cereal-based powders	Na, Mg, P, Cl, K, Ca, Fe and Zn	Influence of measuring times and number of replicates on validation performance characteristics. Effect of number of pellet and measurement time	[24]
ED-XRF, FAAS	Wild edible mushrooms	Cd, Cr, Cu, Fe, Mn, Ni, Se and Zn	Studies concerning bioaccumulation from industrial area	[25]
ED-XRF	Sucrose products	Calcium lactate, magnesium sulfate and yerba mate extract	Results obtained by ED-XRF demonstrated that mineral distribution in the co-crystallized products was uniform	[26]
ED-XRF	Soft- and hard-shelled mud crabs	10 elements: Ca, Cu, K, Mn, Pb, Zn, etc.	To evaluate the food value from the nutritional and safety point of view for human consumption	[27]
ED-XRF	Weld Mexican plants	Elemental composition Cl, K, Mg and Si	Scanning electron microscope equipped with an energy-dispersive X-ray microanalyser	[28]
ED-XRF	Powdered fruit juice	Inorganic and organic composition	Qualitative analysis of artificial powdered fruit juice mixes without pretreatment. PCA showed distinction of the samples with and without artificial sweeteners in their composition	[29]
ED-XRF	Seafood: eight fish species, three cephalopods, and one crustacean	As	The highest concentrations were found in Norway lobster (30.3 ± 8.7 mg/kg wet weight), followed by common octopus (25.9 ± 8.4 mg/kg wet weight). Within fish species, wild gilthead sea bream, anglerfish, and megrim species presented values around 12 mg/kg and in the others the average was lower than 10 mg/kg	[30]
ED-XRF, FAAS	Mussel	Ba, Ca, Cu, Fe, K, Sr and Zn	Cr, Mn, Ni, Cd and Pb were determined by FAAS	[31]
ED-XRF, FAAS	Lobsters	As, Br, Ca, Cl, Cu, Fe, K, S, Se, Sr and Zn	European and American lobsters. Na, Mg, Mn, Cd, Hg and Pb were determined by FAAS	[32]
ED-XRF	*Carcinus maenas* and *Necora puber*	As, Br, Ca, Cl, Cu, Fe, K, S, Se, Sr and Zn		[33]
ED-XRF	Food crustacean tissue	Br, Ca, Cu, Fe, K, Mn, Pb, Se, Sr and Zn		[34]
ED-XRF	Potato starch	P	Calibration was done using 20 potato starch samples, and the results were validated using a second set of 15 samples	[35]
ED-XRF	Food premixes	Cu, Fe and Zn	Fe 500–35,000 mg/kg, Cu 50–4000 mg/kg and Zn 700–32,000 mg/kg. Samples were quantified in 200-s measurements using a rhodium tube	[36]

Table 14.2 (Continued)

Technique	Sample	Elements	Application	Reference
ED-XRF	Rice flour	Ca, P and S		[37]
ED-XRF	Local cooking salt	Pb and other heavy metals		[38]

PCA, principal component analysis; LDA, linear discriminant analysis.

Table 14.3 Examples of WD-XRF studies on the mineral profile of foods

Technique	Sample	Elements	Application	Reference
WD-XRF and LIBS	Spices: cumin		Identified the elemental markers needed for discrimination of select spices back to their country of origin	[39]
WD-XRF	Pistachios	Al, As, Au, Ba, Bi, Br, Ca, Cd, Cl, Cu, Fe, K, La, Mg, Mn, Na, Ni, P, Pb, Rb, S, Si, Sn, Sr, Ti, Zn and Zr	Grown under organic and conventional farming regimes	[40]
WD-XRF	Haricot beans	26 elements such as Al, As, Ba, Bi, Ca, Cl, Cd, Cu, Fe, Hg, K, La, Mg, Mn, Na, Ni, P, Pb, Rb, S, Si, Sn, Sr, Ti, Zn and Zr	Grown under organic and conventional farming regimes	[41]
WD-XRF	Milk, dairy products	Minerals (Ca, Cl, K, Mg, Na, P and S) and trace elements (Br, Cu, Fe, Mn, Ni, Rb, Sr and Zn)	Freeze-dried samples pressed as tablets of 4 g	[42]
WD-XRF	Infant cereal matrices, 88 samples	Macroelements (Na, Mg, P, Cl, K, Ca) and trace elements (Mn, Fe, Zn)	Pellets of 4 g were prepared under 10 tonnes. Calibrations were established with 43 samples, and method validation was made using a second set of 45 samples	[43]
WD-XRF	Transgenic, unmodified soya-foods and popular dairy products	P	Compared with the standard molybdenum blue method. Matrix effects were minimized by using standard reference material. Results indicate the validity of the proposed methods to routine food analysis	[44]

generalized use of WD-XRF; conversely, the reduced price and portability of ED-XRF has encouraged the development of methods suitable for the evaluation of foods *in situ* by direct contact with the solid food or through a container [45], providing semi-quantitative results in many applications. Figure 14.2 summarizes the strength and weakness of the two general approaches for XRF spectrum acquisition and the reader should understand the reasons for the differences between the number of published studies based on each of these techniques.

The traditional semiconductor detector (SD) for XRF, like the Si(Li) SD with high spectral resolution (145 eV, for Mn $K\alpha$), requires liquid nitrogen cooling for good operation. More recently, use of Peltier-cooled silicon detectors (Si-PIN diodes) and silicon drift detectors (SDDs) with thermoelectric cooling and reduced size offer high thermal stability and typical spectral resolutions of 130–160 eV (Mn $K\alpha$) and active zone areas of 10–40 mm^2. Their generalized use has contributed to the reduction in size and price of modern instruments, especially ED-XRF measurements [1].

14.3 Sampling and sample treatment

One of the main advantages of XRF spectrometry compared with alternative techniques for the determination of the mineral profile of foods is its ability to obtain the

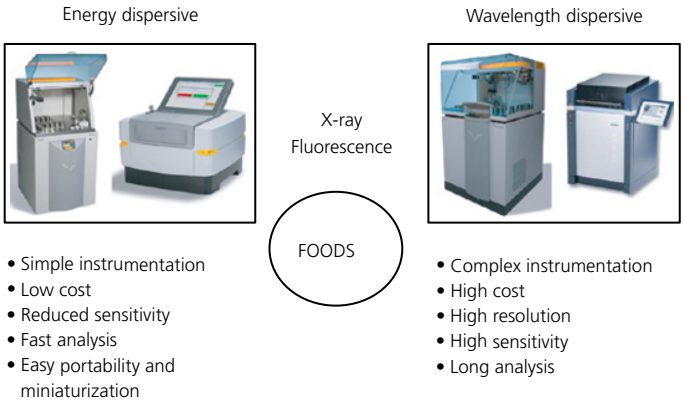


Figure 14.2 X-ray fluorescence instrumentation.

spectra from solid untreated samples and liquid foods directly, and this provides very fast methodologies suitable for use in the field as portable instruments (Table 14.4) [46–53]. However, the easy measurement of samples requires an appropriate calibration of the signals, this being one of the drawbacks of the technique. Additionally, the lack of high sensitivity of XRF, as compared with other commonly used techniques, reduces its direct application in the determination of major and minor components, it being difficult to determine the fluorescence of mineral elements present at trace and ultratrace levels in untreated samples.

Regarding the quantitative applications of XRF, there are numerous examples in geology, mining, metallurgy, arts and archaeology, environmental studies, and forensic and life sciences, with specific monographs on sampling, storage and sample preparation procedures for XRF analysis [54]. However, use of these techniques in food analysis cannot be extrapolated in the same way [1, 55]. Direct analysis of liquid food samples (e.g. water, juices, beverages) requires special containers of stainless steel, polyethylene or Teflon (PTFE) with a thin window of a low-Z material (Mylar®, Kapton® or Prolene®). However, this window produces a background radiation and absorbs the secondary radiation, especially from low-Z elements, thus limiting analysis of those elements at low concentration levels. Because solid samples are easier to analyse by XRF, an alternative is to convert liquid samples to quasi-solid ones by increasing the viscosity, by freezing or transforming into thin films or organic glassy polymers.

Common techniques for XRF analysis of solid food samples are based on the preparation of powders and pellets. Because these samples can exhibit significant heterogeneity, prior treatment based on drying, powdering and homogenizing can be required. Alternatively, lyophilization, dry ashing or wet digestion can provide adequate sample presentation for XRF measurements (Table 14.4).

On the other hand, the fact that XRF can be used directly on untreated samples avoids the use of reagents and acids and makes these methods greener than the traditional flame, electrothermal or plasma-based atomic spectroscopy techniques, drastically reducing the environmental side effects and costs. However, it must be clear that the area of sample employed for each XRF measurement is very small and it can be a problem when we consider the representativeness of the values obtained in such heterogeneous materials. Because of this, a relatively high number of individual measurements must be taken for each sample in order to obtain a complete picture of both mineral profile and heterogeneity of foods.

In summary, the direct application of XRF for evaluating the mineral profile of foods provides a green and reagent- and waste-free methodology that avoids the need for sample treatment steps in the determination of major and minor elements. However, when the measurement of trace or ultratrace elements is required, it is mandatory to perform a rigorous multiparametric chemometric calibration [56] and, in some cases, to extract the mineral elements from the samples and pre-concentrate before measuring their X-ray fluorescence [57]; unfortunately, this nullifies some of the main advantages attributed previously to this technique.

Table 14.4 Studies based on the use of handheld and portable XRF instrumentation for the analysis of foods.

Technique	Sample	Elements	Details	Reference
Handheld ED-XRF	Powdered milk	Ca	Using external standard and standard addition based methods	[47]
Portable XRF	Food, medicinal, and ceremonial items	Pb	Content cut-point of 10 mg/kg	[48]
Handheld ED-XRF	Food and beverage	Pb and other trace metals	Some measurements made in glass containers	[49]
Portable XRF	Beverages	As, Cd, Hg and Pb	Measurements made in cells and the original container	[50]
Portable XRF	Beverage and cocoa powder	Beverage spiked with Cr, Cu and As and cocoa powder spiked with As and Pb	Screening of foods	[51]
Portable-XRF	FDA-regulated products	Toxic elements	Rapid screening of toxic elements in FDA-regulated products	[52]
Portable XRF	Reference materials, flour, bakery products, malted barley and total diet study foods	Br	By using a calibration based on instrumental neutron activation analysis results for Br in reference materials. Accurate quantitative results obtained for mass fractions of about 2–55 mg/kg. Results showed that very short analysis times (<2 min) would be needed to screen foods for Br content at regulatory levels for brominated and enriched brominated flour (24 mg/kg Br) and whole wheat flour and bakery products (36 mg/kg Br). Quantitative results at 75 mg/kg Br level could not be assured because no reference material available	[53]
Handheld XRF	Poisoned food	As, Cr, Cu, Fe, Mo, Ni, Ph and Zn		[54]

14.4 XRF measurement techniques

Basic XRF can be supplied by wavelength dispersion or energy dispersion and it is clear that the first approach involves the use of more complex instrumentation than the second (see Figure 14.2). However, in order to improve the sensitivity of XRF, new developments and high-intensity primary emission sources are required. Total reflection X-ray fluorescence (TXRF), particle-induced X-ray emission (PIXE) and microscopy X-ray fluorescence with synchrotron radiation are some of the modern alternatives developed in this field which can be enhance the analysis of foods.

TXRF is a surface elemental analysis technique suitable for providing data about ultratrace elements in smooth surfaces, being basically an energy-dispersion XRF with a special geometry based on the total external reflection phenomenon discovered by Compton in 1922 [58, 59] and applied by Yoneda and Horiuchi in 1971 to XRF [60]. The use of a critical angle of the incident beam on a sample extended on a flat surface [61] produces reflection of almost all the excitation beam photons and helps to reduce the measurement background by the elimination of sample scattering, thus providing enhancement of the signal-to-noise ratio. This dramatically increases the sensitivity [62], providing a tool for the chemical analysis of small amounts of samples, from microgram to nanogram levels, with a detection limit of the order of micrograms to nanograms per millilitre. The technique has been applied to all fields including food analysis [63]. As shown in Table 14.5 [64–76], TXRF has been used to determine a series of elements in vegetables [64], dietary supplements [67], seafoods [72, 73] and also water samples [75], these measurements being employed for speciation purposes [76] and the authentication of the origin of foods based on trace

Table 14.5 Some examples of total reflection XRF (TXRF) studies in food analysis.

Technique	Sample	Elements	Application	Reference
TXRF, AAS, ICP-MS	Carrots, onions, potatoes, cottage cheese, eggs, honey	As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Na, Ni, Pb, Se and Zn	Quantitative major and trace element analysis	[64]
TXRF	Biological samples and related matrices	F, Cl, Br and I	Determination of halogens in bioanalytical sciences	[65]
TXRF, ICP-OES, FAES, INAA	Rice	36 essential, non-essential and toxic elements	Analysed using four techniques	[66]
TXRF	Medical samples, basic food and dietary supplements	Se		[67]
TXRF	Candies	Br, Cr, Cu, Fe, Mn, Ni, Pb, Rb, Sr, Ti and Zn,	Pb concentration fluctuated in the range of 0.102–0.342 μg/g	[68]
TXRF	Honey: 264 samples	Key elements: Cl, K, Mn and Rb	Determination of the botanical origin of Slovenian honey; principal component analysis (PCA) and regularized discriminant analysis (RDA)	[69]
TXRF	Mineral water, seaweed and dietary supplement tablets	I		[70]
TXRF	Africanized *Apis mellifera* honey: 38 samples	Ba, Br, Ca, Co, Cr, Cu, Fe, Hg, K, Mn, Ni, Ph, Rb, Se, Sr, Ti and Zn,	Means of three replicates: K (109.671 ± 17.487), Ca (14.471 ± 3.9797), Ti (0.112 ± 0.07), Cr (0.196 ± 0.11), Mn (0.493 ± 0.103), Fe (1.722 ± 0.446), Co (0.038), Ni (0.728 ± 0.706), Cu (0.179 ± 0.0471), Zn (0.967 ± 0.653), Se (not detected), Br (not detected), Rb (0.371 ± 0.097), Sr (0.145 ± 0.45), Ba (11.681), Hg (not detected), and Ph (0.863) μg/g	[71]
TXRF	Freshwater mussels	As, Ba, Be, Br, Cd, Cr, Cu, Fe, Mn, Ni, P, Pb, Se, Sr and Zn	Significant site-specific differences were found for the elements As, Ba, Be, Br, Cr, Fe, Mn, Ni, P and Sr. Concentrations of Cd, Cr, Cu, Pb, Se and Zn were below the levels regarded as harmful	[72]
TXRF, AAS	Freshwater fish: muscle and liver tissue	As, Ca, Cd, Cr, Cu, Fe, K, Mn, P, Pb, S, Se and Zn	Ca, Fe, K, Mn, P, Pb, S and Zn showed significantly higher concentrations in the hepatic tissue from the exposed site	[73]
TXRF	Wheat roots	Fe and Pb	Microwave-assisted acidic digestion and TXRF spectrometry	[74]
TXRF	Water samples	20–25 elements	Multi-element determination can be carried out simultaneously for detection limits at the ppt (ng/L) level	[75]
TXRF	Biological matrices: 12 different vegetable foodstuffs	Ca, Cu, Fe, K, Mn, Rb, Sr and Zn	Speciation. It requires only one internal standard and minute samples with simple preparation	[76]

element analysis together with comprehensive chemometric data treatment [69].

PIXE is another non-destructive elemental analysis technique, based on the use of ions for the bombardment of atoms present in a sample in order to cause inner shell ionization. Usually protons with MeV associated energy are used instead of the electron-based X-ray. In fact, an energy-dispersive detector is used to obtain the fluorescence spectra and measure the intensity of the different lines emitted. However, PIXE offers a better

peak-to-noise ratio than classical ED-XRF and thus increases the sensitivity to parts per million levels, being able to determine elements from sodium through uranium at very low levels in a single spectrum and without sample destruction. Proposed for the first time in 1970 by S. Johansson [77] and developed later by R. Akselsson and T.B. Johansson, PIXE is a perfect technique for use in heritage analysis of samples with flat and uniform composition, and for the study of new materials with different layers for which it can provide two-dimensional maps of elemental composition. However, it has also been employed in food analysis (Table 14.6) [78–84], including the direct determination of total mineral elements in edible grains [80], rice and maize flour [81] and canned tuna [79], and also for evaluating the influence of industrial processing on maté tea leaves [82] and the spatial distribution of minerals in wheat grains [78]. Additionally, there are two studies on the rapid digestion of edible foods and plants produced from contaminated areas by using laser-assisted pulsed ultraviolet light photolysis followed by a pre-concentration of metals through complexation as carbamates [83, 84]. Thus new developments in this field should be expected in the next few years, especially relating to the distribution of trace elements in foods.

The modern synchrotron facilities around the world have provided new tools in many basic and applied fields of physics and chemistry, and one of these involves the use of synchrotron radiation, an electromagnetic radiation emitted when charged particles are accelerated radially, as a powerful X-ray excitation radiation to provide XRF measurements from samples [85]. In fact, synchrotron radiation (SR) micro-XRF [86] is possible because of the high intensity and directionality of SR sources based on fast electrons moving through magnetic fields that provide a broad spectrum with a high flux (~10^{13} photons per second) and photons that are highly brilliant (photons s^{-1} milliradian^{-2} mm^{-2} per 0.1% of radiation bandwidth), very stable, and polarizable linear and circularly, thus creating a quasi-monochromatic micro-X-ray beam suitable for reducing the relative contribution of scattered radiation by the samples because of the polarization of the incident radiation and thus providing absolute mass detection limits for elements with atomic number above 14 of between 1000 and 0.1 ag for measuring times of 300 s. This can provide relative detection limits of 0.010 ppm for the determination of elements with an atomic number around 35 and using biological samples with a thickness between 100 μm and 1 mm and with average quantification accuracy in the range of 5% for elements above atomic number 12 determined through their K and L fluorescence radiation. Thus, SR micro-XRF has become a fantastic tool for the spatially resolved distribution and

Table 14.6 Particle-induced X-ray emmision (PIXE) applications in food analysis.

Technique	Sample	Elements	Details	Reference
Micro-PIXE	Wheat grain	Ca, Cd, Cu, Fe, K, Mg, Mn, Mo, Na,P, Pb, S and Zn	Spatial distributions at the tissue level	[78]
PIXE	Brazilian canned tuna fish packed in oil and brine	Cl, Fe, K, Mg, Na, P, S and Zn	Elemental characterization of Brazilian canned tuna fish	[79]
Micro-PIXE	Edible grains: buckwheat	Al, Ca, Cl, Cu, Fe, K, Mg, Mn, P, S, Si, Ti and Zn	Analysis of nutritionally relevant heavy elements in different tissues of the grain	[80]
PIXE	Rice and maize flour	Essential elements		[81]
PIXE	Maté tea leaves	Al, Ca, Fe, K, Mg, Mn, P, Rb, S, Si, Ti and Zn	Influence of the industrial processing	[82]
PIXE	Plant material: wastewater irrigated fruits, vegetables and crops	Co, Cu, Fe, Ni, Pb and Zn	Rapid material digestion by radicals produced with laser-assisted pulsed UV (PUV) photolysis of aqueous solutions containing H_2O_2. Metals pre-concentrated as carbamates	[83]
PIXE, XRF	Edible foods from contaminated agricultural areas	Co, Cu, Fe, Ni, Pb and Zn	UV (PUV)/PIXE and determinations at levels of 1 mg/kg	[84]

speciation of elements present in any type of matrix, suitable for distinguishing valence states of elements like Cr, Fe, Zn, U or Pu, based on the extended X-ray absorption fine structure that provides information on the number and distance of neighbouring atoms by measuring the oscillation from approximately 50 eV above the edge.

In the food field (Table 14.7 and Table 14.8), SR-XRF [87–98] and micro-XRF [99–106] have been applied to the analysis of the composition of berries and honey [98], the presence of metals in fish muscle and viscus [96] and vegetables [95], and recently the presence and accumulation of TiO_2 and other oxide nanoparticles in cucumbers [87] and soybean [89] and speciation of selenium chemical forms [90] and distribution of As and Se [87].

In addition to the aforementioned studies, in the last few years three-dimensional [104, 105] and polarized ED-XRF [106] studies have been applied to the study of the mineral profile of foods. As shown in Table 14.8,

Table 14.7 Applications of XRF in food analysis using synchrotron radiation.

Technique	Sample	Elements	Application	Reference
S-micro-XRF	Cucumber	TiO_2 nanoparticles	TiO_2 accumulation in cucumber fruit and a possible pathway of TiO_2 nanoparticle transfer from soil into the food chain	[87]
S-micro-XRF	Plants, cucumber leaf	Fe	Understand the metabolism and bioavailability through the food chain. Fe (semi)quantitative distribution maps were obtained	[88]
S-micro-XRF	Soybean tissues	ZnO and CeO_2 nanoparticles	Mapping and speciation of CeO_2 and ZnO nanoparticles in soil cultivated soybean	[89]
S-XRF	Rice	Se-methionine, Se-methylcysteine, selenite and selenate	Grain accumulation of selenium species in rice	[90]
S-XRF	Plant parts	Ca	Identifying the relationship between calcium location in the plant cell and nutrient bioavailability	[91]
S-TXRF	Fish	Cr and Zn	Accumulated Cr values are above the limits defined in the Brazilian legislative norm on food	[92]
S-X-ray	Cereal grains	As and Se	Distribution of elements	[93]
S-XRF	Bivalves, oysters, mussels, and clams		Elemental distributions in bivalves	[94]
S-TXRF	Vegetables, leafy vegetables, fruits, cereal and grain	Cr, Cu, Ph and Zn	Some samples were contaminated with concentrations much higher than the reference values	[95]
S-TXRF	Fish	Al, Ba, Ca, Cl, Cr, Cu, Fe, K, Si, Ti, Mn, Ni, P, S, V and Zn	Analysis of muscle and viscus (liver, intestine and stomach) fish samples for environmental monitoring and food safety assessment	[96]
S-TXRF	Soft drinks	20 elements, including Al, As, Cr, Pb, Sb,Ti and Sb	20 elements were detected in the investigated samples, including some trace elements which can be toxic for humans, in soft drinks and in poly(ethylene terephthalate) containers	[97]
S-XRF	Food chain: berries, Iceland moss, cedar kernels, honey	As, Bi, Br, Ca, Co, Cr, Cu, Fe, Ga, Ge, Hg, K, Ti, Mn, Mo, Ni, Nb, Pb, Rb, Se, Sr, Th, U, V, Y, Zn and Zr	Specific features of the multi-element composition of biosubstrates	[98]

Table 14.8 Recent studies on modern methodologies for XRF analysis of foods.

Technique	Sample	Elements	Application	Reference
Micro-XRF, ED-XRF	Lettuce, parsley and ryegrass	Pb	To investigate the localization and the speciation of lead in their edible parts, i.e. leaves	[99]
Micro-XRF, LC-MS	Spineless prickly pear cactus	Se	Selenium accumulation, distribution and speciation	[100]
Micro-XRF	Chicken hotdogs	Fe filings	Food forensic application	[101]
Micro-XRF and micro-XANES	Rice	Se	Localization and speciation of Se	[102]
Micro-ED-XRF probe	Bread and biscuits	Chemical composition	In the frame of the European project 'Nanopathology'	[103]
3D-ED-XRF	Wheat flour	Al, Br, Ca, Cd, Cl, Cu, Fe, K, Mg, Mn, Mo, Na, P, Rb, S, Sr, Zn.	Provenance analysis of wheat flours	[104]
3D-XRF	Spinach leaves	Ba, Br, Ca, Cd, K, Mo, Rb and Zn	Characterize the production locality: XRF technique is suitable for rapid and easy screening analysis of Cd in vegetables at their production sites	[105]
Polarized ED-XRF	Spice plants	Elemental composition	Polarized energy dispersive X-ray fluorescence	[106]

Micro-XANES, micro-X-ray absorption near edge structure.

these techniques have been used for the non-destructive determination of elemental composition of wheat flour, spices and vegetable leaves.

14.5 Calibration and data treatment

Despite the specificity of X-ray transitions, which provide clear qualitative profiles for elements present in different chemical forms in food samples, it is unfortunate that the matrix of foods strongly influences the intensity of the emitted radiation and this affects the ability of the technique to obtain accurate quantitative data. Because of this, careful calibration must be done or, alternatively, samples must be treated at high temperatures with reagents to obtain a comparable structure for both natural samples and prepared standards.

The difficulties found in correctly calibrating X-ray fluorescence measurements is at the centre of any general consideration of this technique as a semi-quantitative tool. However, we are convinced that using deep chemometric study it is possible to model X-ray fluorescence signals to correctly predict both the mineral profile and the concentrations of as many elements as possible present in the food sample, despite the fact that lack of sensitivity limits the number of elements which can be directly determined.

In general, methods available in the literature are based on the use of a limited number of certified reference samples to create the calibration. However, in recent years advances in chemometrics have provided special calibration strategies based on signals corresponding to samples of the same type as those to be analysed and on partial least squares (PLS) or artificial neuronal networks (ANN) algorithms suitable for building the best calibration models that allow XRF signals to be transformed into mineral element concentrations. This is probably the best way to extract as much information as possible from the X-ray spectra of foods in order to obtain a description of the samples with regard to both essential and toxic element contents and to guarantee the safety of foods and the effects of their production processes on their mineral profile. To do this, non-destructive and fast methodologies free from enviromentally deleterious effects are urgently required.

14.6 Future perspectives in food XRF analysis

Previous chapters of this book have examined the available methodologies for establishing the mineral profile of foods at major, minor, trace and ultratrace levels through the use of atomic and ionic methods of analysis, including electrochemical tools. It is therefore clear that we have sufficient technologies with high degrees of sensitivity and selectivity but which involve, in numerous cases, prior sample treatment to obtain appropriate solutions of the mineral elements to be analysed, the only exception being neutron activation analysis where the elemental composition of foods can be directly obtained from untreated samples irradiated with accelerated neutrons in a nuclear reactor. However, this seems an unlikely solution to the problem of transferring technology from the academic world to the real world of everyday analysis of foods in small laboratories. Thus for policy-makers to guarantee the safety of foods and prevent fraudulent practices, it seems mandatory to have simple tools suitable for use by small laboratories in routine analysis for screening purposes, and luckily XRF spectroscopy appears to be such a high-performance tool.

When comparing XRF with the modern use of infrared methodologies, in both the NIR and MIR ranges (see Chapter 15), it can be concluded that the signals provided by XRF are closer to the actual mineral composition of samples than those determined in the infrared range, and this must encourage us to improve the available methodologies to explore the semi-quantitative and quantitative capabilities of low-cost and portable instruments. However, it is also clear that XRF is not a very sensitive technique, especially the ED-XRF portable instrumentation. Therefore we need to explore the geometry of the instruments in order to perform total reflection measurements and also to incorporate gentle and fast sample treatments, especially leaching of target analytes and their pre-concentration in solid matrices, suitable to move the limit of detection from percentage and parts per thousand levels to ppm and ppb levels [107]. Thus we can expect a tremendous increase in the number of papers based on XRF over the next few years with original methodologies suitable for controlling minerals and mineral species in foods.

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Abbreviations

ANN: Artificial neuronal networks
ED-XRF: Energy dispersion X-ray fluorescence
MIR: Middle infrared
NIR: Near infrared
PLS: Partial least squares
PIXE: Particle induced X-ray emission
PTFE: Polytetrafluoroethylene
SD: Semiconductor detector
SDD: Silicon drift detector
Si-PIN: Peltier-cooled silicon detectors
SR: Synchrotron radiation
TXRF: Total reflection X-ray fluorescence
WD-XRF: Wavelength dispersion X-ray fluorescence
XRF: X-ray fluorescence

References

1 Beckhoff B, Kanngießer B, Langhoff N, Wedell R, Wolff H (eds) *Handbook of Practical X-Ray Fluorescence Analysis*. Springer-Verlag, Heidelberg, 2006.
2 Zhao HY, Guo BL, Wei YM, Zhang B. Multi-element composition of wheat grain and provenance soil and their potentialities as fingerprints of geographical origin. *J Cereal Sci* 2013;**57**:391–397.
3 Zaidi MA, Shaheen G, Jehan N, Mansoor A, Yousafzai A. Elemental composition of some arid environment fodder and medicinal plants of Quetta, Balochistan. *J Chem Soc Pakistan* 2010;**32**:71–77.
4 Sastri CS, Iyengar V, Demirel-Gulen S *et al*. Determination of boron, fluorine and some medium Z elements in diet samples. *J Radioanal Nucl Chem* 2004;**259**:515–520.
5 Nas S, Gokalp HY, Sahin Y. K-content and Ca content of fresh green tea, black tea, and tea residue determined by X-ray-fluorescence analysis. *Z Lebensm Unters Forsch A* 1993;**196**:32–37.
6 Nielson KK, Mahoney AW, Williams LS, Rogers VC. Mineral concentrations and variations in fast-food samples analyzed by X-ray-fluorescence. *J Agric Food Chem* 1991;**39**:887–892.

7 Nielson KK, Mahoney AW, Rogers VC. X-ray-fluorescence and atomic-absorption spectrophotometry measurements of manganese, iron, copper, and zinc in selected foods. *J Agric Food Chem* 1998;**36**:1211–1216.

8 Bower NW, Gladney ES, Ferenbaugh RW. Critical comparison of X-ray-fluorescence and combustion: infrared methods for the determination of sulfur in biological matrices. *Analyst* 1986;**111**:105–106.

9 Rethfeld H. Determination of traces of heavy-metals in foods and feeding stuffs of vegetable and animal origin, soil extracts, sewage sludges and floor coatings by means of X-ray-fluorescence analysis. *Fresenius Z Anal Chem* 1982;**310**: 127–130.

10 Scheubeck E, Jorrens C. Preparation of samples for X-ray-fluorescence analysis for determining traces of heavy-metals in foodstuffs. *Siemens Forschungs-Und Entwicklungsberichte, Siemens Research and Development Reports* 1980;**10**:29–33.

11 Menke H. Investigation of a method for determination of lead cadmium, mercury, arsenic and tellurium in food by means of X-ray-fluorescence analysis. *Fresenius Z Anal Chem* 1977;**286**:31–35.

12 King RT. Determination of calcium, potassium, chlorine, sulfur and phosphorus in foodstuffs by X-ray-fluorescence spectrometry. *J Sci Food Agric* 1977;**28**:631–634.

13 Patti F. Quantitative determination of stable strontium by fluorescence x-ray spectrography in biological samples. *Bull Soc Chim Fr* 1970;**11**:4145–4152.

14 Otaka A, Hokura A, Nakai I. Determination of trace elements in soybean by X-ray fluorescence analysis and its application to identification of their production areas. *Food Chem* 2014;**147**:318–326.

15 Chan KW, Khong NMH, Iqbal S, Mansor SM, Ismail M. Defatted kenaf seed meal (DKSM): prospective edible flour from agricultural waste with high antioxidant activity. *LWT Food Sci Technol* 2013;**53**:308–313.

16 Paltridge NG, Palmer LJ, Milham PJ, Guild GE, Stangoulis JCR. Energy-dispersive X-ray fluorescence analysis of zinc and iron concentration in rice and pearl millet grain. *Plant Soil* 2012;**361**:251–260.

17 Paltridge NG, Milham PJ, Ortiz-Monasterio JI *et al*. Energy-dispersive X-ray fluorescence spectrometry as a tool for zinc, iron and selenium analysis in whole grain wheat. *Plant Soil* 2012;**361**:261–269.

18 Imai A, Otaka A, Nakamura S, Kadokura M, Suzuki T, Nakai I. Determination of trace elements in pumpkin seeds by X-ray fluorescence analysis and its application to identifying the geographic origin of individual pumpkins. *J Jpn Soc Food Sci Technol* 2012;**59**:338–347.

19 Aich A, Chakraborty A, Sudarshan M, Chattopadhyay B, Mukhopadhyay SK. Study of trace metals in Indian major carp species from wastewater-fed fishponds of East Calcutta Wetlands. *Aquacult Res* 2012;**43**:53–65.

20 Ekinci N, Dogan B, Han I, Agbaba M. Analysis of infant foods using energy dispersive X-ray fluorescence spectrometry. *Asian J Chem* 2011;**23**:3955–3957.

21 Garshott DM, MacDonald EA, Murray MN, Benvenuto MA, Roberts-Kirchhoff ES. Elemental analysis of a variety of dried, powdered, kelp food supplements for the presence of heavy metals via energy-dispersive X-ray fluorescence spectrometry. In: Benvenuto MA, Roberts-Kirchhoff ES, Murray MN, Garshott DM (eds) *It's All in the Water: Studies of Materials and Conditions in Fresh and Salt Water Bodies*. ACS Symposium Series, Vol. **1086**. American Chemical Society, Washington, DC, 2011, pp. 123–133.

22 Matasin Z, Ivanusic M, Orescanin V, Nejedli S, Gajger IT. Heavy metal concentrations in predator fish. *J Anim Vet Adv* 2011;**10**:1214–1218.

23 Mutsuga M, Sato K, Hirahara Y, Kawamura Y. Analytical methods for SiO_2 and other inorganic oxides in titanium dioxide or certain silicates for food additive specifications. *Food Addit Contam A* 2010;**28**:423–427.

24 Perring L, Monard F. Improvement of energy dispersive X-ray fluorescence throughput: influence of measuring times and number of replicates on validation performance characteristics. *Food Anal Method* 2010;**3**:104–115.

25 Radulescu C, Stihi C, Busuioc G, Gheboianu AI, Popescu IV. Studies concerning heavy metals bioaccumulation of wild edible mushrooms from industrial area by using spectrometric techniques. *Bull Environ Contam Toxicol* 2010;**84**:641–646.

26 Deladino L, Navarro AS, Martino MN. Microstructure of minerals and yerba mate extract co-crystallized with sucrose. *J Food Eng* 2010;**96**:410–415.

27 Mohapatra A, Rautray TR, Patra AK, Vijayan V, Mohanty RK. Trace element-based food value evaluation in soft and hard shelled mud crabs. *Food Chem Toxicol* 2009;**47**: 2730–2734.

28 Raya-Perez JC, Aguirre-Mancilla CL. Elemental composition of several weld Mexican plants. *Rev Chapingo Ser Cienc For Am* 2009;**15**:95–99.

29 Sussulini A, Lima AG, Figueiredo EC *et al*. X-ray scattering information of ED-XRF technique for powdered fruit juice mixes. *X-Ray Spectrom* 2009;**38**:254–257.

30 Anacleto P, Lourenco HM, Ferraria V *et al*. Total arsenic content in seafood consumed in Portugal. *J Aquat Food Prod Technol* 2009;**18**:32–45.

31 Cevik U, Damla N, Kobya AI *et al*. Assessment of metal element concentrations in mussel (M. galloprovincialis) in Eastern Black Sea, Turkey. *J Hazard Mater* 2008;**160**:396–401.

32 Barrento S, Marques A, Teixeira B, Vaz-Pires P, Carvalho ML, Nunes ML. Essential elements and contaminants in edible tissues of European and American lobsters. *Food Chem* 2008;**111**:862–867.

33 Teixeira B, Barrento S, Carvalho ML, Nunes ML, Marques A. Macro and trace elements in edible tissues of Carcinus maenas and Necora puber. *J Sci Food Agric* 2008;**88**: 2451–2459.

34 Mohapatra A, Rautray TR, Vijayan V, Mohanty RK, Dey SK. Trace elemental characterization of some food crustacean tissue samples by EDXRF technique. *Aquaculture* 2007;**270**: 552–558.

35 Nodaa T, Tsudaa S, Moria M *et al*. Determination of the phosphorus content in potato starch using an energy-dispersive X-ray fluorescence method. *Food Chem* 2006;**95**:632–637.

36 Perring L, Andrey D, Basic-Dvorzak M, Hammer D. Rapid quantification of iron, copper and zinc in food premixes using energy dispersive X-ray fluorescence. *J Food Compos Anal* 2005;**18**:655–663.

37 Jayasekera R, Freitas MC. Concentration levels of major and trace elements in rice from Sri Lanka as determined by the k(0) standardization method. *Biol Trace Elem Res* 2005;**103**:83–96.

38 Dim LA, Kinyua AM, Munyithya JM, Adetunji J. Lead and other metals distribution in local cooking salt from the Fofi salt-spring in Akwana, Middle Benue Trough, Nigeria. *J Environ Sci Health B* 1991;**26**:357–365.

39 Hondrogiannis E, Ehrlinger E, Miziolek AW. Spectroscopy methods for identifying the country of origin. In: Drury MA, Crocombe RA (eds) *Next-Generation Spectroscopic Technologies VI*. Proceedings of SPIE, Vol. **8726**. SPIE Press, 2013, Article UNSP 87260Q.

40 Akbaba U, Sahin Y, Turkez H. Element content analysis by WDXRF in pistachios grown under organic and conventional farming regimes for human nutrition and health. *Toxicol Ind Health* 2012;**28**:783–788.

41 Akbaba U, Sahin Y, Turkez H. Comparison of element contents in haricot beans grown under organic and conventional farming regimes for human nutrition and health. *Acta Sci Pol Hortorum Cultus* 2012;**11**:117–125.

42 Pashkova GV. X-ray fluorescence determination of element contents in milk and dairy products. *Food Anal Method* 2009;**2**:303–310.

43 Perring L, Andrey D, Basic-Dvorzak M, Blanc J. Rapid multimineral determination in infant cereal matrices using wavelength dispersive X-ray fluorescence. *J Agric Food Chem* 2005;**53**:4696–4700.

44 Jastrzebska A, Brudka B, Szymanski T, Szlyk E. Determination of phosphorus in food samples by X-ray fluorescence spectrometry and standard spectrophotometric method. *Food Chem* 2003;**8**:463–467.

45 Potts PJ, West M (eds) *Portable X-ray Fluorescence Spectrometry. Capabilities for In Situ Analysis*. RSC Publishing, Cambridge, 2008.

46 Chan JC, Palmer PT. Determination of calcium in powdered milk via X-ray fluorescence using external standard and standard addition based methods. *J Chem Educ* 2013; **90**:1218–1221.

47 Reames G, Charlton V. Lead detection in food, medicinal, and ceremonial items using a portable X-ray fluorescence (XRF) instrument. *J Environ Health* 2013;**75**:16–20.

48 Agostino A, Falcone R. Rapid quantitative analysis of lead and other trace metals in glass containers by handheld ED-XRF. *Glass Technol Eur J Glass Sci Technol A* 2012;**53**:60–64.

49 Anderson DL. Analysis of beverages for Hg, As, Pb, and Cd with a field portable X-ray fluorescence analyzer. *J AOAC Int* 2010;**93**:683–693.

50 Anderson DL. Screening of foods and related products for toxic elements with a portable X-ray tube analyzer. *J Radioanal Nucl Chem* 2009;**282**:415–418.

51 Palmer PT, Jacobs R, Baker PE, Ferguson K, Webber S. Use of field-portable XRF analyzers for rapid screening of toxic elements in FDA-regulated products. *J Agric Food Chem* 2009;**57**:2605–2613.

52 Anderson DL. Determination of bromine in regulated foods with a field-portable X-ray fluorescence analyzer. *J AOAC Int* 2009;**92**:502–510.

53 Ida H, Kawai J. Analysis of wrapped or cased object by a hand-held X-ray fluorescence spectrometer. *Forensic Sci Int* 2005;**151**:267–272.

54 International Atomic Energy Agency. *Sampling, Storage and Sample Preparation Procedures for X-Ray Fluorescence Analysis of Environmental Materials*. IAEA, Vienna, 1997.

55 Aras NK, Ataman OY. X-ray methods. In: *Trace Element Analysis of Food and Diet*. RSC Publishing, Cambridge, 2006, pp. 192–194.

56 Terra J, Antunes AM, Prado MA, Bueno MIMS. Energy value determinations of industrialized foods: the potential of using X-ray spectroscopy and partial least squares. *X-Ray Spectrom* 2010;**39**:167–175.

57 Margui E, Zawisza B, Sitko R. Trace and ultratrace analysis of liquid samples by X-ray fluorescence spectrometry. *Trends Anal Chem* 2014;**53**:73–83.

58 Compton AH. Secondary radiations produced by X-rays, and some of their applications to physical problems. *Bull Natl Res Counc* 1922;**4**:1–56.

59 Compton AH. The total reflection of X-rays. *Philos Mag* 1923;**45**:1121–1131.

60 Yoneda Y, Horiuchi T. Optical flats for use in X-ray spectrochemical microanalysis. *Rev Sci Instrum* 1971;**42**:1069–1070.

61 Wobrauschek P. Total reflection X-ray fluorescence analysis: a review. *X-Ray Spectrom* 2007;**36**:289–300.

62 Alov NV. Total reflection X-ray fluorescence analysis: physical foundations and analytical application (a review). *Inorg Mater Engl Tr* 2011;**47**:1487–1499.

63 Gruber X, Kregsamer P, Wobrauschek P, Streli C. Total-reflection X-ray fluorescence analysis of Australian wine. *Spectrochim Acta B* 2006;**61**:1214–1218.

64 Vincevica-Gaile Z, Klavins M, Rudovica V, Viksna A. Research review trends of food analysis in Latvia: major and trace element content. *Environ Geochem Health* 2013;**35**:693–703.

65 Mello PA, Barin JS, Duarte FA *et al*. Analytical methods for the determination of halogens in bioanalytical sciences: a review. *Anal Bioanal Chem* 2013;**405**:7615–7642.

66 Antoine JMR, Fung LAH, Grant CN, Dennis HT, Lalor GC. Dietary intake of minerals and trace elements in rice on the Jamaican market. *J Food Compos Anal* 2012;**26**:111–121.

67 Stosnach H. Analytical determination of selenium in medical samples, staple food and dietary supplements by means of total reflection X-ray fluorescence spectroscopy. *Spectrochim Acta B* 2010;**65**:859–863.

68 Martinez T, Lartigue J, Zarazua G, Avila-Perez P, Navarrete M, Tejeda S. Total reflection X-ray fluorescence analysis of trace-elements in candies marketed in Mexico. *Spectrochim Acta B* 2010;**65**:499–503.

69 Necemer M, Kosir IJ, Kump P *et al*. Application of total reflection X-ray spectrometry in combination with chemometric methods for determination of the botanical origin of Slovenian honey. *J Agric Food Chem* 2009;**57**:4409–4414.

70 Varga I. Iodine determination in dietary supplement products by TXRF and ICP-AES spectrometry. *Microchem J* 2007; **85**:127–131.

71 Sodre GD, Marchini LC, Zucchi OLAD, Nascimento VF, Otsuk IP, Moreti ACDC. Determination of chemical elements in africanized Apis mellifera (Hymenoptera : Apidae) honey samples from the state of Piaui, Brazil. *Quim Nova* 2007;**30**:920–924.

72 Wagner A, Boman J. Biomonitoring of trace elements in Vietnamese freshwater mussels. *Spectrochim Acta B* 2004;**59**: 1125–1132.

73 Wagner A, Boman J. Biomonitoring of trace elements in muscle and liver tissue of freshwater fish. *Spectrochim Acta B* 2003;**58**:2215–2226.

74 Varga A, Zaray G, Fodor F, Cseh E. Study of interaction of iron and lead during their uptake process in wheat roots by total-reflection X-ray fluorescence spectrometry. *Spectrochim Acta B* 1997;**52**:1027–1032.

75 Klockenkamper R, vonBohlen A. Elemental analysis of environmental samples by total reflection X-ray fluorescence: a review. *X-Ray Spectrom* 1996;**25**:156–162.

76 Gunther K, Vonbohlen A, Strompen C. Element determination by total-reflection X-ray-fluorescence spectrometry at the initial step of element speciation in biological matrices. *Anal Chim Acta* 1995;**309**:327–332.

77 Johansson SAE, Campbell JL, Malmqvist KG (eds) *Particle-Induced X-Ray Emission Spectrometry (PIXE)*. John Wiley & Sons, Inc., New York, 1995.

78 Pongrac P, Kreft I, Vogel-Mikus K *et al*. Relevance for food sciences of quantitative spatially resolved element profile investigations in wheat (Triticum aestivum) grain. *J R Soc Interface* 2013;**10**:Article Number 20130296. doi: 10.1098/rsif.2013.0296.

79 Boufleur LA, Iochims dos Santos CE, Debastiani R, Yoneama ML, Amaral L, Ferraz Dias J. Elemental characterization of Brazilian canned tuna fish using particle induced X-ray emission (PIXE). *J Food Compos Anal* 2013;**30**:19–25.

80 Vogel-Mikus K, Pelicon P, Vavpetic P, Kreft I, Regvar M. Elemental analysis of edible grains by micro-PIXE: common buckwheat case study. *Nucl Instrum Method Phys Res B* 2009;**267**:2884–2889.

81 Mohammed NK, Spyrou NM. The elemental analysis of staple foods for children in Tanzania as a step to the improvement of their nutrition and health. *Appl Radiat Isotopes* 2009;**67**:480–483.

82 Giulian R, Iochims dos Santos CE, Shubeita SM, Manfredi da Silva L, Yoneama ML, Ferraz Dias J. The study of the influence of industrial processing on the elemental composition of mate tealeaves (Ilex paraguariensis) using the PIXE technique. *LWT Food Sci Technol* 2009;**42**:74–80.

83 Mireles A, Solís C, Lagunas-Solar MC *et al*. Enhanced trace element analysis of plant materials after accelerated pulsed UV digestion. *J Radioanal Nucl Chem* 2008;**277**:441–449.

84 Lagunas-Solar MC, Cecilia PU, Solis C, Mireles A. Accelerator-based trace element analysis of foods and agriculture products. *Nucl Instrum Method Phys Res B* 2008;**266**:2391–2395.

85 Lida A. Synchrotron radiation X-ray fluorescence spectrometry. In: Meyers RA (ed.) *Encyclopedia of Analytical Chemistry*. John Wiley & Sons, Inc, 2013, 1–23.

86 Adams F, Vekemans B, Silversmit G, De Samber B, Vincze L. Microscopy X-ray fluorescence analysis with synchrotron radiation sources. In: Vértes A, Nagy S, Klencsár Z, Lovas RG, Rösch F (eds) *Handbook of Nuclear Chemistry*, 2nd edn, Vol. **3**. Springer Science, New York, 2011, chapter 34.

87 Servin AD, Morales MI, Castillo-Michel H *et al*. Synchrotron verification of TiO_2 accumulation in cucumber fruit: a possible pathway of TiO_2 nanoparticle transfer from soil into the food chain. *Environ Sci Technol* 2013;**47**:11592–11598.

88 Terzano R, Alfeld M, Janssens K *et al*. Spatially resolved (semi)quantitative determination of iron (Fe) in plants by means of synchrotron micro X-ray fluorescence. *Anal Bioanal Chem* 2013;**405**:3341–3350.

89 Hernandez-Viezcas JA, Castillo-Michel H, Andrews JC *et al*. In situ synchrotron X-ray fluorescence mapping and speciation of CeO_2 and ZnO nanoparticles in soil cultivated soybean (Glycine max). *ACS Nano* 2013;**7**:1415–1423.

90 Carey AM, Scheckel KG, Lombi E *et al*. Grain accumulation of selenium species in rice (Oryza sativa L.). *Environ Sci Technol* 2012;**46**:5557–5564.

91 Yang J, Punshon T, Guerinot ML, Hirschi KD. Plant calcium content: ready to remodel. *Nutrients* 2012;**4**:1120–1136.

92 Espinoza-Quinones FR, Modenes AN, Palacio SM *et al*. Evaluation of trace element levels in muscles, liver and gonad of fish species from Sao Francisco River of the Parana Brazilian state by using SR-TXRF technique. *Appl Radiat Isotopes* 2010;**68**:2202–2207.

93 Moore KL, Schroder M, Lombi E *et al*. NanoSIMS analysis of arsenic and selenium in cereal grain. *New Phytol* 2010;**185**: 434–445.

94 Jones KW, Bronson S, Brink P *et al*. Bivalve characterization using synchrotron micro X-ray fluorescence. *Acta Phys Pol A* 2009;**115**:477–481.

95 Vives AES, Moreira S, Brienza SMB, Zucchi OLAD, Nascimento VF. Synchrotron radiation total reflection X-ray fluorescence (SR-TXRF) for evaluation of food contamination. *J Radioanal Nucl Chem* 2006;**270**:147–153.

96 Vives AES, Moreira S, Brienza SMB, Zucchi OLAD, Nascimento VF. Analysis of fish samples for environmental monitoring and food safety assessment by synchrotron

radiation total reflection X-ray fluorescence. *J Radioanal Nucl Chem* 2006;**270**:231–236.

97 Zucchi OLAD, Moreira S, Salvador MJ, Santos LL. Multielement analysis of soft drinks by X-ray fluorescence spectrometry. *J Agric Food Chem* 2005;**53**:7863–7869.

98 Koutzenogii KP, Savchenko TI, Chankina OV, Kovalskaya GA, Osipova LP, Bgatov AV. Synchrotron radiation X-ray fluorescence analysis (SRXRF) for measuring the multielement composition of samples of biogenic nature. *J Trace Microprobe Tech* 2003;**21**:311–325.

99 Schreck E, Foucault Y, Sarret G *et al*. Metal and metalloid foliar uptake by various plant species exposed to atmospheric industrial fallout: mechanisms involved for lead. *Sci Total Environ* 2012;**427**:253–262.

100 Banuelos GS, Fakra SC, Walse SS *et al*. Selenium accumulation, distribution, and speciation in spineless prickly pear cactus: a drought- and salt-tolerant, selenium-enriched nutraceutical fruit crop for biofortified foods. *Plant Physiol* 2011;**155**:315–327.

101 St Jeor VL, Muroski AR, McGuire C, Lape A. Micro-X-ray fluorescence in food forensics. In: McNulty I, Eyberger C, Lai B (eds) *AIP Conference Proceedings* 2011;**1365**:411–414. doi: 10.1063/1.3625390.

102 Williams PN, Lombi E, Sun GX *et al*. Selenium characterization in the global rice supply chain. *Environ Sci Technol* 2009;**43**: 6024–6030.

103 Gatti AM, Tossini D, Gambarelli A, Montanari S, Capitani F. Investigation of the presence of inorganic micro- and nanosized contaminants in bread and biscuits by environmental scanning electron microscopy. *Crit Rev Food Sci Nutr* 2009;**49**:275–282.

104 Otaka A, Yanada Y, Hokura A, Matsuda K, Nakai I. Determination of trace elements in wheat flour by X-ray fluorescence analysis and its application to identification of their production area. *Bunseki Kagaku* 2009;**58**:1011–1022.

105 Yanada Y, Hokura A, Matsuda K, Mizuhira M, Nakai I. High-sensitive determination of inorganic elements in spinach leaves by X-ray fluorescence analysis and its application to identification of their production area. *Bunseki Kagaku* 2007;**56**:1053–1061.

106 Uestuenda Z. Polarized energy dispersive X-ray fluorescence applications of spice samples. *Spectrosc Lett* 2009;**42**:7–11.

107 Spietelun A, Marcinkowski L, de la Guardia M, Namiesnik J. Recent developments and future trends in solid phase microextraction techniques towards green analytical chemistry. *J Chromatogr A* 2013;**1321**:1–13.

CHAPTER 15
Vibrational spectroscopy

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Abstract: The use of infrared spectroscopy, in both the middle infrared and near infrared ranges, for the direct determination of the mineral composition of foods has been reviewed in order to evaluate the possibilities offered by vibrational spectroscopy in this field, paying special attention to the chemometric methods employed to build the corresponding models from well-characterized samples and the possibilities offered for the prediction of total mineral content and speciation in foods.

Keywords: infrared, near infrared, NIR, middle infrared, MIR, minerals, metals, elements, food

15.1 Vibrational spectra of foods

Vibrational spectroscopy offers an extended wavenumber range that includes the near (12,500 to 4000 cm^{-1}), middle (4000 to 400 cm^{-1}) and far (400 to 5 cm^{-1}) infrared, which are located between the visible and the microwave ranges, thus offering medium energies suitable for modifying the vibrational and some rotational molecular energy levels. This offers unique fingerprinting information of the bonds of practically all the molecules present in the sample.

As indicated in Figure 15.1, the most important information provided by vibrational spectra comprises the fundamental vibrations in the middle infrared (MIR), the overtones and combination bands of the vibrational modes in the near infrared (NIR), and the vibrational frequencies of both backbone vibrations of large molecules and fundamental vibrations of inorganic or organometallic compounds in the far infrared (FIR). However, the ability to use glass optic elements in the NIR offers improved acquisition of information about foods in an easy and cheap way.

In addition to the extended wavenumber range offered by vibrational spectroscopy, its easy application to the analysis of solid, liquid or dissolved samples and gaseous compounds makes possible the use of infrared methods for the direct determination of many compounds in every type of food [1–6]. The main drawbacks of vibrational spectroscopy are its relatively low sensitivity (percentage and parts per thousand level) compared with ultraviolet/visible (UV/Vis)-based methods and the lack of information provided at the elemental level. However, it is easy to determine the presence of the main compounds of food via their spectra: water (1940 nm wavelength for moisture), proteins (2180 nm), carbohydrates (2100 nm) and lipids (2310 nm) and the absence of specific bands relating to the mineral elements such as the alkaline, alkaline earth and transition metals, metalloids or halogen elements.

Figure 15.2 shows, as an example, the typical infrared spectra of a food like milk with the assignation of the main bands to the major compounds. From these spectra it can be concluded that vibrational spectroscopy offers a unique tool in direct food analysis [1–6] but could create problems in the determination of food mineral profiles. Because of the aforementioned reasons, NIR and MIR have been used in only a few reports that have determined the ash and mineral elements in foods (Table 15.1), the majority being devoted to ash and the major elements and only a small number to trace and ultratrace elements.

However, the mineral profile of foods is an important characteristic for consumer safety and for nutritional reasons. The content of essential elements, such as calcium, iron, zinc, copper, magnesium, lithium, selenium, potassium, chromium and cobalt, must be determined

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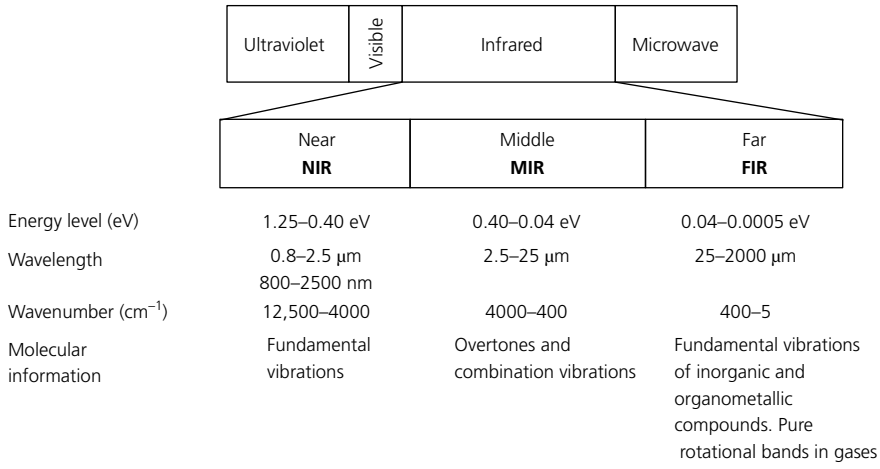


Figure 15.1 Molecular information associated with vibrational spectroscopy as a function of the spectral range considered.

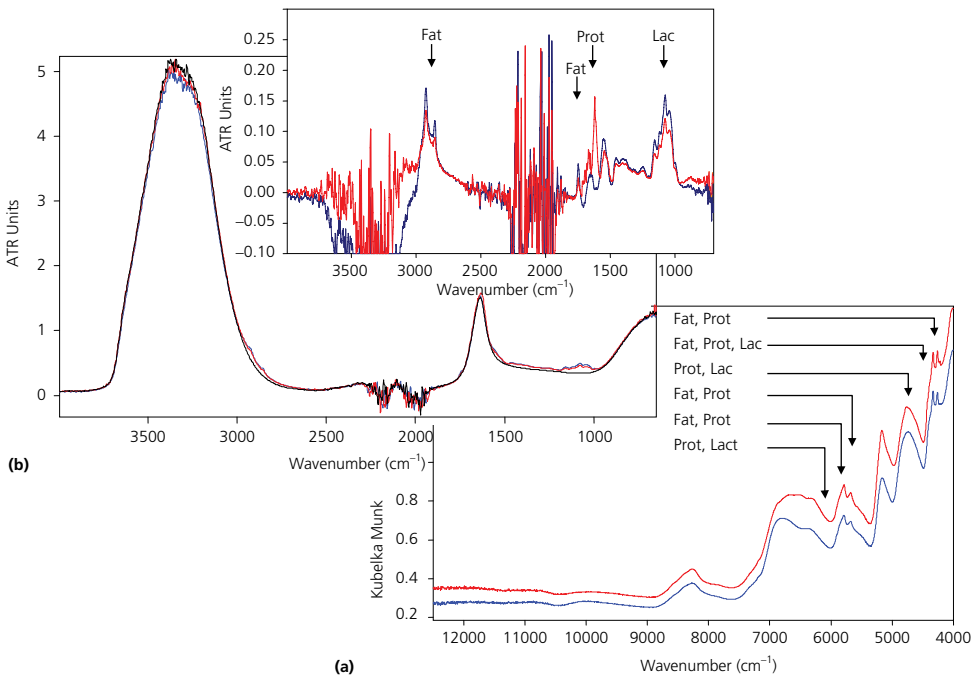


Figure 15.2 Infrared spectra of milk samples: (**a**) NIR diffuse reflectance spectra of two powder milks; (**b**) MIR-ATR spectra of whole and semi-skimmed liquid milk. Fat, regions for fat analysis; Prot, regions for protein; Lac, regions for lactose.

in foods in order to ensure the recommended daily intake established by international organizations [27–29] and to avoid their consumption at excessive levels [30, 31]. On the other hand, several elements like lead, mercury and cadmium are highly toxic and non-degradable and accumulate in organisms through their amplification in the trophic chain, their determination thus being mandatory for safety reasons. Thus the complete mineral profile of foods must be carefully evaluated in order to ensure the absence of those elements that are exclusively toxic and those that are relatively toxic (like As which is present in all organisms but has no specific biological function) and to determine the concentration of the essential elements (Figure 15.3), which can have

Table 15.1 Methods proposed in the literature for the determination of mineral elements in foods by vibrational spectroscopy.

Mineral elements considered	Samples	Measurement	Reference
Ash	Tropical root and tuber crop flours	NIR	[7]
Ashes, Ca, Mg	Seed coats of beans	NIR	[8]
Ash, Ca, K, Na	*Atriplex halimus*	NIR	[9]
Ca	Yoghurt	ATR-FTIR	[10]
Ca, K, Mg, Na, P	Cow milk	MIR	[11]
Ca, Cu, P	Fishmeal	FT-NIR	[12]
Ca, Fe, K, Mn, Na, P, Zn	Alfalfa	NIR (fibreoptic probe)	[13]
Ca, B, Fe, K, Mg, Mn, Na, P, S	Wine	Visible and NIR	[14]
Ca, Fe, K, Mn, Mg, P, S	Red grape homogenates	NIR	[15]
Na	Fermented pork sausage	FT-NIR (contact and remote)	[16]
Na, Ca, K, Mg, Zn	Fresh and freeze-dried cheeses	Visible and NIR	[17]
Na, B, Cu, Fe, Mn, S, Zn	Legumes	NIR	[18]
Fe, Zn	Powdered milk	NIR and MIR	[19]
Fe, Zn, Ca, K, Na	Fresh Iberian pork loin	NIR (fibreoptic probe)	[20]
As	Red crayfish	Visible and NIR	[21]
As	Rice	Visible and NIR	[22]
As	Prostrate amaranth	NIR	[23]
As, Pb	Red paprika powders	FT-NIR	[24]
N, Ca, K, Mg, P, S	Alfalfa and white clover	NIR	[25]
Cu, Ca, Fe, K, Mg, Na	White wine	NIR	[26]

NIR, near infrared spectroscopy; FT, Fourier transform; MIR, middle infrared spectroscopy; ATR, attenuated total reflectance.

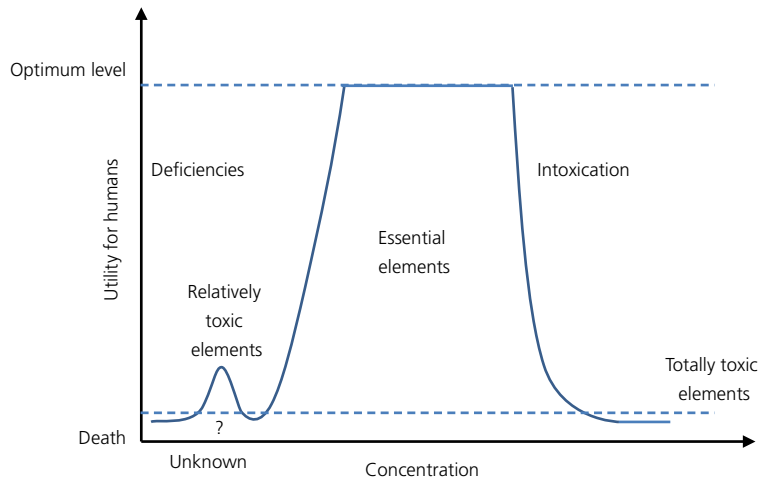


Figure 15.3 The utility function of mineral elements in foods for human health.

deleterious effects on human health when present in high concentrations.

Previous chapters have shown that atomic spectroscopy methods, based on UV/Vis absorption (see Chapter 10) and UV/Vis emission (see Chapter 11) together with inorganic mass spectrometry (see Chapter 12), are the most commonly used tools for determing the mineral composition of foods at major, minor, trace and ultratrace levels, X-ray fluorescence (see Chapter 14) also being suitable for fast characterization of minerals in foods at relatively high concentrations.

Looking at the strategies proposed in the available literature (see Table 15.1), it can be concluded that changes in the size and shape of the characteristic spectral

bands of the major food components provide sufficient information to obtain quantitative relationships between infrared spectra and the concentration of many elements present in the samples, thus offering 'green' alternatives to the methods that involve complex sample preparation with the use of solvents and acids that present risks for both operators and the environment [32–34].

15.2 Sampling, sample treatment and measurement techniques

Vibrational spectroscopy methods are well established in food analysis because of their versatility, which allows sample measurement with no previous treatment or minimal sample preparation and the ability to obtain multicomponent information data from the spectra using multivariate methods. In order to obtain good results it is mandatory to guarantee the reproducibility of measurements, especially in the case of heterogeneous solid samples which, in some cases, require grinding or blending. Alternatively, obtaining a large number of repeat measurements and working with average spectra can increase the representativity and reproducibility of determinations.

Transmission and transflection are the most useful sampling techniques for NIR measurements of liquid samples. For solid samples, diffuse transmission and, especially, diffuse reflection are the techniques commonly employed for routine NIR analysis. Diffuse reflectance (DR) measurements involve detection of the energy that penetrates a material and which undergoes multiple scattering from sample particles. As can be seen in Figure 15.4A, this diffusely reflected radiation, scattered over a large solid angle, is collected and directed to the detector. The use of an integrating sphere permits the collection of this reflected radiation to be maximized, thus providing a good signal-to-noise ratio. Particle size and compaction of samples may affect the scattering process for powders and granulates. However, these broadband particle size effects can be minimized with adequate data pre-processing. Probes and other sampling devices can be employed for diffuse reflection measurements, reducing sample manipulation to a minimum. For quantitative analysis, reflectance (R) measurements are transformed to $\log(1/R)$, offering a spectrum similar to that of absorbance. Using an adequate function, such as Kubelka–Munk, reflectance is transformed to a quantity that varies linearly with concentration.

Transmission is the original and classical measurement mode for obtaining MIR spectra. This technique requires that samples be transparent to infrared radiation and be easily manipulated in order to be placed in the infrared radiation beam. Depending on the nature of the sample (solid, liquid or gas) different sample preparation modes

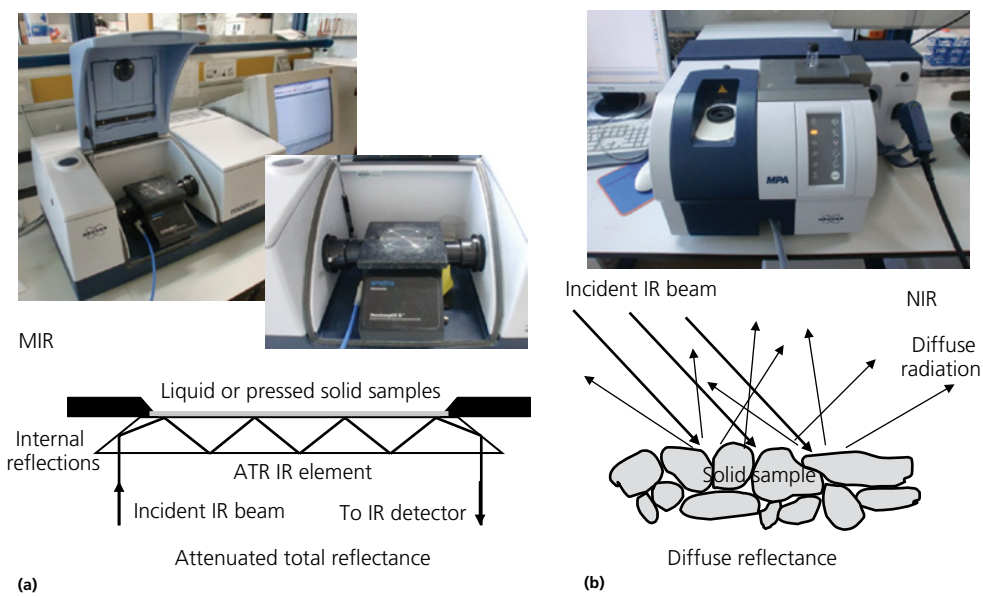


Figure 15.4 Typical measurement modes and instruments used for MIR (**a**) and NIR (**b**) food analysis.

are employed, such as the use of transmission cells, preparation of alkaline halide discs or mineral oil mulls, and direct measurements of thin films of sample. In recent decades the use of reflectance techniques and the development of new versatile and low-cost accessories have generalized the use of infrared spectroscopy in laboratories and control measurements. Internal reflection, also called attenuated total reflection (ATR), has become a widely used sampling technique for both solid and liquid samples, offering tremendous advantages compared with transmission measurements, especially for high absorption or non-transparent samples. This technique is based on the formation of an evanescent wave that penetrates the sample in contact with a high refractive index transparent material in which the infrared radiation is totally internally reflected (Figure 15.4B). The resulting ATR spectrum is equivalent to those obtained for transmission in a very thin film of sample (of the order of fractions of a micrometre). Because the depth of penetration of the evanescent wave inside the sample in contact with the internal reflectance element (IRE) of the ATR accessory is proportional to the wavelength of the radiation, ATR spectra (compared with classical transmission) show decreasing intensity of the characteristic infrared bands when the energy of the radiation increases, thus moving to high wavenumbers.

The development of ATR accessories employing different materials for the IRE, such as ZnSe, Si, Ge or Type IIa diamond, have generalized this measurement technique as in-compartment ATR accessories or as fibreoptic probes. Figure 15.4 shows two typical instruments used for infrared measurements, the NIR machine being equipped with an integrating sphere and fibreoptic probe and the MIR machine with a diamond ATR accessory.

In summary, the ATR sampling technique offers elevated versatility in the analysis of foods and can be applied to a wide variety of samples in both solid and liquid form, especially for samples that show high absorption and which cannot easily be measured in transmittance mode. Moreover, no or reduced sample manipulation is required and the development of ATR accessories with a diamond IRE allows an elevated pressure to be applied to powder samples, thus increasing the sensitivity and reproducibility of measurements.

The regions employed for the direct determination of the mineral compounds of foods covers visible [14, 17, 21, 22], NIR [7–9, 12–26] and MIR [10, 11, 19], with methods based on absorbance and reflectance measurements obtained by direct contact and remote methodologies based on the use of fibreoptic probes [6, 13, 20].

Most of the data presented in Table 15.1 have been made using NIR spectroscopy despite the greater molecular information supplied by MIR, probably because NIR uses simple instrumentation and provides better signal repeatability than that obtained by MIR. This latter point is fundamental when using chemometric models established from the signal of well-characterized samples and suitable for predicting the mineral profile of unknown samples.

15.3 Chemometric data treatment

The success of infrared spectroscopy for the quantification of mineral components in foods is only possible because of the use of chemometric tools. Nearly all research done on this field is made by using partial least squares (PLS) or modified-partial least squares (MPLS) regression models. This indicates how powerful this tool is for the determination of chemical substances in untreated samples. On the other hand, it also indicates that there are few options in the literature other than PLS strategies, which limits the improvement of calculations in cases of unsatisfactory results, indicating that there is a lack of development in this field which hopefully can be improved in the near future.

Variations in spectral pretreatment can provide further help, for example the use of signal derivatives, vector normalization (VN) or multiplicative scatter correction (MSC), but improved results could be also obtained by using sophisticated treatments like artificial neuronal networks (ANN).

Table 15.2 shows the chemometric methods employed for infrared determination of mineral components of foods, also including data pretreatment procedures used and the number of samples considered in each study for both calibration and validation purposes. Spectra data pretreatment procedures include standard normal variate (SNV) [9, 12–14, 17, 19–22, 24, 25], applied to zero-order spectra and also to the first (FD) and second (SD) derivative spectra, de-trending (DT) and MSC, thus confirming the importance of spectra treatment strategies for improving model robustness and the prediction capability of PLS-based methods.

Unfortunately, many of the published studies in this field only evaluate the models through the use of cross-validation strategies, which basically consider the whole

Table 15.2 Chemometric procedures employed for determination of the mineral composition of foods via their infrared spectra.

Sample: analyte(s)	Chemometric method	Spectra pretreatment	Total samples	Calibration set	Validation set	Reference
Alfalfa: Ca, K, P, Fe, Mn, Na, Zn	MPLS, CV	SNV, DT, MSC, FD + SD	120	120	10	[13]
Alfalfa and white clover: N, Ca, K, P, Mg, S	MPLS, CV	SNV, DT	327	—	—	[25]
Atriplex halimus: ash, Ca, K, Na	MPLS, CV	SNV, DT, FD	318	112	—	[9]
Cheeses (fresh and freeze-dried): Ca, K, Na, Mg, Zn	MPLS, CV	Conversion into absorbance, no correction, DT, SNV + DT, MSC, FD + SD	445	362	14	[17]
Cow milk: Ca, K, Mg, Na, P	PLS combined with repeatability file, CV	Conversion into absorbance	1543	100	30	[11]
Fermented pork sausage: Na	PLS	VN, MSC, FD	207	101	—	[16]
Fishmeal: Ca, Cu, P	PLS, KPLS	SNV	120	60	35	[12]
Fresh Iberian pork loin: Ca, Fe, K, Na, Zn	MPLS	SNV, DT, MSC, FD + SD	42	42	8	[20]
Legumes: B, Cu, Fe, Mn, Na, S, Zn,	MPLS, CV	FD + SD	332	262	—	[18]
Milk (powdered): Fe, Zn	PLS, LS-SVM	Three segments, zero polynomials, SNV	7	7	—	[19]
Prostrate amaranth: As	MPLS, CV	FD, SI, SNV, DT	74	74	23	[23]
Red crayfish: As	MPLS, CV	SNV, DT, SD	62	62	—	[21]
Red grape homogenates: Ca, Fe, K, Mg, Mn, P, S	PLS, CV	MSC	209	209	89	[15]
Red paprika powders: As, Pb	PLS	FD, MSC, SNV	33	20	13	[24]
Rice: As	MPLS, CV	SNV, DT, SD	40	—	—	[22]
Seed coats of beans: ash, Ca, Mg	PLS, CV	SNV, FD + SD	90	60	—	[8]
Tropical root and tuber crop flours: ash	PLS, CV, IV	Conversion into absorbance, MSC	472	378	—	[7]
Wine: B, Ca, Fe, K, Mg, Mn, Na, P, S	PLS, CV	SD, SNV	126	63	—	[14]
Wine (white): Ca, Cu, Fe, K, Mg, Na	MLR, PLS	SD	24	24	—	[26]
Yoghurt: Ca	PLS	Elimination of additive artefacts	48	19/32	—	[10]

MLR, multiple linear regression; PLS, partial least squares; CV, cross-validation; IV, independent validation; MPLS, modified partial least squares; KPLS, Kernel partial least squares; LS-SVM, least squares-support vector machine; FD, first derivative; SD, second derivative; SNV, standard normal variate; MSC, multiplicative scatter correction; VN, vector normalization; DT, de-trending; SI, smoothing interval.

population of samples used to build the model and then evaluate model robustness by leaving out one or several samples to make the prediction. In this sense, the most commonly employed method is the so-called 'leave one out' technique which, as the name indicates, excludes from the model those samples employed to build it one by one in order to predict the concentration of each of the samples, this procedure demonstrating that the model is coherent. However, this strategy does not provide information about the ability of the model to correctly evaluate additional samples not employed in creating the model, and thus provides a poor evaluation of the ability of the developed model to be used in the real world. Because of this, in our opinion, evaluation of the prediction capability of PLS infrared methods must be based on external validation, using an independent set of well-known concentration samples not employed during the calibration step.

Only few of the published studies [11–13, 15, 17, 20, 23, 24] have used separate validation sets of samples and, as summarized in Table 15.3, the coefficient of

Table 15.3 Prediction capability of proposed PLS-IR methods for the determination of the mineral concentration in foods.

Sample	Analyte	LV	R^2	SEP	RPD	Reference
Cheese (freeze-dried)	Na	7	0.82	3.17 g/kg		[17]
	Ca	8	0.82	1.99 g/kg		
	K	13	0.64	1.33 g/kg		
	Mg	9	0.48	0.05 g/kg		
	Zn	7	0.89	7.4 mg/kg		
Cheese	Na	9	0.89	1.76 g/kg		[17]
	Ca	10	0.95	0.68 g/kg		
	K	8	0.78	0.14 g/kg		
	Mg	9	0.74	0.03 g/kg		
	Zn	9	0.93	4.1 mg/kg		
Cow milk	Ca		0.91		2.74	[11]
	K		0.76		1.24	
	Mg		0.85		1.68	
	Na		0.8		1.68	
	P		0.89		2.54	
Fishmeal	Ca	7 (PLS)	0.8573			[12]
	P	6 (PLS)	0.8233			
	Cu	5 (PLS)	0.8593			
	Ca	10 (KPLS)	0.9024			
	P	7 (KPLS)	0.8249			
	Cu	8 (KPLS)	0.8521			
Iberian pork loin (ground)*	Fe	5		5.8 mg/kg		[20]
	Zn	5		9.6 mg/kg		
	Ca	5		10.0 mg/kg		
	Na	5		70 mg/kg		
	K	5		436 mg/kg		
Iberian pork loin (fibreoptic)*	Fe	8		8.3 mg/kg		[20]
	Na	8		69 mg/kg		
	K	8		667 mg/kg		
Prostrate amaranth	As	3–4	0.56–0.63		1.26–1.52	[23]
Red grape homogenates	Fe	7	0.3	1.88 mg/kg	1.5	[15]
	Mn	7	0.4	0.88 mg/kg	1.6	
	Ca	6	0.61	79 mg/kg	1.5	
	Mg	8	0.58	29 mg/kg	2.2	
	K	6	0.45	434 mg/kg	1.7	
	P	6	0.38	85 mg/kg	1.7	
	S	7	0.5	34 mg/kg	2.4	

(Continued)

Table 15.3 (Continued)

Sample	Analyte	LV	R^2	SEP	RPD	Reference
Red paprika powder	As	4 (MSC)	0.86	41 µg/Kg		[24]
	As	5 (SNV)	0.87	39 µg/kg		
	Pb	6 (MSC)	0.98	67 µg/Kg		
	Pb	7 (SNV)	0.99	57 µg/Kg		
Seed coats of beans (intact)	Ash	5	0.761	6.54 g/kg	2.03	[8]
	Ca	5	0.821	2.19 g/kg	2.4	
	Mg	7	0.437	0.54 g/kg	1.33	
Seed coats of beans (ground)	Ash	5	0.922	3.8 g/kg	3.49	[8]
	Ca	5	0.922	1.47 g/kg	3.57	
	Mg	8	0.557	0.48 g/kg	1.5	
Tropical root (cassava)	Ash	6	0.94	2.62% m/m	2	[7]
Tropical root (sweet potato)	Ash	5	0.26	0.51% m/m	0.98	[7]
Tropical root (taro)	Ash	6	0.68	0.62% m/m	2.06	[7]
Tropical root (yam)	Ash	7	0.6	0.8% m/m	1.09	[7]
Tropical root (all samples)	Ash	11	0.71	0.7% m/m	1.54	[7]
Wine (validation)	Ca	8		11.9 mg/L	1.5	[14]
	Mg	3		18.29 mg/L	1.5	
	Na	1		9.88 mg/L	2.7	
	K	3		152 mg/L	1.7	
	P	3		39.5 mg/L	2	
	S	3		27.5 mg/L	1.2	
	Fe	8		0.55 mg/L	1.3	
	Mn	2		0.58 mg/L	1.5	
	B	7		1.65 mg/L	1.1	

*Results expressed for a dried sample.

LV, number of factors; R2, coefficient of determination (prediction or validation); SEP, standard error of prediction; RPD, defined as the standard deviation in the validation set divided by the standard error of prediction; PLS, partial least squares; KPLS, Kernel partial least squares; SNV, standard normal variate; MSC, multiplicative scatter correction.

determination for prediction or validation of sample sets (R^2), the standard error of prediction (SEP) and the ratio of the standard deviation in the validation set to the standard error of prediction (RPD), corresponding to each one of the elements evaluated in food samples, have been used as parameters for prediction evaluation of PLS infrared models. This table also indicates the number of factors (latent variables, LV) employed to build the calibration in each case.

The number of latent variables used varies from element to element and is different for each type of sample assayed, from a single factor for the determination of Na in wine by visible and NIR spectrometry [14] up to 13 factors for K determination in freeze-dried cheese samples using MPLS visible/NIR reflectance [17]. The use of a high number of LV is quite astonishing and is probably associated with the fact that mineral element determination in foods by infrared spectroscopy is based more on the interaction between protein or carbohydrate components with the target element than on the presence of specific vibrations related to the mineral element bonds present in the samples, and thus the number of variables to be taken into consideration can be a changing and complex variable that does not depend only on noise and analyte interactions.

The determination of ash in tropical roots [7] and beans [8] involved five to eleven latent variables as a function of the variability of samples considered. However, in this case the use of a high number of variables to evaluate a single parameter in the samples could be associated with the fact that food ash is a complex mixture of inorganic compounds present or produced during food incineration and thus difficult to be modelled based on a reduced set of variables.

Regression coefficients reported in the literature vary from 0.26 in the case of ash determination in sweet

potato roots [7] to 0.95 for the determination of Ca in cheese samples [17] and this parameter provides a good indication of model robustness and of the difficulties found in many studies,which make it far from useful in real-world applications.

Reported SEP values vary from as low as 0.58 mg/L and 0.88 mg/kg for the determination of Mn in wine [14] and red grape homogenates [15], respectively, to as high as 3.17 g/kg for Na determination in freeze-dried cheese [17].

Reported RPD values vary between 0.98 for ash prediction in sweet potato [7] and 3.57 for Ca determination in ground seed coats of beans [7] and root mean squared error of prediction (RMSEP) was reported in only two studies, one concerning the determination of Ca, P and Cu in fishmeal [12] and the other investigating the amount of Pb and As in red paprika powder [24]. In the case of RMSEP, values of 0.06 g/kg for Cu, 2.0 g/kg for Ca and 2.5 g/kg for P were obtained for fishmeal and 39–41 μg/kg for As and 57–67 μg/kg for Pb in red paprika powder, these representing the aforementioned strong differences depending on mineral element concentration and on the complexity of samples considered and models built.

15.4 Mineral profile of foods using vibrational spectroscopy

The majority of the reported studies, summarized in Table 15.1, concern the determination of the total mineral content of a reduced number of elements and also prediction of the ash content of foods [7–9]. Ash, or ashes, together with the concentration of Ca and Mg or K, have been determined by NIR in tropical roots [7], seeds of beans [8] or *Atriplex halimus* [9].

Calcium, followed by potassium, magnesium, sodium, iron and zinc, have been by far the most studied elements in foods using infrared spectroscopy, some of these elements being determined in liquid samples like wine or milk, yoghurt, fruits, meat and fish products, vegetables and dry foods, showing the versatility of infrared spectroscopy and its ability to provide quantitative results in many different cases. However, it must be also accepted that, as a function of the size of the prediction errors reported in Table 15.3, it is clear that the majority of published studies originate from the academic world and only a minority of the available procedures seem suitable in the real world.

15.5 Speciation of trace elements based on vibrational spectroscopy

Feo and Aller [35] showed the ability of MIR spectroscopy to evaluate the presence of mercury species as methylmercury, ethylmercury and phenylmercury in a sample such as whole *Escherichia coli* strain K-12 cells. This was based on direct measurement of the quotient between absorbance at different wavenumbers of the middle FTIR spectra, obtained in transmission mode, from untreated whole bacterial cells deposited as films by direct evaporation of water and using wavenumbers between 500 and 1500 cm^{-1}, relating to nucleic acids and hydrogen bond regions. The proposed method provided a limit of detection of 0.02–0.20 mg/mL as a function of the mercury species and wavenumbers considered, with recovery percentages of 97–102% for a spiked level of 10 mg/mL of each of the mercury species considered, thus opening the possibility for use of the technique for speciation purposes in cells and foods.

Inorganic arsenic was determined in red crayfish [21] using reference data obtained by hydride generation atomic absorption spectrometry after freeze-drying sample digestion with hydrochloric acid and solvent extraction into chloroform after reduction overnight with HBr and hydrazine sulfate and back-extraction into HCl. NIR spectra of freeze-dried ground samples were modelled by MPLS and the coefficient of determination obtained by cross-validation was 0.84, being the quotient between the standard error of cross-validation and the standard deviation of data 0.38. Five variables were used in the selected calibration equations. As the authors of the study concluded, NIR provides a direct and fast methodology for identifying samples having low, medium or high inorganic arsenic concentrations, thus providing a potential screening tool for speciation that avoids the systematic use of chromatography procedures based on the specific chemical behaviour of As compounds during sample pretreatment. This important information should be considered with regard to the new regulations concerning foods, which are moving away from determining the total content

of toxic elements to the content of their toxic chemical forms.

15.6 Future trends in food mineral composition based on vibrational spectra

Infrared spectroscopy offers interesting possibilities as a 'green' alternative for the determination of mineral elements in foods. However, to perform quantitative predictions of major and trace elements in untreated liquid and solid foods, it seems absolutely necessary to move from the academic level of many of the published works to the real world. To do this, there must be appropriate selection of an extended set of samples to build calibrations, together with advances in chemometric treatments, to improve available models in order to enhance their prediction capabilities. Additionally, it is clear that vibrational methods must be explored in depth to evaluate their capabilities as screening tools, for both total element and speciation purposes, which is particularly important in cases like calcium speciation to confirm the potential availability of this element in foods on the market.

The development of powerful handheld and portable instruments, based on the use of infrared radiation, can provide on-line or real-time tools for use in rapid food characterization. This idea is especially interesting in the case of in-process manufacturing of foods fortified with minerals to permit optimization of production and to ensure quality and homogeneity of products, enabling timely correction measures during manufacturing [36].

Finally, Raman spectroscopy, a technique used widely used in food analysis for investigations related to the structure of biological molecules relevant to foodstuffs, the identification and quantitation of particular components of food, the non-destructive *in situ* analysis of food and agricultural products or evaluation of changes and quality of foods during processing [37–39], has been demonstrated to be valid as a non-destructive analytical tool for direct determination of metals in sediments [40]. Unfortunately this facility has not been exploited in the case of the mineral composition of foods and, in this sense, we think that additional efforts must be made to incorporate this technique into the arsenal of analytical tools available to mineral analysis in foods.

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Abbreviations

ANN: Artificial neuronal networks
ATR: Attenuated total reflection
CV: Cross-validation
DR: Diffuse reflectance
DT: De-trending
FD: First derivative
FIR: Far infrared
IR: Infrared
IRE: Internal reflectance element
LV: Latent variables
MIR: Middle infrared
MPLS: Modified-partial least squares
MSC: Multiplicative scatter correction
NIR: Near infrared
PLS: Partial least squares
***R*:** Reflectance
RMSEP: Root mean squared error of prediction
RPD: Ratio of the standard deviation to the standard error of prediction
SD: Second derivative
SEP: Standard error of prediction
SNV: Standard normal variate
UV/Vis: Ultraviolet/visible
VN: Vector normalization

References

1 Li-Chan ECY, Ismail AA, Sedman J, van de Voort FR. Vibrational spectroscopy of food and food products. In: Chalmers JM, Griffiths PR (eds) *Handbook of Vibrational Spectroscopy*. John Wiley & Sons, Ltd, Chichester, 2002.
2 Li-Chan ECY, Griffiths PR, Chalmers JM (eds) *Applications of Vibrational Spectroscopy in Food Science*. John Wiley & Sons, Ltd, Chichester, 2010.
3 Burns DA, Ciurczak EW (eds) *Handbook of Near-Infrared Analysis*, 3rd edn. CRC Press, Boca Raton, FL, 2008.

4 Sun DW (ed.) *Infrared Spectroscopy for Food Quality Analysis and Control*. Academic Press, New York, 2009.

5 Ozaki Y, McClure WF, Christy AA (eds) *Near Infrared Spectroscopy in Food Science and Technology*. John Wiley & Sons, Inc., Hoboken, NJ, 2007.

6 Siesler HW, Ozaki Y, Kawata S, Heise HM (eds) *Near-Infrared Spectroscopy: Principles, Instruments, Applications*. Wiley-VCH, Weinheim, 2006.

7 Lebot V, Champagne A, Malapa R, Shiley D. NIR determination of major constituents in tropical root and tuber crop flours. *J Agric Food Chem* 2009;**57**:10539–10547.

8 Plans M, Simo J, Casanas F, Sabate J. Near-infrared spectroscopy analysis of seed coats of common beans (*Phaseolus vulgaris* L.): a potential tool for breeding and quality evaluation. *J Agric Food Chem* 2012;**60**:706–712.

9 Andueza D, Muñoz F, Murray I. The prediction of chemical composition and in vitro digestibility of damples of *Atriplex halimus* by NIR. In: Ben Salem H, Nefzaoui A, Morand-Fehr P (eds) *Nutrition and Feeding Strategies of Sheep and Goats under Harsh Climates*. CIHEAM, Zaragoza, 2004.

10 Moros J, Inon FA, Khanmohammadi M, Garrigues S, de la Guardia M. Evaluation of the application of attenuated total reflectance-Fourier transform infrared spectrometry (ATR-FTIR) and chemometrics to the determination of nutritional parameters of yogurt samples. *Anal Bioanal Chem* 2006;**385**: 708–715.

11 Soyeurt H, Bruwier D, Romnee JM *et al*. Potential estimation of major mineral contents in cow milk using mid-infrared spectrometry. *J Dairy Sci* 2009;**92**:2444–2454.

12 Masoum S, Alishahi A, Shekarchi M, Farahmand H. Evaluation of the mineral contents in fish meal by FTIR using PLS and Kernel PLS. *Iranian J Math Chem* 2011;**2**:31–38.

13 Gonzalez-Martin I, Hernandez-Hierro JM, Gonzalez-Cabrera JM. Use of NIRS technology with a remote reflectance fibre-optic probe for predicting mineral composition (Ca, K, P, Fe, Mn, Na, Zn), protein and moisture in alfalfa. *Anal Bioanal Chem* 2007;**387**:2199–2205.

14 Cozzolino D, Kwiatkowski MJ, Dambergs RG *et al*. Analysis of elements in wine using near infrared spectroscopy and partial least squares regression. *Talanta* 2008;**74**:711–716.

15 Cozzolino D, Cynkar W, Shah N, Smith P. Quantitative analysis of minerals and electric conductivity of red grape homogenates by near infrared reflectance spectroscopy. *Comput Electron Agric* 2011;**77**:81–85.

16 Collell C, Gou P, Picouet P, Arnau J, Comaposada J. Feasibility of near-infrared spectroscopy to predict a(w) and moisture and NaCl contents of fermented pork sausages. *Meat Sci* 2010;**85**:325–330.

17 Lucas A, Andueza D, Rock E, Martin B. Prediction of dry matter, fat, pH, vitamins, minerals, carotenoids, total antioxidant capacity, and color in fresh and freeze-dried cheeses by visible-near-infrared reflectance spectroscopy. *J Agric Food Chem* 2008;**56**:6801–6808.

18 Cozzolino D, Moron A. Exploring the use of near infrared reflectance spectroscopy (NIRS) to predict trace minerals in legumes. *Anim Feed Sci Technol* 2004;**111**:161–173.

19 Wu D, He Y, Shi J, Feng S. Exploring near and mid-infrared spectroscopy to predict trace iron and zinc contents in powdered milk. *J Agric Food Chem* 2009;**57**:1697–1704.

20 Gonzalez-Martin I, Gonzalez-Perez C, Hernandez-Mendez J, Alvarez-Garcia N. Mineral analysis (Fe, Zn, Ca, Na, K) of fresh iberian pork loin by near infrared reflectance spectrometry: determination of Fe, Na and K with a remote fibre-optic reflectance probe. *Anal Chim Acta* 2002;**468**:293–301.

21 Font R, del Rio-Celestino M, Velez D, de Haro-Bailon A, Montoro R. Visible and near-infrared spectroscopy as a technique for screening the inorganic arsenic content in the red crayfish (*Procambarus clarkii* Girard). *Anal Chem* 2004;**76**:3893–3898.

22 Font R, Velez D, del Rio-Celestino M, de Haro-Bailon A, Montoro R. Screening inorganic arsenic in rice by visible and near-infrared spectroscopy. *Microchim Acta* 2005;**151**: 231–239.

23 Font R, del Rio M, Velez D, Montoro R, de Haro A. Use of near-infrared spectroscopy for determining the total arsenic content in prostrate amaranth. *Sci Total Environ* 2004;**327**: 93–104.

24 Moros J, Llorca I, Cervera ML, Pastor A, Garrigues S, de la Guardia M. Chemometric determination of arsenic and lead in untreated powdered red paprika by diffuse reflectance near-infrared spectroscopy. *Anal Chim Acta* 2008;**613**: 196–206.

25 Moron A, Cozzolino D. Determination of macro elements in alfalfa and white clover by near-infrared reflectance spectroscopy. *J Agric Food Chem* 2002;**139**:413–423.

26 Sauvage L, Frank D, Stearne J, Millikan MB. Trace metal studies of selected white wines: an alternative approach. *Anal Chim Acta* 2002;**458**:223–230.

27 World Health Organization. *Trace Elements in Human Nutrition and Health*. World Health Organization, Geneva, 2001.

28 Elmadfa I (ed.) *European Nutrition and Health Report 2009*. S. Karger AG, Basel, 2009.

29 National Research Council. *Dietary Reference Intakes Research Synthesis: Workshop Summary*. National Academies Press, Washington, DC, 2006.

30 Reilly C. *Metal Contamination of Food: Its Significance for Food Quality and Human Health*. Blackwell Science, Malden, MA, 2002.

31 Fraga CG. Relevance, essentiality and toxicity of trace elements in human health. *Mol Aspects Med* 2005;**26**:235–244.

32 Cervera ML, de la Guardia M, Dutta S, Das AK. An overview of green analytical techniques in the spectrometric analysis of environmental and biological samples. *Spectrosc Lett* 2009;**42**:284–295.

33 de la Guardia M, Garrigues S (eds) *Challenges in Green Analytical Chemistry*. RSC Publishing, Cambridge, 2011.

34 de la Guardia M, Garrigues S (eds) *Handbook of Green Analytical Chemistry*. John Wiley & Sons Ltd, Chichester, 2012.

35 Feo JC, Aller AJ. Speciation of mercury, methylmercury, ethylmercury and phenylmercury by Fourier transform infrared spectroscopy of whole bacterial cells. *J Anal Atom Spectrom* 2001;**16**:146–151.

36 Hassel S, Rodriguez-Saona LE. Application of a handheld infrared sensor for monitoring the mineral fortification in whole grain cornmeal. *Food Anal Method* 2012;**5**:571–578.

37 Ozaki Y. Raman spectroscopy. In: Mossoba MM (ed.) *Spectral Methods in Food Analysis: Instrumentation and Application*. Marcel Dekker, New York, 1998.

38 Viereck N, Salomonsen T, van den Berg F, Engelsen SB. Raman applications in food analysis. In: Amer MS (ed.) *Raman Spectroscopy for Soft Matter Applications*. John Wiley & Sons, Inc., Hoboken, NJ, 2008.

39 Hassing S, Jernshøj KD, Christensen LS. Raman spectroscopy: a non-destructive and on-site tool for control of food quality? In: Kapiris K (ed.) *Food Quality*. InTech, Rijeka, Croatia, 2012. doi: 10.5772/32462

40 Villanueva U, Raposo JC, Castro K, de Diego A, Arana G, Madariaga JM. Raman spectroscopy speciation of natural and anthropogenic solid phases in river and estuarine sediments with appreciable amount of clay and organic matter. *J Raman Spectrosc* 2008;**39**:1195–1203.

CHAPTER 16
Ion chromatography

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Abstract: The use of ion chromatography in food analysis has been reviewed, paying special attention to the sampling and sample pretreatment of different types of foods, from milk and infant formulas to fruits, vegetables and cereals, meat, seafood, and also stressing the main strategies and applications for anion determination and speciation in foods.

Keywords: ion chromatography, food analysis, anions and cations, speciation

16.1 Physicochemical basic of ion chromatography

The physicochemical basis for ion chromatography (IC) is the exchange rate of ions in solution or by ions in a solid phase depending on the electric charge of the ion exchanged (which thus distinguishes cationic and anionic chromatography). In this sense, the properties of solid phases on which the process takes place are important. These properties will result in the separation of the different components. Therefore, the exchange must not be irreversible, thus avoiding the separation of analytes, but should slow down passage of some compounds relative to others such that there is sufficient separation to identify them individually with the appropriate detection system.

Table 16.1 summarizes some of the most common compounds used as the basis of ion exchange, as well as their chemical structure and the linked functional groups [1]. For use in a chromatographic column these exchangers must satisfy some requirements, such as regular and uniform shape, constant size at different conditions of pressure and solvation. In conjunction with the appropriate eluent, this leads to suitable conditions for the separation of analytes.

Figure 16.1 shows a diagram of an ion chromatograph, which can be divided into seven clearly differentiated parts. First, it is necessary to use a container to store the eluent, also called carrier solution. This solution will be responsible for carrying the sample and the standards throughout the system towards the detector. The composition and concentration of the carrier will depend on the ions to be separated.

The impulsion system will allow solution displacement at the established flow rate. The flow rate should be fast enough to obtain adequate productivity, but without unduly increasing the pressure of the column, and slow enough to allow time for the separation of all analytes passing through the chromatographic column. Flows generally used are close to 1 mL/min. Impulsion systems employed can transport the carrier isocratically, without varying its concentration, or can be programmed to vary the carrier concentration by a gradient thereof.

Injection of the sample, or standards, to the system is usually performed by a six-way valve, either manually or via an autoinjector. The volume used is typically a few tens of microlitres. The injected bulk, driven by the carrier, passes through the chromatographic column and separation of the analytes takes place.

The output of the chromatographic column can be coupled to the required detector: UV/Vis-spectrophotometer, fluorimeter, mass spectrometry (MS), inductively coupled plasma (ICP), flame atomic absorption spectrometry (FAAS) or conductivity. For conductivity detection, it is advisable to pass the exiting solution to a suppressor charge system before detection. This achieves a better signal-to-noise ratio. Lastly, the signal is converted to concentration units through the computer that control the chromatograph.

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Table 16.1 Principal ion exchanger materials.

Ion exchange base	Chemical structure	Key features
Condensation resin	Phenol	Strongly acidic cation exchanger: sulfonic acid
	Formaldehyde	Weakly acidic cation exchanger: carboxylic acid, phosphonic acid
Polymerization resin	Styrene-divinylbenzene	Strongly basic anion exchanger: quaternary ammonium
	Methacrylate-divinylbenzene	Weakly basic anion exchanger: primary amine, secondary amine, tertiary amine
Chelating resin		Containing O, N or S in the chelate forming groups
Anphoteric resin		Containing both acidic and basic groups
Cellulose	Hydrophilic cellulose	Acidic groups: carboxymethyl, sulfoethyl
Agarose	Hydrophilic agarose	Basic groups: aminoethyl, diethylaminoethyl
Inorganic	Zeolites	Inorganic cation exchanger: zeolites
	Acid salts of polyvalent metal	Inorganic cation exchanger: zirconium phosphate, titanium tungstate, nickel hexacyanoferrate(II)
	Hetero-polyacids salts	Inorganic cation exchanger: ammonium molybdophosphate
	Oxides of trivalent and tetravalent metal ions	Inorganic cation/anion exchanger: depending on the pH solution
Papers and layers		Impregnation of paper with ion-exchange reagents
		Resin powder fixed by an adhesive to the sheet
Membranes		Sheets with appropriate mechanical and electrical properties
Monolitic	Continuous bed of silica or polymer	Functionalized cross-linked strands

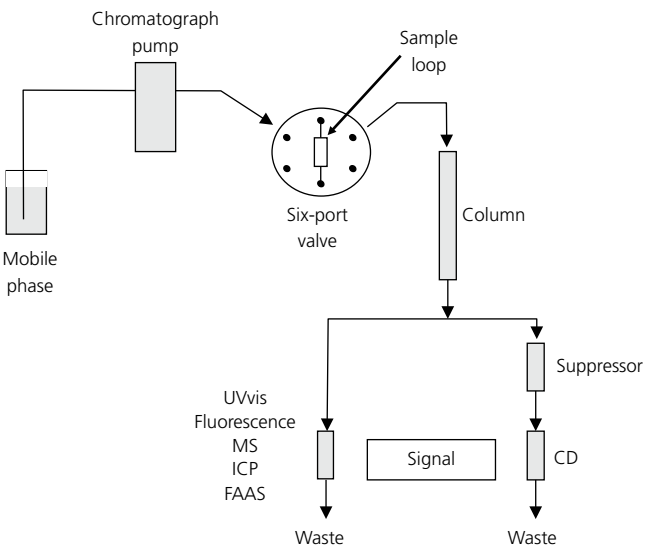


Figure 16.1 Ion chromatograph.

16.2 Ion chromatography for the analysis of cations and anions in food

The different strategies developed for IC in order to determine elements present as cations and anions in food samples depend on their nature as well as on the species to be determined and their concentration levels. This chapter reviews and discusses the literature up to the present on such analytical techniques. In fact, nearly 100 papers published in 49 journals during the period 2001–2013 have been thoroughly reviewed.

The chapter has been systematically organized so that the IC literature on the determination of inorganic cations and anions in food has been divided into four main topics:

(i) food samples analysed and elements of interest; (ii) sampling and sample treatment approaches for analyte extraction; (iii) measurement techniques for cation and anion determination; and (iv) advantages and drawbacks of IC in comparison with other analytical techniques used to obtain the mineral profile of foods. The literature has been classified on the basis of the chemical species determined, and in order to make available a complete picture of the different procedures applied, the sections have been organized with regard to the sample matrix. In this way, the reader can quickly find information regarding both the desired analyte and sample matrix.

It is interesting to note that the majority of the developed methodologies has focused on determination of perchlorate, chloride, nitrate, nitrite, sulfate, sulfite, phosphate or thiocyanate, although several speciation applications for arsenic and selenium have been found. To a lesser degree, methods have been reported for the determination of cations, both alkali and alkaline earth elements (Na, K, Mg, Ca) and transition metals (Co, Cu, Fe, Mn, Ni).

The application of IC to food analysis has been reviewed by a number of authors. Yashin and Yashin [2] presented the analytical capabilities of IC using an amperometric detector for the determination of anthropogenic and natural pollutants and special additives in food products and beverages. Michalski and Kurzyca [3] presented a critical analysis of methods based on IC for determining nitrate, nitrite and ammonium ions in environmental samples and food products. Michalski *et al*. [4] focused on the application of IC coupled with ICP-MS detection in the determination of selected halides and metal/metalloid species for food product quality control.

A general review of the literature on IC in food analysis from 2001 to the present reveals that the number of scientific papers published on this topic has grown in a linear way, focused mainly on anion determination. As can be see in Figure 16.2 the speciation studies, mainly on nitrogen, chloride, phosphorus, arsenic and selenium, have attracted much more attention than cation determinations.

Regarding the journals in which papers about IC applications in food analysis have been published (Table 16.2), it can be appreciated that they correspond to journals devoted to general analytical chemistry (*Analytica Chimica Acta*, *Talanta*), food chemistry (*Journal of Agricultural and Food Chemistry*, *Food Chemistry*, *Journal of AOAC International*, *Food Additives and Contaminants*), chromatography (*Journal of Chromatography A*, *Chinese Journal of Chromatography*) and a great variety of different journals with subjects as diverse as atomic physics, electroanalytical techniques, education and the environment.

With regard to the main strategies developed in IC for improving the determination of cations and anions in foods, we would stress those aspects related to (i) the use of extraction and clean-up method for precise determination of target analytes; (ii) the development of pre-concentration procedures based on solid-phase extraction (SPE); (iii) the hyphenation between ion chromatography and spectrophotometry, fluorimetry or mass detectors, particularly useful for speciation in ICP-MS; or (iv) post-column derivatization.

The literature on IC applied to the determination of inorganic cations and anions in food samples can be classified into eight groups: (i) infant formulas and baby food analysis; (ii) beverages, including wine and beer analysis; (iii) cereals (grain, flour and bread); (iv) dairy product (milk, yoghurt and cheese); (v) fruits, such as orange, mandarin, lemon, apples, grapefruit or strawberries; (vi) meat products, both fresh and cured; (vii7) sea products, including fish, molluscs and crustaceans; and (viii) vegetables, including edible leafy and root vegetables. Figure 16.3 shows the distribution of the literature in this field as a function of the type of samples analysed. Vegetables and dairy products are the main subject of application, comprising 51% of the papers published, followed distantly by beverages, sea products and baby food analyses, which comprise 9%, 9% and 8% of the total studies published, respectively.

With regard to the mineral species determined, common anions are by far the most studied in food, comprising 77% of the determinations performed. This percentage increases to 91% when considering arsenic and selenium inorganic species. Figure 16.4 shows that anion determination studies in food using IC have been applied to the periodic table elements of groups VA (N, P, As), VIA (S, Se) and VIIA (F, Cl, Br, I). As can be seen, determination of N and Cl species comprise the majority, with 60% of determinations. The individual anionic species perchlorate, nitrate and nitrite comprise 21%, 13% and 12%, respectively, and are thus the most studied species. The inorganic arsenic species are also a group to be taken into account. However, in these cases, because of the low concentrations of As(III) and As(V) in foods, it is necessary to use highly sensitive detection systems, such as MS or atomic fluorescence spectroscopy.

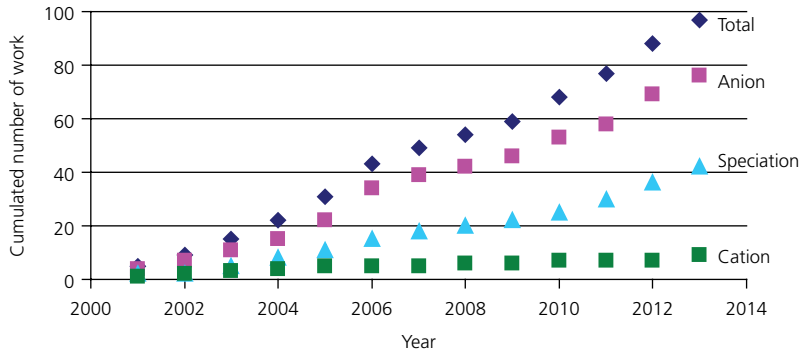


Figure 16.2 Evolution of the scientific literature on ion chromatographic applications in food analysis published during the twenty-first century. (See insert for colour representation of the figure.)

Table 16.2 Scientific journals which have published papers on food analysis by ion chromatography.

Journal title	Papers
Food Chemistry	9
Analytica Chimica Acta	8
Journal of Agricultural and Food Chemistry	7
Journal of AOAC International	6
Talanta	6
Food Additives and Contaminants	5
Chinese Journal of Chromatography	4
Bunseki Kagaku	3
Journal of Chromatography A	3
Analytical Sciences	2
Chemosphere	2
Environmetl Science and Technology	2
Journal of Analytical Atomic Spectrometry	2
Journal of Food and Drug Analysis	2
Korean Journal of Food Science and Technology	2
Meat Science	2
American Laboratory	1
Analytical Chemistry	1
Analytical Letters	1
Critical Reviews in Analytical Chemistry	1
Critical Reviews in Food Science and Nutrition	1
Doktorsavhandlingar vid Chalmers Tekniska Hogskola	1
Environment International	1
Environmental Health Perspectives	1
Environmental Monitoring and Assessment	1
Environmental Pollution	1
European Food Research and Technology	1
Fleischwirtschaft	1
International Journal of Food Science and Technology	1
Journal of Radioanalytical and Nuclear Chemistry	1
Journal of Analytical Chemistry	1
Journal of Animal Science	1
Journal of ASTM International	1
Journal of Automated Methods and Management in Chemistry	1
Journal of Dairy Science	1
Journal of Environmental Monitoring	1
Journal of Exposure Science and Environmental Epidemiology	1
Journal of Food Science	1
Journal of Food Science and Technology	1
Journal of Forensic Sciences	1
Journal of the Food Hygienic Society of Japan	1
Journal of Liquid Chromatography and Related Technologies	1
LWT Food Science and Technology	1
Microchimica Acta	1
Nutrition Bulletin	1
Polish Journal of Environmental Studies	1
Quintessence International	1
Science of the Total Environment	1

The available literature published in the twenty-first century on anion and cation determination has been revised and summarized in Tables 16.3, 16.4 and 16.5. In these tables, papers are organized as a function of the food matrix in order to make available for the reader the information concerning both the analyte and the sample. Tables summarize only the main quantitative information about the techniques employed, including parameters such as dynamic range, limit of detection, coefficient of variation and accuracy, evaluated through the recovery percentage of the species determined in spiked samples.

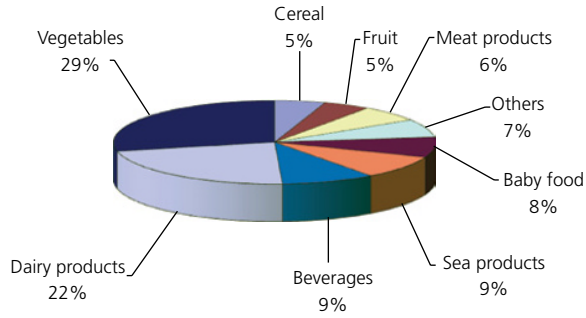


Figure 16.3 Distribution of published papers on determination of mineral components in food by means of ion chromatography as a function of the matrix considered for the period 2001–2013. (See insert for colour representation of the figure.)

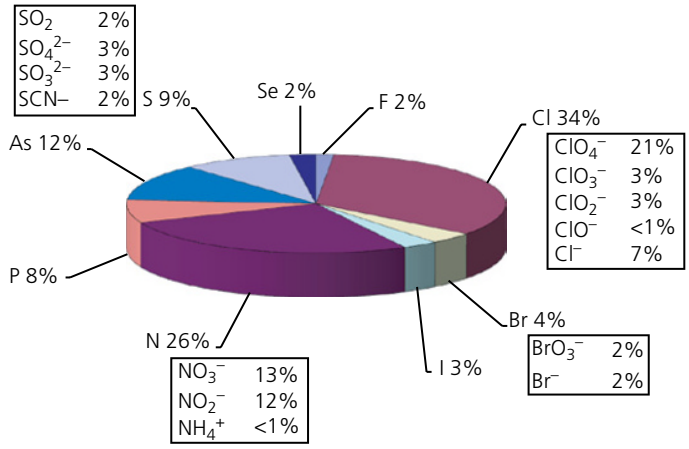


Figure 16.4 Distribution of anionic species determination in food samples by mean of ion chromatography. (See insert for colour representation of the figure.)

16.3 Sampling and sample treatment

Sample preparation and preservation is important in food analysis. Furthermore, the sample treatment must ensure the quantitative lixiviation of analytes of interest. Moreover, during these steps, contamination and losses can be a serious source of error. In the following sections the specific sample preparation methods suggested in the literature for each kind of sample are discussed.

16.3.1 Infant formulas and baby food

Krynitsky *et al*. [5] extracted the perchlorate anion from 10 g portion sample with a mixture of 5 mL of 1% acetic acid and 15 mL of acetonitrile. The purpose of the acetonitrile in the extraction solution for infant foods was to precipitate proteins and also to prevent a cloudy supernatant. Supernatant was clarified by passing through a graphitized carbon SPE that removes pigments and renders a water-clear eluant ready for injection in IC.

Her *et al*. [6] prepared a 67% (w/w) sample solution by dissolving, in 10 mL ultrapure water, 20 g of powered milk infant formula spiked with $NaCl^{18}O_4$ to a final concentration of 10 µg/L and vortexed for 30 s. The authors tested different extraction solutions to determine optimal conditions for perchlorate extraction: (i) MetOH 20 mL + 1% acetic acid 5 mL; (ii) ACN 17 mL + 1% acetic acid 5 mL + EtOH 3 mL; and (iii) ACN 20 mL + 1% acetic acid 5 mL. Sample were shaken at 260 rpm for 10 min and centrifuged at 4000 rpm for 10 min. All EtOH and ACN in the aqueous solution were evaporated at a temperature of 95°C for 30 min. Therefore, 10 mL of extract was passed through the SPE cartridge, and then filtered by a 0.2-µm pore size syringe filter.

Niemann and Anderson [7] dissolved in water a 5-g portion of sample and added one drop of the anti-foaming agent dodecyl alcohol. From the solution was weighed a

Table 16.3 Literature on anion determination in foods by ion chromatography.

Matrix	Element	Species	Technique	Injection volume	Linearity (*r*)	LOD (µg/L)	CV (%)	*R* (%)	Reference
Alfalfa	Cl	Chloride	IC-UV		0.1–5 mg/L	10			[45]
		Chlorite				10			
		Chlorate				2000			
Allium tuberosum	N	Nitrite	IC-UV						[68]
		Nitrate							
Apples	Cl	Chloride	IC-UV		0.1–5 mg/L	10			[45]
		Chlorite				10			
		Chlorate				2000			
Baby food	N	Nitrite	IC-UV/Vis (525 nm)					82–107	[87]
		Nitrate							
Beer	Cl	Chloride	IC-CD	20 µL					[13]
	N	Nitrate							
	P	Phosphate							
	S	Sulfate							
Beer	Cl	Perchlorate	IC-ESI-MS/MS	100 µL		0.005	0.9–9	95–102	[14]
Beers	S	Sulfites	HPIC-CD						[76]
Berries	Cl	Perchlorate	IC-CD	1000 µL	2.5–500 µg/L	50 ng/g			[44]
Beverages	Cl	Chloride	IC-suppresed CD	25 µL					[12]
		Chlorate							
Bread	Br	Bromate	IC-ICPMS	500 µL		0.3	2	90	[24]
Bread	Br	Bromate	IC-UV (+KI 352 nm)	300 µL	5.0–30 µg/L (0.9999)	1.5	0.04		[23]
Broccoli	Br	Bromide	IC						[72]
Cabbage	N	Nitrite	IC-UV						[68]
		Nitrate							
Cabbage	Cl	Perchlorate	IC-CD	20 µL	0.10–20.0 µg/L	0.2	2.5		[29]
Canned food	Cl	Perchlorate	IC-ESI-MS/MS	100 µL		0.005	0.9–9	95–102	[14]
Canned vegetables	N	Nitrite	IC-AWD	100 µL	0.5–100 mg/L (>0.999)	200	0.5 (50 mg/L)	96–104	[47]
		Nitrate			1.0–100 mg/L (>0.999)	300	0.6 (50 mg/L)	94–108	
Cantaloupe	Cl	Perchlorate	IC-CD	50–500 µL	1.00–50.0 ng of perchlorate	3–6 µg/kg	10.6	93–96	[65]
Cantaloupe	Cl	Chloride	IC-UV		0.1–5 mg/L	10			[45]
		Chlorite				10			
		Chlorate				2000			
Cantaloupe	Cl	Perchlorate	IC-MS/MS	50 µL		2.0 µg/kg	0.7–7.0 (10 µg/L)	107–114	[31]
Carrot	N	Nitrite	IC-UV						[68]
		Nitrate							
Carrot	Cl	Perchlorate	IC-CD	20 µL	0.10–20.0 µg/L	0.2	2.5		[29]

Carrots	Cl	Perchlorate	IC-CD	50–500 µL	1.00–50.0 ng of perchlorate	3–6 µg/kg	10.6	93–96	[65]
Cheese	Cl	Perchlorate	IC-CD	20 µL	0.1–20 µg/L	0.2	2.5		[30]
Chinese cabbage,	N	Nitrite Nitrate	IC-UV						[68]
Cod	P	Phosphate P_2O_7 P_3O_{10} P_3O_9	IC	10 µL	1–100 mg/L				[61]
Cucumber	N	Nitrite Nitrate	IC-UV						[68]
Cured ham	N	Nitrite Nitrate	IC-CD	20 µL	0.1–5 mg/L 0.01–2.5 mg/L				[49]
Cured meat	N	Nitrite Nitrate	IC-AWD	100 µL	0.5–100 mg/L (>0.999) 1.0–100 mg/L (>0.999)	200 300	0.5 (50 mg/L) 0.6 (50 mg/L)	96–104 94–108	[47]
Cured meat	N	Nitrite Nitrate	IC-MS						[50]
Dairy	N	Nitrite NItrate	IC-CD	25 µL	0.9999 0.9998	1.5 mg/kg 3.2 mg/kg	<8.5 <6.7	99 98	[40]
Dairy milk	Cl	Perchlorate	IC-MS/MS	100 µL	0.1–50 µg/L	0.12			[6]
Dairy products	F	Fluoride	IC-CD						[94]
Dairy products	Cl	Perchlorate	IC-ESI-MS						[64]
Dairy products	N	Nitrite Nitrate	IC-UV (225 nm)	200 µL	0.005–0.50 mg/L 0.05–1.50 mg/L			84–104	[89]
Dairy products	N	Nitrite Nitrate	IC-CD	25 µL	0.9999 0.9998	1.5 mg/kg 3.2 mg/kg	<3.0 <3.9	98.7 98.3	[48]
Dried vegetables	S	SO_2	IC-CD	25 µL	0.051–32.1 mg/L SO_2	13	0.7–8.2	77–105	[17]
Dry products	Cl	Perchlorate	IC-MS/MS			3.0 µg/kg		80–120	[5]
Fish	Cl	Perchlorate	IC-CD	1000 µL	2.5–500 µg/L	50 ng/g			[44]
Fish	P	Polyphosphates	IC						[90]
Fish	Cl	Perchlorate	IC-CD	20 µL	0.10–20.0 µg/L	0.2	2.5		[29]
Fish products	N	Nitrite NItrate	IC-CD	25 µL	0.9999 0.9998	1.5 mg/kg 3.2 mg/kg	<8.5 <6.7	99 98	[40]
Fishery products	P	Phosphates	IC-ECD						[60]
Flour	Br	Bromate	IC-CD	500 µL	5–1000 µg/L (0.9998)	0.5	6.4	86–110	[26]
Flour	Br	Bromate	IC-UVvis			0.5	<9.2	63–105	[93]
Food	Cl N S	Chloride Nitrite Nitrate Sulfate	IC-CD	100 µL	0.999	300–600	0.4–1.0 (n = 6)		[85]

(Continued)

Table 16.3 (Continued)

Matrix	Element	Species	Technique	Injection volume	Linearity (*r*)	LOD (μg/L)	CV (%)	*R* (%)	Reference
Food	Cl	Perchlorate	IC						[82]
Food	Cl	Chloride	IC/non-suppressed	100 μL					[41]
	N	Nitrite	CD						
	S	Nitrate							
		Sulfate							
Food	S	SO_2	IC-CD	25 μL	0.051–32.1 mg/L SO_2	13	0.7–8.2	77–105	[17]
Food	I	Iodide	IC-UV			0.01 μg/g	1.2		[95]
Food	Br	Bromate	IC-UV (+KI 352 nm)	300 μL	5.0–30 μg/L (0.9999)	1.5	0.04		[23]
Foodstuffs	N	Nitrite	IC-Fluorescence	50 μL	1–25 mg/L		2.8		[51]
		Nitrate	(exc270–em355)		3–65 mg/L				
Foodstuffs	Br	Bromide	IC-CD	50 μL	1.4×10^{-8} to 1.0×10^{-3} M	0.2 mg/kg	3		[71]
Fresh food	Cl	Perchlorate	IC-ESI-MS/MS	100 μL		0.005	0.9–9	95–102	[14]
Fresh meat	Cl	Perchlorate	IC-CD	20 μL	0.1–20 μg/L	0.2	2.5		[30]
Fresh meat	N	Nitrite	IC-AWD	100 μL	0.5–100 mg/L (>0.999)	200	0.5 (50 mg/L)	96–104	[47]
		Nitrate			1.0–100 mg/L (>0.999)	300	0.6 (50 mg/L)	94–108	
Fresh meat	S	Sulfites	IC-CD suppresed	25 μL	0.9998	2.7 mg/kg SO_2	<8	85–92	[52]
Fresh meat	S	Sulfites	IC-CD	25 μL		3.0 mg/kg			[53]
Fresh meat	N	Nitrite	IC		0.2–12 mg/L	1.5 mg/kg	2.4–8	97–100	[46]
		Nitrate			0.6–20 mg/L	3.2 mg/kg	2.6–6.7	98–99	
Fresh meat	N	Nitrite	IC-CD	25 μL	0.9999	1.5 mg/kg	<3.0	98.7	[48]
		Nitrate			0.9998	3.2 mg/kg	<3.9	98.3	
Fresh milk	I	Iodine	IC-DA		10–160 μg/L (0.9991)	1	3.9 (1 mg/L)	89.0	[9]
					0.4–5 mg/L (0.9986)		1.8 (2 μg/L)	86.0	
								84.1	
Fresh vegetables	N	Nitrite	IC-AWD	100 μL	0.5–100 mg/L (>0.999)	200	0.5 (50 mg/L)	96–104	[47]
		Nitrate			1.0–100 mg/L (>0.999)	300	0.6 (50 mg/L)	94–108	
Fruit	Cl	Perchlorate	IC, IC-MS						[42]
Fruit yoghurt	F	Fluoride	IC						[92]
Fruits	Cl	Perchlorate	IC-MS/MS			1.0 μg/kg		80–120	[5]
Fruits	Cl	Perchlorate	IC-MS/MS	60 μL		22.5 ng/kg	0.2–9.8	80–115	[43]
Garlic	N	Nitrite	IC-UV						[68]
		Nitrate							
Garlic	S	Sulfite	IC	50 μL					[75]
Grapefruit	Cl	Perchlorate	IC-CD	20 μL	0.10–20.0 μg/L	0.2	2.5		[29]

Greek Kiwi wine	Cl	Chloride	IC						[15]
	P	Phosphate							
	S	Sulfate							
Green onion	N	Nitrite	IC-UV						[68]
		Nitrate							
Green pepper	N	Nitrite	IC-UV						[68]
		Nitrate							
Herring roe	Cl	Chlorite	IC-CD	50 µL		5 mg/kg	4.2	88 ± 4	[63]
Honey	Br	Bromide	IC		0.5–10 mg/L	500	<5	90–95	[81]
	Cl	Chloride			10–80 mg/L (chloride)				
	N	Nitrite							
	P	Nitrate							
	S	Phosphate							
		Sulfate							
Infant foods	Cl	Perchlorate	IC-MS/MS			2.0 µg/kg		80–120	[5]
Infant formula	I	Iodine	IC-DA		10–160 µg/L (0.9991)	1	3.9 (1 mg/L)	89.0	[9]
					0.4–5 mg/L (0.9986)		1.8 (2 µg/L)	86.0	
								84.1	
Infant formula	I	Iodide	IC-UV	1 mL	1–50 ng (0.9999)	0.04 mg/kg	3–5	91–104	[7]
	SCN	Thiocyanate	Pre-concentration column		6–300 ng, (0.9998)		6–14	80–110	
Infant formula	Cl	Perchlorate	IC-MS/MS	100 µL	0.1–50 µg/L	1.0 µg/kg			[6]
Infant formula	Cl	Perchlorate	ID-IC-MS/MS			0.4		94–110	[8]
Infant formula fruit	N	Nitrite	IC					87–104	[86]
		Nitrate							
Infant formula Vegetable	N	Nitrite	IC					91–104	[86]
		Nitrate							
Leafy vegetable	Cl	Perchlorate	IC-ESI-MS						[64]
Leafy vegetables	N	Nitrite	IC-CD	25 µL	0.9999	1.5 mg/kg	<8.5	99	[40]
		NItrate			0.9998	3.2 mg/kg	<6.7	98	
Leafy vegetables	N	Nitrite	IC-CD	25 µL	0.9999	1.5 mg/kg	<3.0	98.7	[48]
		Nitrate			0.9998	3.2 mg/kg	<3.9	98.3	
Lemon	Cl	Perchlorate	IC-CD	20 µL	0.10–20.0 µg/L	0.2	2.5		[29]
Lettuce	N	Nitrite	IPC-UV (220 nm)	20 µL	0.03–1 mg/L	30			[67]
		Nitrate			0.01–0.5 mg/L	10			
Lettuce	N	Nitrite	IC-UV						[68]
		Nitrate							
Lettuce	Cl	Perchlorate	IC-MS/MS	50 µL		1.0 µg/kg	0.7–7.0 (10 µg/L)	93–107	[31]
Lettuce	Cl	Perchlorate	IC-CD	50–500 µL	1.00–50.0 ng of perchlorate	3–6 µg/kg	10.6	93–96	[65]

(Continued)

Table 16.3 (Continued)

Matrix	Element	Species	Technique	Injection volume	Linearity (*r*)	LOD (μg/L)	CV (%)	*R* (%)	Reference
Lettuce	Cl	Chloride	IC-UV		0.1–5 mg/L	10			[45]
		Chlorite				10			
		Chlorate				2000			
Lettuce	Cl	Perchlorate	IC-CD	20 μL	0.10–20.0 μg/L	0.2	2.5		[29]
Light salami	N	Nitrite	IC-Fluorescence	50 μL	1–25 mg/L		2.8		[51]
		Nitrate	(exc270–em355)		3–65 mg/L				
Mandarin	Cl	Perchlorate	IC-CD	20 μL	0.10–20.0 μg/L	0.2	2.5		[29]
Meat	N	Nitrite	IC-CD	25 μL	0.9999	1.5 mg/kg	<8.5	99	[40]
		NItrate			0.9998	3.2 mg/kg	<6.7	98	
Meat products	N	Nitrite	IC-UV	0.05 mg/L	1–20 mg/L	20 μL	1.57 (10)		[88]
		Nitrate		0.08 mg/L			2.43 (10)		
Meat products	N	Nitrite	IC-CD	25 μL	0.9999	1.5 mg/kg	<3.0	98.7	[48]
		Nitrate			0.9998	3.2 mg/kg	<3.9	98.3	
Milk	Cl	Perchlorate	IC-CD	1 mL		0.5		96–102	[32]
Milk	Cl	Perchlorate	IC-MS/MS	50 μL		3.0	0.7–7.0 (10 μg/L)	99–101	[31]
Milk	Cl	Iodide	IC-MS	1000 μL			3–6	>94	[35]
	I	Perchlorate							
	SCN	Thiocyanate							
Milk	Cl	Perchlorate	IC-ESI-MS						[64]
Milk	I	Iodide	IC-MS						[83]
	Cl	Perchlorate							
	SCN	Thiocyanate							
Milk	Cl	Perchlorate	IC-ESMS		50.0–50,000 ng/L (0.995)	0.01			[21]
Milk	Cl	Perchlorate	IC-MS						[33]
Milk	F	Fluoride	IC-PED (pulse electrochemical detector)	25 μL	10–2000 μg/L	3.8	0.24–1.02	97–108	[37]
Milk	Cl	Perchlorate	IC-CD	20 μL	0.10–20.0 μg/L	0.2	2.5		[29]
Milk	Cl	Perchlorate	IC-CD	20 μL	0.1–20 μg/L	0.2	2.5		[30]
Milk	N	Nitrite	IC			70			[93]
		Nitrate				330			
Milk	Cl	Perchlorate	IC-MS/MS			0.12	<20	98–105	[22]
Milk	Cl	Perchlorate	IC-MS/MS			0.015			[36]
Mushrooms	Br	Bromide	IC-CD	50 μL	1.4×10^{-8} to 1.0×10^{-3} M	0.2 mg/kg	3		[71]

Onion	N	Nitrite Nitrate	IC-UV						[68]
Orange	Cl	Perchlorate	IC-CD	20 μL	0.10–20.0 μg/L	0.2	2.5		[29]
Oranges	Cl	Chloride Chlorite Chlorate	IC-UV		0.1–5 mg/L	10 10 2000			[45]
Pickles	N	Nitrite Nitrate	IC-UV			10 5	Good	Good	[69]
Potato	N	Nitrite Nitrate	IC-UV						[68]
Potato products	S	Sulfites	HPIC-CD						[76]
Powder milk	I	Iodine	IC-DA		10–160 μg/L (0.9991) 0.4–5 mg/L (0.9986)	1	3.9 (1 mg/L) 1.8 (2 μg/L)	89.0 86.0 84.1	[9]
Powdered milk	I SCN	Iodide Thiocyanate	IC-UV Pre-concentration column	1 mL	1–50 ng (0.9999) 6–300 ng (0.9998)	0.04 mg/kg	3–5 6–14	91–104 80–110	[7]
Powdered milk	N	Nitrite Nitrate	IC			70 330			[93]
Preserved foods	S	SO_2	IC-CD	25 μL	0.051–32.1 mg/L SO_2	13	0.7–8.2	77–105	[17]
Pumpkin	N	Nitrite Nitrate	IC-UV						[68]
Radish	N	Nitrite Nitrate	IC-UV						[68]
Red cabbage	Cl	Perchlorate	IC-CD	20 μL	0.10–20.0 μg/L	0.2	2.5		[29]
Rice	Cl	Perchlorate	IC-ESMS		50.0–50,000 ng/L (0.995)	0.01			[21]
Rice	Cl	Perchlorate	IC-MS/MS			0.27 μg/kg	<20	98–105	[22]
Sausage	N	Nitrite Nitrate	IC-AWD	100 μL	0.5–100 mg/L (>0.999) 1.0–100 mg/L (>0.999)	200 300	0.5 (50 mg/L) 0.6 (50 mg/L)	96–104 94–108	[47]
Seafoods	P	Phosphate Pyrophosphate Metaphosphate Total phosphorus	IC-CD supressor		0.3–60 mg/L	2.1–2.3 mg/kg	1.6–2.6	82–100	[62]
Seaweed	Cl	Perchlorate	IC-MS/MS			0.07 μg/kg	<20	98–105	[22]

(Continued)

Table 16.3 (Continued)

Matrix	Element	Species	Technique	Injection volume	Linearity (*r*)	LOD (μg/L)	CV (%)	*R* (%)	Reference
Seaweeds	Br	Bromide	IC-CD	20 μL	>0.999	2–50	<2	97–103 Cl	[59]
	Cl	Chloride						87–105SO_4^{2-}	
	F	Fluoride							
	N	Nitrite							
	P	Nitrate							
	S	Phosphate							
		Sulfate							
Shellfishes	N	Nitrite	IC-CD	25 μL	0.9999	1.5 mg/kg	<3.0	98.7	[48]
		Nitrate			0.9998	3.2 mg/kg	<3.9	98.3	
Shrimps	P	Polyphosphates	IC						[90]
Shrimps	S	Sulfites	IC-CD suppresed	25 μL	0.9998	2.7 mg/kg SO_2	<8	85–92	[52]
Soybean	N	Nitrite	IC-UV						[68]
		Nitrate							
Spinach	N	Nitrite	IPC-UV (220 nm)	20 μL	0.03–1 mg/L	30			[67]
		Nitrate			0.01–0.5 mg/L	10			
Spinach	N	Nitrite	IC-UV						[68]
		Nitrate							
Spinach	Cl	Perchlorate	IC-CD	50–500 μL	1.00–50.0 ng of perchlorate	3–6 μg/kg	10.6	81	[65]
Spinach	Cl	Perchlorate	IC-CD	20 μL	0.10–20.0 μg/L	0.2	2.5		[29]
Spinach.	Cl	Chloride	IC-CD		Up to 25–30 mg/kg	0.1 mg/kg	0.09–0.12		[74]
	N	Nitrate				0.1 mg/kg			
	P	Phosphate				0.2 mg/kg			
	S	Sulfate				0.2 mg/kg			
Sprouts	N	Nitrite	IC-UV						[68]
		Nitrate							
Sprouts	Cl	Chloride	IC-UV		0.1–5 mg/L	10			[45]
		Chlorite				10			
		Chlorate				2000			
Strawberries	Cl	Chloride	IC-UV		0.1–5 mg/L	10			[45]
		Chlorite				10			
		Chlorate				2000			

Sugar	Cl	Chloride	HPAEC-CD	25 µL	0.05–10 mg/L	50	7.6	104.3	[79]
	N	Nitrite			0.1–5.0	60	6.1	93.4	
	P	Nitrate			0.2–10.0	10	2.2	110.1	
	S	Phosphate			0.1–20.0	210	3.2	99.2	
		Sulfate			0.2–5.0	40	2.8	94.0	
Taro	P	H_2PO_4	IC-CD		50–1000 µM				[77]
Tomato	Cl	Perchlorate	IC-CD	20 µL	0.10–20.0 µg/L	0.2	2.5		[29]
Tomatoes	Cl	Chloride	IC-UV		0.1–5 mg/L	10			[45]
		Chlorite				10			
		Chlorate				2000			
Leafy vegetables	Cl	Perchlorate	IC-CD	1000 µL	0.5–100 µg/L	0.2			[66]
Vegetables	Cl	Perchlorate	IC-MS/MS			2.0 µg/kg		80–120	[5]
Vegetables	Cl	Perchlorate	IC, IC-MS						[42]
Vegetables	Cl	Perchlorate	IC-MS/MS	60 µL		22.5 ng/kg	0.2–9.8	80–115	[43]
Vegetables	Cl	Perchlorate	IC-MS/MS			0.16 µg/kg	<20	98–105	[22]
Vegetation	Cl	Perchlorate	IC-CD	1000 µL	2.5–500 µg/L.	50 ng/g			[44]
Vinegar	Cl	Chloride	IC-CD	100 µL	> 0.999		0.53–1.03		[84]
	N	Nitrite							
		Nitrate							
Wine	Cl	Perchlorate	IC-ESI-MS/MS	100 µL		0.005	0.9–9	95–102	[14]
Wine	Cl	Chloride	HPIC						[16]
	N	Nitrate							
	P	Phosphate							
	S	Sulfate							
Wine	S	SO_2	IC-CD	25 µL	0.051–32.1 mg/L SO_2	13	0.7–8.2	77–105	[17]
Wines	S	Sulfites	HPIC-CD						[76]
Yoghurt	Cl	Perchlorate	IC-CD	20 µL	0.1–20 µg/L	0.2	2.5		[30]

Table 16.4 Literature on arsenic and selenium species determination in food by ion chromatography.

Matrix	Element	Species	Technique	Injection volume	Linearity (*r*)	LOD (µg/L)	CV (%)	*R* (%)	Reference
BCR-627	Se	SeMet, Se(IV), Se(VI)	IC-ICPMS	100 µL	Se 1.5–20 µg/L	Se 0.7	<5	Se 57–97	[57]
	As	AsB, As(III), DMA, mMA, As(V)			As 0.5–40 µg/L	As 0.1		As 82–94	
Biscuit	As	As(III), As(V)	IC-ICPMS	250 µL	As 0.05–5.0 µg/L	As 0.4 µg/kg	2.5–3.7	102	[11]
		MMA, DMA				As 0.3 µg/kg	1.9–2.7	100	
Bread	As	As(III), As(V)	IC-ICPMS	250 µL	As 0.05–5.0 µg/L	As 0.4µg/kg	2.5–3.7	102	[11]
		MMA, DMA				As 0.3 µg/kg	1.9–2.7	100	
Cereals	As	As(III), DMA, mMA, As(V)	IC-DRC-ICPMS	200 µL	0.1–10 ng/mL (0.9990)	0.009	<5	94–105	[20]
	Se	SeCys2, SeMet, Se(IV), Se(VI)				0.030			
Chicken liver	As	As(III), As(V), mMA, DMA HAMPA, HACPA,Rox	IC-ICPMS	50 µL		0.07–0.18 µg/kg			[54]
Corn snakes	As	As(III), As(V)	IC-ICPMS	250 µL	As 0.05–5.0 µg/L	As 0.4 µg/kg	2.5–3.7	102	[11]
		MMA, DMA				As 0.3 µg/kg	1.9–2.7	100	
Crustacean (shrimp)	As	As(III), mMA, DMA, As(V) AsB, AsC, TMAO, TMAs	IEC-ICPMS	100 µL	0–20 µg/L	As 19–75 µg/kg	3–14	82–104	[56]
DOLT-3	Se	SeMet, Se(IV), Se(VI)	IC-ICPMS	100 µL	Se 1.5–20 µg/L	Se 0.7	<5	Se 57–97	[57]
	As	AsB, As(III), DMA, mMA, As(V)			As 0.5–40 µg/L	As 0.1		As 82–94	
Edible oil	As	As(III), As(V), mMA DMA, AsB, AsC	IC-ICPMS	100 µL		0.008–0.024	<8	90–105	[78]
Fish (saithe, salmon, smoked salmon, tuna and canned tuna)	As	As(III), mMA, DMA, As(V) AsB, AsC, TMAO, TMAs	IEC-ICPMS	100 µL	0–20 µg/L	As 19–75 µg/kg	3–14	82–104	[56]
Fish (canned tuna)	Se	SeMet, Se(IV), Se(VI)	IC-ICPMS	100 µL	Se 1.5–20 µg/L	Se 0.7	<5	Se 57–97	[57]
	As	AsB, As(III), DMA, mMA, As(V)			As 0.5–40 µg/L	As 0.1		As 82–94	
Fish oil	As	As(III), As(V), mMA, DMA TMAO, TMAs, AsB, AsB2, AsC	IC-ICP-MS			5 ng/g		85–100	[55]
Food composites	As	AsB	IC-ICPMS	200 µL		0.07	3–5	78–96	[80]
		AsC				0.1	7–14	98–102	
		As(III)				0.08	4–5	92–102	
		As(V)				0.2	4–14	89–108	
		DMA				0.04	3–5	94–97	
		MMA				0.04	3–5	103–106	

Infant cereals	As	As(III), As(V) MMA, DMA	IC-ICPMS	250 µL	As 0.05–5.0 µg/L	As 0.4 µg/kg As 0.3 µg/kg	2.5–3.7 1.9–2.7	102 100	[11]
Infant food (rice-based, mixed rice, milk-based)	As	As(III) As(V) DMA MMA	IC-ICPMS			5 ng/g 10 ng/g 5 ng/g 5 ng/g		94–128	[10]
Infant food (purée of peaches, purée of pears, purée of carrot)	As	As(III) As(V) DMA MMA	IC-ICPMS			5 ng/g 10 ng/g 5 ng/g 5 ng/g		94–128	[10]
Infant food (purée of sweet potatoes, purée of squash)	As	As(III) As(V) DMA MMA	IC-ICPMS			5 ng/g 10 ng/g 5 ng/g 5 ng/g		94–128	[10]
Infant food (purée of green beans, apple sauce)	As	As(III) As(V) DMA MMA	IC-ICPMS			5 ng/g 10 ng/g 5 ng/g 5 ng/g		94–128	[10]
Kale	Se	Se(IV), Se(VI), MeSeCys SeCys2, SeMet	IPLC-ICPMS	50 µL					[73]
Milk	Se	SeMet, SeCys, Se(IV)	HPLC-ICPMS	100 µL	Standard addition (0.990)	Se 1 ng/mL		91 ± 5	[34]
Mollusc (mussel, oyster, scallop)	As	As(III), mMA, DMA, As(V) AsB, AsC, TMAO, TMAs	IEC-ICPMS	100 µL	0–20 µg/L	As 19–75 µg/kg	3–14	82–104	[56]
Mussel	As	AsB AsC TMAO $TMAs^+$	IC-UVox-HGAFS	100 µL		5 pg 12 pg 4 pg 6 pg	<3		[58]
Oyster	As	AsB AsC TMAO $TMAs^+$	IC-UVox-HGAFS	100 µL		5 pg 12 pg 4 pg 6 pg	<3		[58]

(Continued)

Table 16.4 (Continued)

Matrix	Element	Species	Technique	Injection volume	Linearity (*r*)	LOD (µg/L)	CV (%)	*R* (%)	Reference
Pasta	As	As(III), As(V) MMA, DMA	IC-ICPMS	250 µL	As 0.05–5.0 µg/L	As 0.4 µg/kg As 0.3 µg/kg	2.5–3.7 1.9–2.7	102 100	[11]
Rice	As	As(III) AsB DMA MMA As(V)	IC-ICPMS	20 µL	5–50 µg/L	0.107 0.084 0.120 0.121 0.101	5.9 3.9 2.7 5.2 5.4		[19]
Rice-based raw materials and finished products	As	As(III), As(V), mMA DMA, AsB	IC-ICPMS			0.007–0.02 mg/kg	0.2–0.6	93–122	[18]
Seafood-based raw materials and finished products	As	As(III), As(V), mMA DMA, AsB	IC-ICPMS			0.007–0.02 mg/kg	0.2–0.6	93–122	[18]
Shark, marlin	Se As	SeMet, Se(IV), Se(VI) AsB, As(III), DMA, mMA, As(V)	IC-ICPMS	100 µL	Se 1.5–20 µg/L As 0.5–40 µg/L	Se 0.7 As 0.1	<5	Se 57–97 As 82–94	[57]
Tropaeolum majus (capuchinas)	As	As(III) Phenylarsonic acid *p*-Aminophenylarsonic *o*-Aminophenylarsonic acid Phenylarsine oxide 3-Nitro-4-hydroxyphenylarsonic acid	IC-ICPMS	40 µL	As 10–200 µg/L r >0.99 (n = 5)	0.1–1 µg/L	<2–14		[70]
Wheat flour	As	As(III), As(V) MMA, DMA	IC-ICPMS	250 µL	As 0.05–5.0 µg/L	As 0.4 µg/kg As 0.3 µg/kg	2.5–3.7 1.9–2.7	102 100	[11]

Table 16.5 Literature on cation determination in food by ion chromatography.

Matrix	Element	Technique	Injection volume	Linearity (*r*)	LOD (µg/L)	CV (%)	*R* (%)	Reference
Bovine liver	Pu, Am, Np	IC-ICPMS	350 µL	(<0.995)	20 ng/g		100 ± 20	[28]
Buffalo milk (14 samples)	Li, Na, K, Mg, Ca	HPIC-CD						[39]
Cabbage	Pu, Am, Np	IC-ICPMS	350 µL	(<0.995)	20 ng/g		100 ± 20	[28]
Feedstuff	Li, Na, K, NH_4, Mg, Ca	IC-CD					99–102	[96]
Infant formulas	Cu, Ni, Zn, Co, Mn, Fe	IC	50 µL		5			[91]
Infant formulas	Co, Cu, Fe, Mn, Ni, Zn	IC-UV/Vis (500 nm)	50 µL	Range 1: 10–100 ppb Range 2: 100–1000 ppb Range 3: 1–5 ppm	5	0.3–3.0		[27]
Honey	Na, K, NH_4, Ca, Mg	IC		0.1–10 µg/mL 50–300 µg/mL (K)	<500 ng/mL	<5	90–95	[81]
Lamb	Pu, Am, Np	IC-ICPMS	350 µL	(<0.995)	20 ng/g		100 ± 20	[28]
Maize flour	Co, Cu, Fe, Mn, Ni, Zn	IC-UV/Vis (500 nm)	50 µL	Range 1: 10–100 ppb Range 2: 100–1000 ppb Range 3: 1–5 ppm	5	0.3–3.0		[27]
Milk	Pu, Am, Np	IC-ICPMS	350 µL	(<0.995)	20 ng/g		100 ± 20	[28]
Milk	Ca	HPIC-CD	20 µL	1–10 mg/L			100	[38]
Mozzarella cheese (14 samples)	Li, Na, K, Mg, Ca	HPIC-CD						[39]
Ryegrass	Pu, Am, Np	IC-ICPMS	350 µL	(<0.995)	20 ng/g		100 ± 20	[28]
Sorghum red flour	Co, Cu, Fe, Mn, Ni, Zn	IC-UV/Vis (500 nm)	50 µL	Range 1: 10–100 ppb Range 2: 100–1000 ppb Range 3: 1–5 ppm	5	0.3–3.0		[27]
Sorghum white flour	Co, Cu, Fe, Mn, Ni, Zn	IC-UV/Vis (500 nm)	50 µL	Range 1: 10–100 ppb Range 2: 100–1000 ppb Range 3: 1–5 ppm	5	0.3–3.0		[27]
Wine (15 samples)	Li, Na, K, Mg, Ca	HPIC						[16]

15-g aliquot inside an ultrafiltration unit and centrifuged at 4500 rpm and 30°C for 60 min. Ultrafiltrate was cleaned up on a 6-mL graphitized carbon SPE column, previously conditioned with MetOH and 10 mM HNO_3, at a flow rate of 1–2 mL/min. The first fraction (3 mL) was discarded and the next 1.5-mL fraction collected for further chromatographic analysis.

Other studies have been carried out on infant formulas for perchlorate determination [8], iodide and thiocyanate determinations [9] and arsenic speciation [10, 11].

16.3.2 Beverages, including wine and beer

Beverages

Jackson *et al*. [12] have described that hypochlorite is fragile when added to most common beverages and typically breaks down over time. When a beverage was adulterated with bleach, but no hypochlorite is detected, it is still possible to demonstrate the addition of bleach because the pH of the adulterated product will be higher than the pH of the control. A chlorine or organic odour may be noted and, additionally, elevated chloride and chlorate may be observed by IC as compared with the control.

Beer

No extensive sample treatments are needed to determine anions in beer, although the foam should be removed. Bruce [13] decanted the beer 20 times to facilitate the removal of carbon dioxide before sonication for 15 min. After that, beer sample was diluted 1 : 20 with deionized water and injected directly into the ion chromatograph. El Aribi *et al*. [14] prepared the sample by taking 5 mL of beer and adding 25 mL of distilled water. After mixing the sample with water, it was shaken for 1 min and then centrifuged at 9500 rpm for 30 min at room temperature. Samples were then filtered using a 0.2-μm pore size nylon-mesh syringe filter.

Wine

Usually wine samples do not require laborious treatments. Thus it may be enough to use a simple adequate dilution with high purity water and filter through cartridges of polyvinylpolypyrrolidone and Millipore filter (0.45 μm) [15, 16] or 0.2-μm pore size nylon-mesh syringe filter [14] before direct injection to the ion chromatograph.

However, Zhong *et al*. [17] presented an elaborate experimental design to facilitate the extraction of the analytes of interest. Figure 16.5 shows the distillation system employed for the determination of total SO_2 in wines. Sodium carbonate added to the samples was utilized to release the bonded sulfite and generate large volumes of carbon dioxide. Thus sulfur dioxide and all species of S(IV) were distilled and trapped effectively in NaOH. This distillation system could provide satisfactory recoveries within 3 min without blowing nitrogen or air. The procedure described is as follows: 5.0 g of sample were put into distillation tubes, following by addition of 10 mL of 5% (w/v) Na_2CO_3, being vortex-mixed for 2 min. After that, 20 mL of 9.0 M H_2SO_4 were added to the mixture, and then connected rapidly to a distillation tube and a 100 mL acceptor tube with 25 mL of 0.3 M NaOH. The distillation process was completed after 100 mL collection solutions were obtained. After that, the collected solution was purified with LC-SCX cartridges for ion chromatography analysis.

Prusisz *et al*. [16] have determined Li, Na, K, Mg and Ca in wine samples by IC. Only filtration and dilution were required.

16.3.3 Cereals (grain, flour and bread)

Rice analysis has been carried out for arsenic speciation [18–20] and perchlorate determination [21, 22] by using MS detection. Moreover, the determination of bromate has been performed mainly in bread by ultraviolet (UV) [23] and MS detection [24] and in flour by UV [25] and conductimetric detection [26].

An easy perchlorate extraction procedure was employed by Shi *et al*. [21]. Rice samples were air dried, ground and homogenized with a 40-mesh sieve; 5.0 g of rice powder was weighed inside a 50 mL conical flask and then extracted with 20 mL water by shaking at 200 rpm for 2 hours at room temperature. The mixture was centrifuged for another 25 min at 6000 rpm. After that, the supernatant solution was filtered through 0.22-μm nylon membrane and injected to the chromatograph.

Tsai and Jiang [20] used a microwave-assisted extraction procedure for arsenic and selenium extraction in rice and wheat flour. Approximately 0.5 g of cereal samples were weighed in 15 mL polyethylene centrifuge tubes; 10 mg amylase, 50 mg Protease XIV, and 10 mL of 15 mM ammonium citrate (pH 7.5) were added. The centrifuge tube was put into a 1-L beaker containing 450 mL of water. The microwave system was programmed to maintain the water temperature at 70°C for

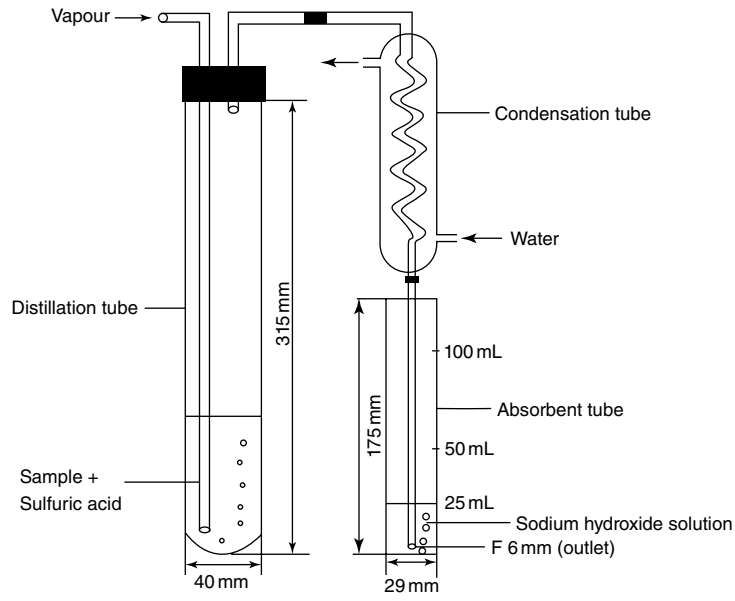


Figure 16.5 The system employed for SO_2 distillation. From Zhong *et al.* [17]. Reproduced with permission of Elsevier.

30 min. After microwave heating, samples were allowed to cool and directly centrifuged for 10 min at 1870 *g*. The supernatants were directly filtered through a PVDF filter of 0.22-μm porosity, and then injected into the IC separation system.

Shi *et al.* [26] used a sonication-assisted extraction procedure for bromate extraction in flour and related samples which were ground into flour. About 5 g were weighed in a 250 mL conical flask and diluted with 250 mL of distilled water. The sample was mixed thoroughly before sonication for 30 min at room temperature. After treatment, samples were centrifuged for 10 min at 3000 rpm and the supernatant solution collected. The supernatant solution (15 mL) was passed through a 0.22-μm nylon membrane and OnGuard II RP column. In cases where the chloride concentration in the sample solution was higher than 50 mg/L, the first 3 or 6 mL were discarded on the basis of the size of the OnGuard column (1.0 or 2.5 mL), and the following solution was collected for injection to the IC.

Fredrikson *et al.* [27] developed two different sample treatments for maize, white sorghum and red sorghum flours, a leaching treatment and a microwave digestion, for Co, Cu, Fe, Mn, Ni and Zn determination. For 0.4 g dry samples, leaching with 4 mL of 1 M HCl was used for an incubation time of 12 hours. After the incubation was added 1 mL of 20 g/L ascorbic acid and water until 10 mL total volume. This solution was further incubated for 2 hours by sonication and thorough vortex mixing followed by centrifugation for 10 min at 10,000 *g*. The filtrate was then injected into the IC system. Microwave sample digestion was done by mixing 0.4 g of dry sample, 3 mL of H_2O, 0.75 mL of concentrated HNO_3 and 0.15 mL of concentrated HCl in a Teflon vial. The sample was digested to a transparent solution in the microwave oven by a continuous temperature program reaching 180°C in 15 min; this temperature was kept constant for 20 min. After this treatment samples were cooled to room temperature, and decanted into test tubes with 10 mL volume level premarked. The Teflon vial was washed, and the washing water and sample liquid were pooled in the test tube and diluted to a final volume of 10 mL. Before injection, 0.1 mL of ascorbic acid (20 g/L) was added to 0.9 mL of sample.

Bromate was extracted from bread with water. The clean-up procedure included the use of a 0.2-μm filter, a C18 cartridge for defatting, a silver cartridge to remove halogen anions, a centrifugal ultrafiltration unit to remove proteins, and a cation-exchange cartridge to remove silver ions [24]. Each specimen was cut into small pieces and homogenized in a blender. The crumbs (10 g) were placed in 100-mL glass beakers and stirred with 50 mL water for 15 min, with a magnetic stirrer. The bread extracts were centrifuged at 8000 *g* for

10 min, and the supernatant filtered through filter paper. The filtrates were passed through 0.2-μm cellulose acetate syringe filter, an activated C18 cartridge and a silver cartridge, attached in tandem in that order. The presence of chloride and bromide may affect the baseline noise and the resolution of the bromate peak in the IC/IC-PMS determination.

A similar procedure to the above was employed by Wang *et al*. [23] for bromate determination in bread, although the determination was carried out by using a conductimetric detector (CD). Samples (200 g) were sliced and dried in an oven at 50°C and then milled before weighing. About 10 g of sample were accurately weighed in 250-mL conical flask and 100 mL of water added. Samples were mixed thoroughly before centrifugation for 20 min at 3000 rpm, and the supernatant solutions (15 mL) filtered through 0.22-μm nylon membranes and OnGuard II (Ag and H) columns successively. The first effluent (6 mL) was discarded and the following solution was collected for the analysis by IC-UV.

Evans *et al*. [28] employed microwave-assisted digestion for Pu, Am and Np extraction from ryegrass. Samples were mineralized in closed-vessel microwave digestion using concentrated HNO_3 as reagent in Teflon or quartz vessels. The first stage of the digest involved weighing 0.5 g of sample into the digestion vessels with 5 g of concentrated HNO_3. This was followed by a stepped heating program (0–600 W for CEM microwave and 0–1000 W for Multiwave) for a total digestion time of 1.3 and 1 hours, respectively. Where determination of Pu was the primary objective, samples were oxidized using a mixture of concentrated HNO_3 and H_2O_2 to leave a single Pu oxidation state. However, this leads to poor Am recovery. Good Am recovery was only obtained by omitting the oxidation step.

16.3.4 Dairy product (milk, yoghurt and cheese)

Milk

Milk is a complex liquid and it is necessary to remove the proteins and lipids before injection to the chromatograph. Some studies have proposed acetonitrile for this purpose. In a 50-mL conical flask were placed 5 mL of milk sample, 5 mL of deionized water and 20 mL of acetonitrile. After shaking by hand for 2 min the mixture was transferred into a centrifuge tube and centrifuged at 6000 rpm for 20 min, or at 2000 rpm for 5 min, at room temperature. Supernatant was filtered through a 0.22-μm pore size nylon membrane before injection to the ion chromatograph and perchlorate determined by conductimetry [29, 30] or MS [6, 21, 31] detectors. Ethanol was also employed for the coagulation of proteins [32, 33].

Many studies have been devoted to the determination of perchlorate in milk by IC with MS detection [6, 21, 31, 33–36]. In some of them, milk samples were spiked with stable isotope-labelled perchlorate ($Cl^{18}O_4^-$) [6, 33, 35]. Dyke *et al*. [35] spiked a 20-mL milk aliquot with 100 μL of a 1 mg/L solution of the isotopically labelled perchlorate standard. Elimination of milk lipids was carried out by ultracentrifugation at 16,000 rpm at 15°C for 20 min. Lipids separate into the top layer on centrifugation while some material also deposits at the bottom. The liquid in the intermediate layer, the resulting 'skim milk', can be easily decanted; 10 mL of this liquid were filtered and centrifuged at 5000 rpm at 15°C for 90 min in order to significantly reduce the background and baseline signal. Her *et al*. [6] employed 9.6 mL of milk, spiked with $NaCl^{18}O_4$, for analysis; 5 mL of 1% acetic acid and 20 mL acetonitrile were added to the sample, shaking at 200 rpm for 20 min and centrifuging at 4000 rpm for 5 min, in order to separate lipids and proteins. Next, the supernatant was processed through C18 SPE and then passed through a prewashed 25-mm, 0.2-μm pore polytetrafluoroethylene (PTFE) syringe filter prior to analysis. Sanchez *et al*. [33] spiked 0.5 mL of milk sample with the stable isotope $NaCl^{18}O_4$. Proteins were precipitated by addition of 3 mL EtOH, centrifuged (3016 *g*, –5°C) for 35 min, and supernatant transferred to a clean tube and evaporated to dryness under a stream of nitrogen at 60°C. Sample were resuspended in 1.0 mL of deionized water and added to a preconditioned C18 SPE cartridge. Finally, 1 mL of this solution was transferred for IC-MS/MS analysis.

Although less sensitive and less specific than MS, the conductimetric detector has also been used for the determination of perchlorate in liquid milk [32]. A 5-mL sample of milk was mixed with 10 mL EtOH. Samples were then allowed to stand for 24 hours refrigerated at –4°C. After centrifugation at 3700 rpm for 20 min at –5°C, the supernatant was collected. Each sample was then evaporated to 4 mL by warm nitrogen evaporation and then processed through 2-g C18 SPE cartridges, prewashed sequentially with 2 mL of *n*-hexane, 2 mL of acetone, and 2 mL of ultrapure water, and the effluent was then passed through a second 1-g C18 cartridge.

After the C18 clean-up, the required amount of ultrapure water was added as needed to return the samples to the original volume of 5 mL. Further, the extract was injected to the IC-CD.

Bierla *et al*. [34] used a proteolytic digestion for the simultaneous determination of SeCys, Se(IV) and SeMet by MS. A 0.1-g freeze-dried sample was mixed with 2 mL urea and sonicated. An amount of 5 ng of ^{77}Se-labelled selenomethionine was added and the solution was then incubated for 2 hours at 25°C in the dark with dithiothreitol and iodoacetamide to alkylate cysteine and selenocysteine residues and to break the Se–Se, S–S and Se–S bonds. After that, an aliquot of 1 mL of protease solution (20 mg protease in 0.1 M Tris buffer pH 7.5) was added and the sample was incubated for 17 hours at 37°C. Proteolysed samples were frozen and freeze-dried. The lyophilate was dissolved in 1 mL of water and centrifuged.

Single drop microextraction (SDME) with *in situ* generation of volatile hydrogen fluoride has been developed for fluoride determination in milk samples [37]. The headspace SDME is illustrated in Figure 16.6. A 3.0-mL sample was placed inside the sample vial with a PTFE septum and stirred with a magnetic bar. A 15-µL volume of Na_2CO_3 was drawn into a microsyringe. The syringe was fixed above the vial and the needle was introduced through the septum. After injecting 1.0 mL of 0.05 M H_2SO_4 through the septum, the microdrop containing 9 mM Na_2CO_3 aqueous solution was exposed to the headspace of the sample stirred at 600 rpm for 15 min. After extraction, the remaining drop was retracted back into the microsyringe. The needle was removed from the headspace, then the extract solution was put in a 2-mL measuring flask and made to a constant volume and then introduced into the chromatograph for subsequent analysis. Potential interferents as, macromolecules, most small organic and inorganic compounds, remain in the sample solution. Additionally, the main advantage of this method is that sample clean-up and environmentally friendly preconcentration can be completed in a single step.

Evans *et al*. [28] employed microwave-assisted digestion for Pu, Am and Np extraction from milk using the system proposed for ryegrass. Powdered milk samples were analysed for perchlorate [6], thiocyanate [7] and selenium speciation [34]. For selenium speciation analysis, freeze-dried samples were required in order to obtain appropriate detection limits. The milk powders were defatted by solid–liquid extraction with cyclohexane. Milk powder was dissolved prior to analysis in aqueous chromatographic buffer [34].

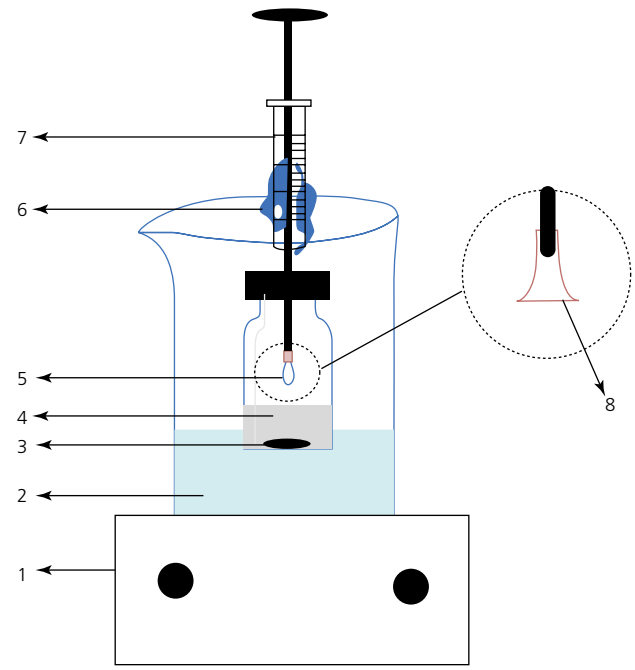


Figure 16.6 The system employed for the single drop microextraction of fluoride in milk samples. 1, Magnetic stirrer; 2, water; 3, magnetic stirrer bar; 4, sample solution; 5, microdrop; 6, ice bag; 7, microsyringe; 8, flared PTFE tube. From Yiping and Caiyun [37]. Reproduced with permission of Elsevier.

Niemann and Anderson [7] prepared 5% (w/w) slurry of milk powdered infant formula by weighing 5 g of sample and dissolving in 100 mL of water. An aliquot of 15 g was weighed and centrifuged at 4500 rpm at 30°C for 60 min. Ultrafiltrate was cleaned up on a 6-mL graphitized carbon SPE column, conditioned with MetOH and 10 mM HNO_3, with flow rate through the column maintained at 1–2 mL/min. The first fraction (3 mL) was discarded, the second fraction (1.5 mL) was collected in an autosampler vial, and 500 µL were analysed chromatographically.

Her *et al*. [6] prepared 67% (w/w) slurry of milk-based powdered infant formula by weighing 20 g of sample and dissolving in 10 mL of ultrapure water. The mixture was spiked with $NaCl^{18}O_4$, and extracted using a combination of EtOH, acetic acid and/or acetonitrile. After shaking at 260 rpm for 10 min and centrifuged at 4000 rpm for 10 min, all EtOH and acetonitrile in the aqueous phase were evaporated at 95°C for 30 min. Each sample was passed through SPE cartridges, and then filtered through a 0.2-µm pore size syringe filter for injection in the ion chromatograph.

Rahimi-Yazdi *et al*. [38] developed an IC-CD procedure for calcium determination in milk samples. All calcium species must be changed to the Ca^{2+} soluble form. In centrifuge tubes a 666-µL milk aliquot was acidified with 200 µL of 1 M HCl to precipitate the proteins and to dissolve the micellar calcium phosphate; 466 µL of water were added and the mixture was centrifuged at 4500 *g* for 15 min at room temperature. The supernatant was collected (666 µL), brought to 50 mL with water and injected into the system.

Brescia *et al*. [39] have determined Li, Na, K, Mg and Ca in buffalo milk and mozzarella cheese samples by high-performance ion chromatography with conductimetric detection. For these determinations, milk and mozzarella ash (600°C) samples were dissolved in 50 mL of 0.1 M HCl.

Cheese

A 4-g portion of the dairy product was mixed with 40 mL of ultrapure water. The mixture was vortexed for 1 min and then centrifuged for 5 min at 1500 *g* at room temperature. The supernatant was filtered through Whatman paper No. 40 and then 2 mL of filtrate were filtered through Anotop 10 LC 0.2 µm prior to chromatographic analysis [40].

Yoghurt

Yoghurt samples were prepared by adding 5 g of sample to 20 mL deionized water, and then mixing for 20 min and centrifugating at 2000 rpm. After that, 100 µL of supernatant were injected into the chromatograph [41].

16.3.5 Fruits

High water content fruits, such as orange, lemon, mandarin, grape, strawberries and apple, have been analysed for perchlorate [5, 29, 42, 43], chlorite [44, 45] and different chlorine species [45]. Perchlorate was determined by MS. In the procedure developed by Krynitsky *et al*. [5], 10 g chopped fruit were mixed in a centrifuge tube with 100 µL of 3.0 µg/mL internal standard solution. After addition of 20 mL of 1% acetic acid, capped tubes were shaken by hand for 2 min and then centrifuged at 2000 rpm for 5 min. Supernatant was passed through a preconditioned (6 mL acetonitrile followed by 6 mL 1% acetic acid) ENVI-Carb SPE column and collected in a clean centrifuge tube. Approximately, 1.0 mL was syringe-filtered for IC-MS/MS analysis. In the procedure proposed by Wang *et al*. [43], approximately 100 g of each sample were homogenized in a food processor. Analytical samples were prepared by weighing 10 g of each homogenized food inside polypropylene centrifuge tubes. To each sample, 20 µL of 10.0 mg/kg $^{18}O_4$-labelled perchlorate was added as internal standard to correct matrix effects on measured signals. Subsequently, 10 mL of deionized water were added to serve as extraction reagent. After shaking the samples on a vortex for 1 min, samples were centrifuged at 3000 rpm for 25 min. An aliquot of the supernatant was then filtered through a 0.20-µm Acrodisc nylon syringe filter into a 2-mL vial. The filtered samples were stored at –20°C until IC-MS/MS analysis.

Sungur and Sangün [29] developed a procedure for conductimetric perchlorate determination. Bulk samples were cut into small (<2 cm) pieces and chopped in a food processor. Samples for analysis were prepared by weighing 10 g of each food into separate polypropylene centrifuge tubes; 20 mL of ultrapure water were added and after mixing, centrifugation and filtration, the extract was ready for IC-CD.

Trinetta *et al*. [45] have studied the residual by-products generated in the process of fruit disinfection with ClO_2. After ClO_2 treatment, food surfaces were immediately rinsed with water to remove any remaining by-product.

Fruits were rinsed with 400 mL distilled water in a glass beaker and shaken for 10 min at 150 rpm. The rinsing water was then collected and analysed for chloride, chlorate, and chlorite using IC.

16.3.6 Meat products

Usually meat products have been analysed mainly for nitrate and nitrite determination in fresh meat [40, 46–48], cured meat and sausage [47, 49–51] and sulfite determination in fresh meat [52, 53]. Additionally, an application for arsenic speciation in fresh meat has been published [54].

Fresh meat

Yu *et al*. [47] extracted nitrite and nitrate anions from fresh meat using ultrasound. The extract was centrifuged at 2000 *g* for 5 min and the supernatant was passed through a 0.45-μm membrane filter and a C18 cartridge to remove organic interference. To remove the excess of chloride, which interferes with nitrite peak, the effluent was treated by Dowex 50W-X8 cation exchange resins in the Ag^+ form.

Iammarino and di Taranto [46] and Iammarino *et al*. [40] developed an easy and quick procedure for nitrite and nitrate, and sulfite [53] extractions from fresh meat. A 5-g portion of the sample, homogenized by mincing machine, was mixed with 100 mL of ultrapure water and placed for 5 min at 70°C. The mixture was then centrifuged for 5 min at 1500 *g* at room temperature. The supernatant was filtered through Whatman paper No. 40 and 2 mL of the filtrate were passed through Anotop 10 LC 0.2 μm, prior to injection into the IC-CD chromatograph. No further clean-up step was required.

Conklin *et al*. [54] developed a procedure for arsenic speciation in chicken liver by IC/ICP-MS. This study tried to link the different species of arsenic with the feeding of chickens with feed treated with roxazone. Liver portions (0.4–0.55 g) were weighed in polypropylene centrifuge tubes and homogenized for 30–90 s in 3.0 mL of 0.625% TMAH in water using an Omni-Prep multi-sample homogenizer set at 24,000 rpm. The homogenates were vortex mixed for an additional 15–30 min, diluted with 6.5 mL water, remixed briefly, and then transferred to Centriprep Ultracel YM-30 centrifugal ultrafilters. Homogenates were centrifuged at 2000 *g* for at least 1 hour to obtain more than 3 mL filtrate. A portion of the filtrate was transferred to nitric acid-washed glass autosampler vials for analysis.

Evans *et al*. [28] employed microwave-assisted digestion for Pu, Am and Np extraction from liver and lamb samples. Samples were mineralized inside closed microwave digestion vessels using concentrated HNO_3 as reagent; 0.5 g of sample were mixed with 5 g of concentrated HNO_3 and digested using a heating program (0–600 W for CEM microwave and 0–1000 W for Multiwave system) for a total digestion time of 1.3 and 1 hour, respectively. Where determination of Pu was the primary objective, samples were oxidized using a mixture of concentrated HNO_3 and H_2O_2 to leave a single Pu oxidation state. However, this leads to poor Am recovery. Good Am recovery was only obtained by omitting the oxidation steps.

Cured meat and sausage

A IC-fluorimetric detection procedure was developed by Stalikas *et al*. [51] for nitrate and nitrite in salami, in which 70 mL of warm double distilled water were added to 10 g of salami. The mixture was then homogenized in a blender for 1 min and heated at 70°C for 15 min. After cooling to room temperature, the sample was centrifuged at 1370 *g* for 10 min and the supernatant removed and filtered successively through Whatman paper No. 40 and a graphite filter. The filtrate was then collected in a volumetric flask and diluted up to 100 mL before analysis.

Iammarino *et al*. [52] determined sulfites in fresh sausage and hamburger by IC-CD. A 4-g portion of the homogenized sample was mixed with 40 mL of a stabilizing solution (containing 50 mM NaOH, 10 mM fructose and 0.1 M EDTA) in a horizontal shaker for 30 min. After centrifugation for 5 min at 1200 rpm at room temperature, the extract (~2 mL) was filtered on Whatman paper No. 40 and then on Anotop 10 LC 0.2 μm prior to chromatographic analysis. No further clean-up step was required.

Nitrite and nitrate determinations in cured ham were carried out by Armenteros *et al*. [49]. A 5-g portion of minced sample was homogenized 1 : 10 (w/v) with 50 mL ultrapure water in a stomacher for 10 min at 4°C. Then samples were centrifuged at 12,000 *g* for 20 min at 4°C and the supernatant was filtered through glass wool and immediately frozen at –20°C until use. Thawed samples were filtered through 0.45-μm nylon membrane filters before being analysed by IC-CD.

16.3.7 Sea product (including fish, mollusc or crustacean)

Arsenic and phosphorus have been the most studied elements in these kinds of samples. Nevertheless, different detection systems have been employed: arsenic has been determined primarily by ICP-MS [18, 55–57] and HGAFS [58], while phosphorus species have been determined only with the conductimetric detector [59–62]. To a lesser extent, chlorite [63], perchlorate [22], nitrate and nitrite [40, 48, 59], sulfite [52] and selenium species [57] have also been determined.

Simon *et al*. [58] determined arsenic species in oyster and mussel samples by IC coupled to HGAFS. A 0.5-g portion of dried sample was placed in a polypropylene tube with 10 mL of MetOH. Magnetic stirring was applied for 15 min and then the solution was diluted to 25 mL in order to compensate for any solvent evaporation. The extract was filtered through a 0.2-μm filter and diluted before injection in the IC system.

An arsenic speciation extraction procedure in fish oil was proposed by Kohlmeyer *et al*. [55]. A 5-g portion of fish oil sample was diluted in 10 mL hexane and 10 mL of MetOH and 1 mL of ultrapure water was added. After shaking for 1 hour, the MetOH/H_2O layer was separated from the hexane by centrifugation. The hexane layer was once again extracted with 10 mL of MetOH/H_2O (1 : 3). The MetOH/H_2O fractions were pooled and filled up to 50 mL with ultrapure water. The extract was analysed by IC/ICP-MS after five times dilution with water.

Leufroy *et al*. [56] used a methanol/water microwave-assisted extraction for arsenic species determination in fish. Portions of 0.150 g freeze-dried samples were separately weighed in microwave digestion vessels and 10 mL of a MetOH/H_2O mixture were added. The closed vessels were heated and maintained at 80°C for 6 min in a microwave system. After cooling, suspensions were transferred into 50-mL polyethylene flasks, filled to volume with ultrapure water and centrifuged at 3500 rpm for 5 min. Supernatants were filtered through a 0.45-μm Millipore syringe filter and five times diluted before injection. Dilution of the extracts reduced matrix effects, resulting in similar retention times for a given species in standard solutions and various seafood matrices.

Reyes *et al*. [57] have developed a microwave-assisted enzymatic extraction of arsenic and selenium species from fish. A 200-mg portion of sample, together with 20 mg of pronase E and 5 mg of lipase, were weighed into individual Teflon digestion vessels; 10 mL of 50 mM phosphate buffer (pH 7.25) were added and a magnetic stir bar was placed into each vessel for thorough mixing of solvent with the sample. The microwave vessels were sealed and irradiated at 37°C for 30 min. After microwave heating, vessels were allowed to cool to room temperature and extracts were centrifuged for 10 min at 4000 rpm. The supernatants were filtered through 0.22-μm fibreglass filters followed by a 1+3 dilution with double deionized water. The extracts were stored at 4°C in a cold room for analysis within 48 hours until analysis by IC/ICP-MS. Blanks were prepared along with the samples in each batch.

Different species of phosphorus were determined in cod muscle by IC-CD [61]. A 5-g portion of the thoroughly minced sample was weighed in a 50-mL centrifuge tube and 25 mL of deionized water was added and mixed for 1 min using a vortex mixer. Then, the phosphate species were extracted by ultrasonic leaching for 10 min. Fish meat was removed by centrifugation at 5525 *g* for 20 min at 4°C. The supernatant was collected and transferred to a 50-mL centrifuge tube. In order to precipitate the soluble proteins, 5 mL of 0.20 g/mL trichloroacetic acid solution were added and mixed for 30 s. The solution was centrifuged again at 5525 *g* for 15 min at 4°C. The supernatant was collected and adjusted to pH 8.5 to prevent phosphate degradation. Then it was finally diluted to 45 mL and stored in ice. Before injection to the system, samples were filtered through a 0.45-μm Millex syringe.

Kawasaki *et al*. [63] developed a procedure for chlorite determination in herring roe treated with sodium chlorite. This compound was extracted with 9 mM Na_2CO_3. After centrifugation, the supernatant was filtered through a 0.2-μm nylon filter and the filtrate deproteinized by ultrafiltration. The chloride ion was removed with an OnGuard Ag cartridge column and the eluate was analysed with IC-CD.

Gómez-Ordóñez *et al*. [59] have proposed a dry ashing procedure for conductimetric determination of fluoride, chloride, nitrite, nitrate, sulfate and phosphate in seaweeds by ion chromatography. Seaweed samples (0.5 g) were incinerated in a muffle furnace at 550°C for 16 hours and weighed. Ash content was calculated and expressed as a percentage of the seaweed sample dry weight. Just before injection to the chromatographic system, ashes were milled with pestle and mortar and an aliquot (25 mg) dissolved in 50 mL ultrapure water. To separate particulate matter,

filtration was performed through Whatman No. 44 cellulose filter. Then all samples were appropriately diluted in ultrapure water and adjusted to a final volume of 10 mL to give an approximate concentration of 100 mg/L, poured into autosampler polyethylene tubes and analysed by ion chromatography.

16.3.8 Vegetables (including leafy and roots edible vegetables)

Vegetables are the most studied samples for anion determination by IC. Perchlorate in leafy edible vegetables, as lettuce, spinach and cantaloupe, and root edible vegetables, such as carrot, cabbage and red cabbage, have been determined using MS detection [5, 22, 31, 42, 43, 64] and conductimetric detection [29, 65, 66].

Nitrate and nitrite determination have been conducted jointly in leafy vegetables by UV detection [47, 67–69] or CD detection [40]. To a lesser extent, chloride, together with chlorite and chlorate [45], arsenic [70], bromide [71, 72], selenium [73], sulfate and phosphate [74], and sulfite [75, 76] have also been determined.

Leafy edible vegetables

Krynitsky *et al*. [31] determined perchlorate in lettuce and cantaloupe by using MS detection. A 100-g portion of chopped edible commodity was homogenized for 6 min with 150 mL of 10 mM HNO_3. The contents plus two 20-mL dilute acid rinses were filtered under vacuum through a glass fibre filter and the final volume of extract was noted. An aliquot drawn into a syringe was filtered (0.45-μm nylon) into a graduated tube which already contained 100 μL of 0.10 μg/mL internal standard solution, until the 1.0 mL mark was reached. The contents of the tube were mixed by gentle rotation and injected for IC.

Sanchez *et al*. [66] analysed 22 leafy vegetables. Samples were frozen and completely freeze-dried within 48 h. A 600-mg portion of freeze-dried product was weighed into centrifuge tubes and 15 mL of deionized water was added. Tubes were boiled for 30 min and their content was placed in a refrigerator overnight with occasional gentle shaking. Tubes were then centrifuged for 30 min and the supernatants were filtered sequentially through wipes and 0.2-μm syringe filters. A 2-mL portion of the above extract was reacted with 1000 mg of DD-alumina. Vials were gently agitated two or three times over a 24-hour period after which 18 mL of deionized water were added to the mixture. After the solution was stirred and settled, it was filtered through another 0.2-μm membrane syringe filter and the resulting solution was stored in the freezer until analysis by IC-CD for perchlorate determination.

Niemann *et al*. [65] determined perchlorate in spinach, lettuce and cantaloupe by conductimetric detection. A 100-g test portion of thawed, thoroughly mixed sample and 10 g of Celite were blended with 150 mL of 10 mM HNO_3 for 6 min at high speed. The contents plus two 20-mL rinses were filtered under vacuum through a rinsed glass fibre filter. A 25-mL portion was poured into a beaker and stirred magnetically while cation-exchange resin was added until pH 2 was reached, after which stirring continued for 15 min. After settling, supernatant was poured onto a conditioned ENVI-Carb SPE column placed on a vacuum manifold, two 2-mL fractions were collected, and an aliquot of the second fraction was analysed by IC-CD.

Determination of perchlorate in vegetables was carried out by Krynitsky *et al*. [5]. Into a centrifuge tube, 10 g of edible portion chopped commodity was weighed and 100 μL of 3.0 μg/mL internal standard solution and 20 mL of 1% acetic acid were added. The capped tube was shaken by hand for 2 min and then centrifuged at 2000 rpm for 5 min. Supernatant was passed through a preconditioned (6 mL of acetonitrile followed by 6 mL of 1% acetic acid) ENVI-Carb SPE column and collected in a clean centrifuge tube. Approximately 1.0 mL was syringe-filtered for IC-MS/MS analysis.

Sungur and Sangün [29] determined perchlorate in cabbage, carrot, lettuce and spinach. A 4-g portion of homogenized sample was mixed with 200 mL of ultrapure water and placed at 70°C for 5 min. After cooling the mixture was filtered through Whatman No. 41 paper and then 3 mL of filtrate were purified by an alumina neutral cartridge, previously activated with 3 mL of ultrapure water. The cartridge eluate was filtered through Anotop 10 LC 0.2 μm prior to chromatographic analysis. Dilution with ultrapure water was necessary for samples with nitrate concentrations higher than 625 mg/kg.

Yu *et al*. [47] used ultrasonic extraction of nitrite and nitrate from vegetables. The extract was then centrifuged at 2000 *g* for 5 min and the supernatant was passed through a 0.45-μm membrane filter and a C18 cartridge to remove organic interferences. The effluent was treated by a Dowex 50W-X8 cation exchange resin in the Ag^+ form to remove the excess of chloride that interferes with nitrite peak.

Spinach and lettuce were analysed by Butt *et al.* [67]. A 50-g portion of each sample was homogenized in an electrical blender with 50 mL of double deionized distilled water at 60°C for 5 min and further ultrasonically homogenized for 15 min. A 25-mL portion was centrifuged for 10 min and the supernatant solution was decanted. A 5-mL solution was passed through a 0.25-μm Millipore syringe filter and then through a C18 sample clean-up column for direct injection into the liquid chromatograph for nitrate and nitrite determination.

Extraction experiments for arsenic species from plant materials were carried out by Schmidt *et al.* [70]. For the extraction, 200 mg of plant materials, intact leaves or homogenized tissue of leaves, were weighed into 15-mL polypropylene centrifuge tubes. Samples were extracted with 10 mL of different extracting agents for 4 hours in a single extraction step. Deionized water and 0.1 M Na_2HPO_4 were used as extracting agents. The extraction procedure was assisted by ultrasonication for 10 min in an ultrasound water bath. Finally, the extraction residues were removed from the extract solution by filtration through a 0.45-μm cellulose acetate membrane filter. The first filtrate and the washing solution were combined for total arsenic and arsenic species determination by IC/ICP-MS.

Extraction experiments for selenium species from kale were carried out by Chan *et al.* [73]. A 30-mg portion of ground sample was treated with 2 mL of 30 mM Tris-HCl solution containing 2% sodium dodecyl sulfate (SDS) and 0.1 mM phenylmethylsulfonyl fluoride. The mixture was stirred for 2 hours, and subsequently centrifuged at 4°C and 5000 rpm for 15 min. The supernatant was filtered through a 0.22-μm polyvinylidene fluoride (PVDF) filter. Half of the filtrate was distributed into two vials, one of which was injected onto the SEC column and the other was submitted for IPRP-HPLC/ICP-MS analysis after further filtering through a 5-kDa cut-off membrane.

Evans *et al.* [28] employed microwave-assisted digestion for Pu, Am and Np extraction from cabbage. Samples were mineralized by closed-vessel microwave digestion using concentrated HNO_3 as the reagent in Teflon or quartz vessels. A 0.5-g portion of sample was accurately weighed into the digestion vessels, 5 g of concentrated HNO_3 added and the vessels sealed. Stepped heating program (0–600 W for CEM microwave system and 0–1000 W for Multiwave system) for a total digestion time of 1.3 and 1 hours, respectively, were employed. For Pu determination samples were oxidized using a mixture of concentrated HNO_3 and H_2O_2 to leave a single Pu oxidation state. However, good Am recovery was only obtained by omitting the oxidation step.

Root edible vegetables

Sample portions of approximately 50 mg were extracted with 1 mL of 0.1% Tween-20 in a boiling water bath for 30 min. Then the supernatants were filtered and diluted with pure water and solutions passed through the IC-CD for $H_2PO_4^-$ determination [77].

Edible oil

Chu and Jiang [78] developed a microwave-assisted extraction procedure for arsenic speciation in used and fresh soybean oils. Oil samples (0.500 g each) were weighed into 15-mL polyethylene centrifuge tubes and 10 mL of 0.5% v/v HNO_3 in 80% v/v methanol were added. Tubes were then put into a beaker and exposed to microwave heating, programmed to maintain the temperature at 80°C for 120 min. After microwave heating, solutions were allowed to cool and directly centrifuged at 0°C for 10 min at 4000 *g*. The supernatant was decanted and then dried at 70°C by rotary evaporation for 20 min to remove most of the methanol. The extracts were diluted to 5 mL with pure water and then injected into IC/ICP-MS for arsenic determination.

Mushrooms

Di Narda *et al.* [71] have described a procedure suitable for direct analysis of samples for bromide determination by IC. Dried mushrooms (0.5–1 g) were introduced into a digestion vessel and to this was added small volumes of a silver nitrate solution and a volume of acid oxidizing mixture suitable to achieve good mineralization. After the microwave treatment step, digestion vessels were cooled and the content (precipitate and supernatant) transferred into a glass centrifuge tube and centrifuged at 4000 rpm for 5 min. The supernatant (containing HNO_3, H_2O_2 and excess $AgNO_3$) was removed and the precipitate was washed carefully with water to eliminate as much residual nitric acid as possible and then solubilized with ammonia. Finally, the resulting solution was passed through a cation-exchange column containing sodium-loaded Dowex 50WX8-400 to trap (and hence eliminate) the cationic complex $[Ag(NH_3)_2]^+$ from the bromide sample, thus making the eluate suitable for subsequent analysis.

Sugar

Sugar samples were diluted with ultrapure water at a ratio of 10 g to 100 mL. Before injection they were filtered through membrane filters with a pore diameter of 0.45 μm [79].

16.4 Measurement techniques

IC offers interesting possibilities for the sequential determination of most common anions, for example chloride, nitrate, nitrite, sulfate and phosphate, and of those that are found infrequently, for example arsenic or selenium species in a single sample. The determination of arsenic species and perchlorate in food has been particularly important in recent years and methods have been developed for their determination in a variety of foods.

Tables 16.6 and 16.7 summarize the instruments, trade mark, columns and eluents employed for anionic and cationic species determination in food samples. Dionex equipment is the most commonly employed, being covered in 57% of published papers. Other commercial instruments are also employed, but at lower levels, for example Agilent (14%), Metrohm (9%), Shimadzu (7%) and Hitachi (3%). Regarding the detectors employed, conductimetric detection is by far the most commonly used for individual and multielement determinations.

16.4.1 Anion determination

Arsenic species

Six species of arsenic, As(III), As(V), mMA, DMA, AsB and AsC, were determined using the coupling of ion chromatography with ICP-MS [80]. Separation of the arsenic species was performed on two consecutive anion-exchange columns, Hamilton PRP-X100 and PRP-X200, connected in series with PEEK tubing and eluted with ammonium carbonate and methanol buffer in the ratio 94 : 6 (v/v). After every 75–100 samples it was necessary to regenerate the columns to ensure reproducible performance of the separation. The cation-exchange column was back-flushed with 0.1 M HNO_3 for 1 hour followed by deionized water, while the anion-exchange column was back-flushed with 0.1 M NaOH for 1 hour followed by deionized water.

Simon *et al*. [58] employed atomic fluorescence detection IC for determination of four arsenic species (AsB, AsC, TMAO, $TMAs^+$). Atomic fluorescence spectrometry can be a good alternative to ICP-MS, with advantages of low cost of investment and operation. While inorganic species are separated by anion exchange, organoarsenic compounds have mainly a neutral or cationic character and thus cation-exchange chromatography appears to be the most suitable tool for their separation and thus a Hamilton PRP-X200 cation-exchange column was employed. Authors proposed on-line UV photo-oxidation for the analysis of organoarsenic species by coupling cation-exchange chromatography and atomic fluorescence spectrometry with hydride generation (Figure 16.7). For photo-oxidation, a PTFE tube was wrapped around a Philips TUV-15 lamp (253.7 nm). HCl and $NaBH_4$ solutions, used for hydride generation, were added at a flow rate of 0.3 mL/min using a peristaltic pump through a T-joint. Another channel of this pump was required to remove waste from the gas–liquid separator.

Kohlmeyer *et al*. [55] achieved the separation of eight arsenic species (As(III), As(V), mMA, DMA, AsB, AsC, TMAO, $TMAs^+$) within 18 min via the IonPac AG7-AS7 anion-exchange column. A nitric acid gradient (pH 3.4–1.8) and benzene-1,2-disulfonic acid dipotassium salt, as ion-pairing reagent in the mobile phase, were applied for separation of anionic, neutral and cationic arsenic species in a single run. Detection was carried out by ICP-MS.

Pearson *et al*. [19] achieved the separation of five arsenic species (As(III), As(V), mMA, DMA, AsB) with a C18 reversed-phase column Chromolith RP-18e. A 25-cm length of PEEK tubing was used to connect the chromatographic column to ICP-MS. The eluent employed for separation was 2.5 mM tetrabutyl ammonium bromide/10 mM phosphate buffer (pH 5.6) and 1.0% (v/v) MetOH.

Six species of arsenic (As(III), PAO, PAA, *o*-APAA, *p*-APAA, Rox) were determined using IC with ICP-MS [70]. The separation of the arsenic species was performed on two consecutive anion-exchange columns (IonPac AS7, 4 × 250 mM) assembled in a HPLC Agilent 1100 and by a binary gradient elution based on 0.4 mM HNO_3 and 50 mM HNO_3 with 0.05 mM benzene-1, 3-disulfonic acid (BDSA). The eluent flow rate was set at 0.750 mL/min.

Reyes *et al*. [57] developed an IC/ICP-MS procedure for the separation and quantification of five species of arsenic (As(III), As(V), mMA, DMA, AsB). The separation was achieved by using the columns MetrosepTM Anion Dual 3 and MetrosepTM Anion Dual 3 guard

Table 16.6 Instrumental systems employed for IC anion determination in food samples.

Analyte	Sample	Detection	Eluent	Instrument	Comments	Reference
As(III), PAO, PAA, *o*-APAA, *p*-APAA, Rox	*Tropaeolum majus*	IC-ICPMS	(A) 0.4 mM HNO_3 (B) 50 mM HNO_3 + 0.05 mM BDSA 750 μL/min	Agilent 1100	The separation of the arsenic compounds was performed on two consecutive anion exchange columns (IonPac AS7, 4 × 250 mm). Binary gradient elution	[70]
As(III), As (V), MMA, DMA	Cereals	IC-DRC-ICPMS	(A) 0.5 mM ammonium citrate in 1% v/v methanol (pH 4.5) (B) 15 mM ammonium citrate in 1% v/v methanol (pH 8.0)	Hitachi L6000	Hamilton PRP-X100, 10 μm particles, 4.1 mm i.d. × 250 mm length Gradient elution mode: (i) 0–1.5 min, 100% (A); (ii) 1.5–2.5 min, 100% (A) up to 100% (B); (iii) 2.5–8 min, 100% (B)	[20]
As(III), As(V), MMA, DMA, AsB, AsC	Edible oil	IC-ICPMS	$(NH_4)_2CO_3$ and methanol at pH 8.5	Hitachi L6000	Hitachi model L-6100 (Intelligent pump), Hamilton PRP-X100 anion exchange Column: 10 μm particle, 4.1 mm i.d. × 250 mm length Gradient elution allowed the chromatographic separation of all species in less than 8 min	[78]
As(III), As(V), MMA, DMA, HAMPA, HACPA, Rox	Chicken liver	IC-ICPMS	(A) 100 mM TMAH with 1% (v/v) methanol (B) 1% (v/v) methanol in water 1 mL/min	Agilent 1200	Column Dionex IonPac AS18 (4 × 250 mm) with guard AG18 (4 × 50 mm) Elution gradient: 0–17 min, 45%(A); 17.1–42 min, 70% (A); 42.1–50 min, 45% (A)	[54]
As(III), As(V), MMA, DMA, AsB	Rice, seafood	IC-ICPMS				[18]
As(III), As(V), MMA, DMA, AsB, AsC, TMAO, TMAs	Fish oil	IC-ICP-MS	HNO_3 gradient	Dionex DX300	Sample extracts and acid digests were determined by IC/ICP-MS, with a Dionex DX300 gradient pump hyphenated to an Agilent 7500s ICP-MS via an anion-exchange column (IonPac AG7þAS7) A nitric acid gradient (pH 3.4–1.8) and benzene-1, 2-disulfonic acid dipotassium salt as ion-pairing reagent in the mobile phase were applied for separation of anionic, neutral and cationic arsenic species in a single run within 18 min	[55]
As(III), As(V), MMA, DMA, AsB, AsC, TMAO, TMAs	Fish, mollusc, crustacean	IEC-ICPMS	(A) 0.8 mM HNO_3, 1% MeOH, pH 3.8 (B) 500 mM HNO_3, 1% MeOH, pH 1.4 1.35 mL/min	Dionex Ultimate 3000	Guard column: IonPac AG7 (50 × 4 mm, 10-μm particles, Dionex) Analytical column: IonPac AS7 (250 × 4 mm, 10-μm particles, Dionex) Gradient program: (i) 0–3 min, 99% (A); (ii) 3–5 min, 10% (A); (iii) 5–12 min, 80% (A)	[56]

As(III), As(V), MMA, DMA	Biscuit, bread, corn snacks, infant cereals, pasta, wheat flour	IC-ICPMS	26 mM $NH_4H_2PO_4$, pH 6.2 (adjusted with aqueous ammonia) 1 mL/min	Dionex ICS-3000	The chromatographic column is a Hamilton PRP-X100 (150 × 4.1 mm, 5 μm). Column temperature at 30°C. Isocratic elution allowed the chromatographic separation of four species in 10 min	[11]
As(III), As(V), MMA, DMA, AsB, AsC	Food composites	IC-ICP-MS	Ammonium carbonate/ methanol buffer, 94 : 6 (v/v)	Waters 600S	Lyophilization of food followed by prewashing with acetone and extraction by sonication with 50/50 methanol/water. The columns used were Hamilton PRP-X100 (10 μm, 4.1 × 250 mm i.d.) and PRP-X200 (10 μm, 4.6 × 150 mm i.d.) connected in series with PEEK tubing	[80]
As(III), As(V), MMA, DMA, AsB	Rice	IC-ICPMS	2.5 mM tetrabutyl ammonium bromide/ 10 mM phosphate buffer (pH 5.6) and 1.0% (v/v) methanol	Speck&Burke	A Speck Analytical HPLC pump Model SA103 was used to deliver the mobile phase. C18 reversed phase column Chromolith RP-18e 100 × 4.6 mm i.d. Sample injection was performed using a six-way Rheodyne injector. A 25-cm length of PEEK tubing (250 mm i.d.; 1/1600 o.d.) was used to connect the chromatographic column to the nebulizer	[19]
As(III), As(V), MMA, DMA, AsB	Fish: canned tuna, shark, marlin DOLT-3 BCR-627	IC-ICPMS	(A) 5 mM NH_4NO_3 (B) 50 mM NH_4NO_3, 2% (v/v) methanol, pH 8.7 1 mL/min	Metrohm	The chromatography system consisted of two 818 IC pumps, a 762 IC interface, and an 838 IC autosampler. MetrosepTM Anion Dual 3 column (100 × 4.0 mm, 6 μm) and MetrosepTM Anion Dual 3 guard column (1.7 × 3.5 mm, 0.2 μm) Gradient program: (i) 0 min, 0% (B); (ii) 2 min, 0% (B); (iii) 7 min, 100% (B); (iv) 9 min, 100% (B); (v) 9.5 min, 0% (B); (vi) 12 min, 0% (B)	[57]
As(III), As(V), MMA, DMA	Infant food	IC-ICPMS			Sample treatment with trifluoroacetic acid at 100°C was an efficient and mild method for extraction of arsenic species present in different food matrixes	[10]
AsB, AsC, TMAO, $TMAs^+$	Oyster, mussel	IC-UVox-HGAFS	KNO_3	Dionex P580	The chromatographic column is a Hamilton PRP-X200 (250 × 4.1 mm) cation-exchange column, protected by a column guard packed with the same stationary phase	[58]
Bromate	Bread	IC-ICPMS	30 mM $(NH_4)_2CO_3$ 1.0 mL/min	Agilent 1100 system	The clean-up procedure included a 0.2-μm filter, a C18 cartridge for defatting, a silver cartridge to remove halogen anions, a centrifugal ultrafiltration unit to remove proteins, and a cation-exchange cartridge to remove silver ions. An Agilent 7500 omega was used as the ICP-MS systems, and it was connected to the IC by a 500 × 0.3 mm i.d. ethylene tetrafluoroethylene (ETFE) tube. Column GL-IC-A15G/GL-IC-A15. Column temperature 40°C	[24]

(Continued)

Table 16.6 (Continued)

Analyte	Sample	Detection	Eluent	Instrument	Comments	Reference
Bromate	Flour products	IC-UV	NaOH gradient elution		Bromate was separated using an IonPac AS19 column and detected by UV after post-column reaction to form triiodide	[25]
Bromate	Flour	IC-CD	Linear gradient KOH 1.0 mL/min	Dionex ICS2000	IC equipped with a dual-piston (in series) pump, a Dionex IonPac AS19 analytical column (4 × 250 mm), and an IonPac AG19 guard column (4 × 50 mm). The detection was performed with a Dionex DS6 conductivity detector. Suppression of the eluent was achieved with a Dionex anion ASRS electrolytic suppressor (4 mm) in the autosuppression external water mode. Performed at 30°C	[26]
Bromate	Food Bread	IC-UV (+KI 352 nm)	20 mM KOH 0.8 mL/min	Dionex ICS2000	The KOH eluent was achieved by a KOH eluent generator (Dionex). Column temperature at 25°C	[23]
Bromide	Foodstuffs Mushrooms	IC-CD	1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$ 2 mL/min	Dionex 2000i	Guard column 5-cm Dionex AG4A. Column 25-cm Dionex AS4A. Micromembrane suppressor Dionex AMMS-1 regenerated with 12.5 mM H_2SO_4. Detector Dionex CD20 conductance detector. Isocratic separation at room temperature	[71]
Bromide	Honey	IC-CD	8 mM Na_2CO_3/1 mM $NaHCO_3$ 1 mL/min	Dionex ICS1000	Equipped with an IonPac AS14A (Dionex) column. Conductivity system was equipped with a ASRS-ULTRA suppression mode (Dionex)	[81]
Bromide	Seaweeds	IC-CD	3.2 mM Na_2CO_3/1 mM $NaHCO_3$ 0.70 mL/min	Metrohm IC861	Equipped with a pump (IC-818), an eluent degasser (IC-837) and a liquid handling unit (IC-833) with a 0.45-μm filter that required a minimal volume of 10 mL for the samples. Detection was performed with a conductivity detector (IC-819). Separation was performed in a Metrosep A Supp 5–250 column (250 × 4 mm, 5-μm particle size). All measurements were carried out at 32°C (column temperature)	[59]
Bromide	Broccoli	IC				[72]
Chlorate	Beverages	IC-CD	9 mM Na_2CO_3 1.3 mL/min	Dionex DX500	With a GP40 gradient pump, an ED40 conductivity detector, Dionex IonPAC AG9-HC guard and AS9-HC analytical column	[12]
Chlorate	Tomatoes, oranges, apples, strawberries, lettuce, alfalfa sprouts, cantaloupe	IC-UV	2 mM Na_2CO_3/0.75 mM $NaHCO_3$ 2 mL/min	Dionex	IC-pack column	[45]

Chloride	Beer	IC-CD	Na_2CO_3/$NaHCO_3$	Metrohm	Sonication for 15 min. The beer was then diluted 1 : 20 with deionized water. The modular system used for the analysis of beer comprised the Metrohm 709 IC Pump, Metrohm 732 IC Detector, Metrohm 733 IC Separation Center, Metrohm 752 Pump Unit and Metrohm 762 IC interface. Metrohm A SUPP 5 analytical column	[13]
Chloride	Honey	IC-CD	8 mM Na_2CO_3/1 mM $NaHCO_3$ 1 mL/min	Dionex ICS1000	Equipped with an IonPac AS14A (Dionex) column. Conductivity system was equipped with an ASRS-ULTRA suppression mode (Dionex)	[81]
Chloride	Seaweeds	IC-CD	3.2 mM Na_2CO_3/1 mM $NaHCO_3$ 0.70 mL/min	Metrohm IC861	Equipped with a pump (IC-818), an eluent degasser (IC-837) and a liquid handling unit (IC-833) with a 0.45-μm filter that required a minimal volume of 10 mL for the samples. Detection was performed with a conductivity detector (IC-819). Separation was performed in a Metrosep A Supp 5–250 column (250 ×4 mm, 5-μm particle size). All measurements were carried out at 32°C (column temperature)	[59]
Chloride	Beverages	IC-CD	9 mM Na_2CO_3 1.3 mL/min	Dionex DX500	With a GP40 gradient pump, an ED40 conductivity detector, Dionex IonPAC AG9-HC guard and AS9-HC analytical column	[12]
Chloride	Spinach processing	IC-CD	0.65 mM $NaHCO_3$/1.5 mM Na_2CO_3	Dionex Model 2000i	Dionex AS4A column (P/N37041) anion separator column and a guard column containing the same resin (AG4A P/N 37042, for anions) with autosampler Model 035274 Dionex. The detector used was a CMD conductivity cell with 0.01 N H_2SO_4 regenerant for anions. The pump pressure was 900 psi. The results showed the anions to increase in the water during washing of raw spinach and during the freezing and canning processes	[74]
Chloride	Wine	HPIC			Sample preparation required only filtration and dilution where necessary	[16]
Chloride	Vinegar	IC-CD	0.35 mM salicylic acid 0.1 mM sodium salicylate 0.8 mL/min (pH 3.5)		CarbonIC BI-01 (4.6 mm i.d. × 100 mm) column coated with cetylmethylammonium ion. Temperature 40°C	[84]
Chloride	Greek Kiwi wine	IC-CD		Dionex Series 4500i	The system was equipped with an anion trap column (P/N 037151), a guard column OmniPac PAX-500, P/N 042153 (4.0 mm i.d. × 50 mm) and an analytical or separation column OmniPac PAX-500, P/N 042152 (4.0 mm i.d. × 250 mm) Dionex	[15]

(Continued)

Table 16.6 (Continued)

Analyte	Sample	Detection	Eluent	Instrument	Comments	Reference
Chloride	Tomatoes, oranges, apples, strawberries, lettuce, alfalfa sprouts, cantaloupe	IC-UV	2 mM Na_2CO_3/0.75 mM $NaHCO_3$ 2 mL/min	Dionex	IC-pack column	[45]
Chloride	Sugar	HPAEC-CD	(A) 100 mM NaOH (B) Ultrapure water 1.5 mL/min	Dionex ICS3000	Separation was conducted with the use of the IonPac AS11-HC 4 × 250 mm column and the ASRS-ULTRA II 4 mm conductivity suppressor produced by Dionex Time and composition program (eluents A and B expressed as percent v/v): (i) 0 min, 1% (A) and 99% (B); ii) from 0 to 17 min. 5% (A) and 95% (B); (iii) from 17 to 25 min, 25% (A) and 75% (B); (iv) from 25 to 32 min,40% (A) and 60% (B); (v) from 32 to next run, 1% (A) and 99% (B)	[79]
Chloride	Food	IC-CD	2.0 mM benzoic acid/ 1.2 mM TAMAHP 1 mL/min (pH 4.4)		Column Carbon IC BI-01	[85]
Chloride	Food	IC-CD	(1) 0.35 mM salicilic acid/ 0.l mM sodium salicylate (pH 3.5), 0.8 mL/min (2) 2.0 mM benzoic acid/1.2 mM Tris aminomethane (pH 4.4), 1.0 mL/min	Toa DKK	The ion chromatographic equipment consisted of a Model ICA-5120 pump, a Model ICA-5220 conductivity detector, a Model ICA-5410 column oven (Toa DKK). Carbon IC BI-01 (100 mm × 4.6 mm i.d.)	[41]
Chlorite	Herring roe	IC-CD		Dionex 50	IP 25, AS 50 Dionex. CD 25 Dionex. Analytical column IonPac AS9-HC (4 × 250 mm). Guard column AG9-HC (4 × 50 mm)	[63]
Chlorite	Tomatoes, oranges, apples, strawberries, lettuce, alfalfa sprouts, cantaloupe	IC-UV	2 mM Na_2CO_3/0.75 mM $NaHCO_3$ 2 mL/min	Dionex	IC-pack column	[45]
Fluoride	Fruit yoghurt	IC			Fluoride levels were the same for all yoghurts tested	[92]

Fluoride	Seaweeds	IC-CD	3.2 mM Na_2CO_3/1 mM $NaHCO_3$ 0.70 mL/min	Metrohm IC861	Equipped with a pump (IC-818), an eluent degasser (IC-837) and a liquid handling unit (IC-833) with a 0.45-μm filter that required a minimal volume of 10 mL for the samples. Detection was performed with a conductivity detector (IC-819). Separation was performed in a Metrosep A Supp 5–250 column (250 × 4 mm, 5-μm particle size). All measurements were carried out at 32°C (column temperature)	[59]
Fluoride	Dairy products	IC-CD			Optimized post-column reaction conditions were found for fluoride measurements; the xylenol orange–zirconium complex gave the highest sensitivity	[94]
Fluoride	Milk	IC-PED	9 mM Na_2CO_3 1 mL/min	Dionex ICS1000	Headspace single-drop microextraction and ion chromatography for the pre-concentration and determination of fluoride. The method lies in the *in situ* HF generation and subsequent sequestration into an alkaline microdrop (15 μL) exposed to the headspace above the stirred aqueous sample. Samples were separated on a Dionex IonPac AS9-HC anion-exchange column (250 × 4.0 mm i.d.) coupled with an AG9-HC guard column (50 ×4.0 mm i.d.). A Dionex anion micromembrane suppressor (ASRS-ULTRAII 4 mm) was installed between the conductivity detector and the separation column	[37]
Iodide	Milk	IC-MS	100 mM NaOH 1.00 mL/min	Dionex DX-600	IC with a GS50 gradient pump, an ASRS-Ultra 4-mm suppressor and a CD25 conductivity detector. A TAC-LP1 (4 × 35 mm) anion concentrator was used as a pre-concentration column (PCC). The wash solution (5 mM NaOH at a flow rate of 0.85 mL/min) washes off the less strongly retained anions such as chloride, nitrate and sulfate from the PCC. After 2.5 min of washing, the injector is switched to admit the main eluent, which has considerably greater eluting power, to the PCC. The analyte anions of interest are now eluted to the separation column	[35]
Iodide	Milk	IC-MS	100 mM NaOH 1.00 mL/min.	Dionex DX-600	Biomonitorig	[83]

(Continued)

Table 16.6 (Continued)

Iodide	Powdered milk Infant formula	IC-UV Pre-concentration column	65 mM NaOH 1.0 mL/min	Thermo Finnigan	HPLC column-switching was plumbed with a Dionex IonPac 4 × 50 mm AG16 guard column (used as an enrichment pre-column) in place of the injection loop (reverse-flush mode) and a 4 × 250 mm AS16 analytical column. Columns were cleaned at 1.0 mL/min starting with a gradient from water to 20 : 80 water/ACN in 5 min, holding for 20 min, followed by a 5-min gradient to 1.5 mL/min and 100 mM NaOH, and holding for 20 min. Mobile phase was generated from 100 mM NaOH and water (65 : 35)	[7]
Iodide	Food	IC-UV				[95]
Iodine	Infant formula milk, powdered milk, fresh milk	IC-DA	0.04 M NaOH			[9]
Nitrate	Cured ham	IC-CD	1.0 mM $NaHCO_3$/3.2 mM Na_2CO_3 plus 0.3% of acetone (v/v) 0.7 mL/min	Metrohm IC716	Using an Advance Sample Processor (IC-838, Metrohm). The column was a Metrosep A Supp 5 (4.0 × 250 mm) from Metrohm, operating at room temperature. Isocratic elution	[49]
Nitrate	Beer	IC-CD	Na_2CO_3/$NaHCO_3$	Metrohm	Sonication for 15 min. The beer was then diluted 1 : 20 with deionized water. The modular system used for the analysis of beer comprised the Metrohm 709 IC Pump, Metrohm 732 IC Detector, Metrohm 733 IC Separation Center, Metrohm 752 Pump Unit and Metrohm 762 IC interface. Metrohm A SUPP 5 analytical column	[13]
Nitrate	Spinach, lettuce	IPC-UV (220 nm)	NaH_2PO_4 5 mM (+0.5 M H_3PO_4) pH 2	Wescan Ion Analyzer model 260	Including conductivity detector Wescan 213–505. A low-capacity anion column model 269.029. Using tetraethylammonium as ion-pairing reagent	[67]
Nitrate	Baby food	IC-UV/Vis (525 nm)			Nitrate was reduced to nitrite on-line by post-column reduction using vanadium(III) chloride and heat. Nitrite reacted with Griess reagent to produce a dye that was detected at 525 nm	[87]
Nitrate	15 vegetables	IC-UV				[68]
Nitrate	Honey	IC-CD	8 mM Na_2CO_3/1 mM $NaHCO_3$ 1 mL/min	Dionex ICS1000	Equipped with an IonPac AS14A (Dionex) column. Conductivity system was equipped with an ASRS-ULTRA suppression mode (Dionex)	[81]

Nitrate	Seaweeds	IC-CD	3.2 mM Na_2CO_3/1 mM $NaHCO_3$ 0.70 mL/min	Metrohm IC861	Equipped with a pump (IC-818), an eluent degasser (IC-837) and a liquid handling unit (IC-833) with a 0.45-μm filter that required a minimal volume of 10 mL for the samples. Detection was performed with a conductivity detector (IC-819). Separation was performed in a Metrosep A Supp 5–250 column (250 × 4 mm, 5-μm particle size). All measurements were carried out at 32°C (column temperature)	[59]
Nitrate	Fresh meat	IC-CD	(A) 2 mM NaOH (B) 21 mM NaOH 0.5 mL/min	Dionex	Composed of a GP50 quaternary gradient pump, an electrochemical detector (model ED40) set to conductivity mode IonPac AS11-HC column (250 × 4 mm i.d., particle size 13 μm). Gradient elution	[46]
Nitrate	Meat, dairy, fish products, leafy vegetables	IC-CD	9 mM Na_2CO_3 1.0 mL/min	Dionex	Composed of a GP50 quaternary gradient pump, an electrochemical detector (model ED40 set to conductivity mode) equipped with a temperature compensated conductivity cell IonPac AS9-HC anion exchange column (250 × 4 mm i.d., particle size 13 μm) associated with a pre-column AG9-HC. Total run time, 20 min	[40]
Nitrate	Dairy, fish, fresh meat, leafy vegetables, meat products, shellfish	IC-CD	9 mM Na_2CO_3 1.0 mL/min	Dionex	Separations were accomplished using an IonPac AS9-HC anion exchange column (250 × 4 mm i.d., 13 μm) associated with a pre-column AG9-HC (Dionex). Dionex anion self-regenerating suppressor set to 50 mA (ASRS II, 4 mm) was used for the electrochemical suppression. Isocratic elution. Total run time, 20 min	[48]
Nitrate	Pickles	IC-UV	10 mM ammonium phosphate		The octadecyl-bonded silica (ODS) column coated with cetyltrimethylammonium bromide (CTAB) was used to separate anions	[69]
Nitrate	Spinach processing	IC-CD	0.65 mM $NaHCO_3$/1.5 mM Na_2CO_3	Dionex Model 2000i	Dionex AS4A column (P/N37041) anion separator column and a guard column containing the same resin (AG4A P/N 37042, for anions) with autosampler Model 035274 Dionex. The detector used was a CMD conductivity cell with 0.01 N H_2SO_4 regenerant for anions. The pump pressure was 900 psi. The results showed the anions to increase in the water during washing of raw spinach and during the freezing and canning processes	[74]
Nitrate	Baby foods	IC-CD				[86]

(Continued)

Table 16.6 (Continued)

Analyte	Sample	Detection	Eluent	Instrument	Comments	Reference
Nitrate	Meet products	IC-UV (210 nm)	1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$ 1.0 mL/min	Metrohm IC850	Column, Shodex IC SI-90 4E	[88]
Nitrate	Wine	HPIC			Sample preparation required only filtration and dilution where necessary	[16]
Nitrate	Cured meat	IC-MS	NaOH		Nitrate can be separated from interfering compounds with a high-capacity anion-exchange column. The described IC/MS method is robust, sensitive to nitrate concentrations as low as 0.10 mg/kg, and can determine sugar phosphates that are useful for monitoring meat freshness	[50]
Nitrate	Vinegar	IC-CD	0.35 mM salicylic acid 0.1 mM sodium salicylate 0.8 mL/min (pH 3.5)		CarbonIC BI-01 (4.6 mm i.d. × 100 mm) column coated with cetylmethylammonium ion. Temperature, 40°C	[84]
Nitrate	Light salami Foodstuffs	IC-F (exc270–em355)	Citric acid–NaOH 0.08 M 1.5 mL/min	Shimadzu	Anionic column (Shim-pack IC-A1) connected in series with a pre-column (Shim-pack IC-GA1), both being thermostatted at 40°C. Detection was carried out with an RF-551 fluorescence detector (Shimadzu). Subsequent to the ion separation, the mobile phase merged with a 30 mM phosphate solution of tryptophan (5 μg/mL), flowing at 1 mL/min. Detects the suppression of fluorescence of the tryptophan due to the elution of the target ions	[51]
Nitrate	Sugar	HPAEC-CD	(A) 100 mM NaOH (B) Ultrapure water 1.5 mL/min	Dionex ICS3000	Separation was conducted with the use of the IonPac AS11-HC 4 × 250 mm column and the ASRS-ULTRA II 4 mm conductivity suppressor produced by Dionex Time and composition program (eluents A and B were expressed as percent v/v): (i) 0 min, 1% (A) and 99% (B); (ii) from 0 to 17 min, 5% (A) and 95% (B); (iii) from 17 to 25 min, 25% (A) and 75% (B); (iv) from 25 to 32 min, 40% (A) and 60% (B); (v) from 32 to next run, 1% (A) and 99% (B)	[79]
Nitrate	Milk	IC				[93]
Nitrate	Food	IC-CD	2.0 mM benzoic acid/1.2 mM TAMAHP 1 mL/min (pH 4.4)		Column Carbon IC BI-01	[85]

Nitrate	Food	IC-CD	(1) 0.35 mM salicylic acid/0.l mM sodium salicylate (pH 3.5), 0.8 mL/min (2) 2.0 mM benzoic acid/1.2 mM Tris aminomethane (pH 4.4), 1.0 mL/min	Toa DKK	The ion chromatographic equipment consisted of a Model ICA-5120 pump, a Model ICA-5220 conductivity detector, a Model ICA-5410 column oven (Toa DKK). Carbon IC BI-01 (100 mm × 4.6 mm i.d.)	[41]
Nitrate	Cured meat, fresh meat, canned vegetables, fresh vegetables, sausage	IC-AWD	1.5 mM KHP 1.2 mL/min	Shimadzu IC-6A	Column: Shim-pack IC-A1 (4.6 mm i.d. × 100 mm stainless-steel) packed with an anion-exchange resin on polymethacrylate support incorporating a quaternary ammonium base. Column and detector are set at 40°C	[47]
Nitrate	Dairy products	IC-UV (225 nm)			An AS 19 column was used as the analytical column	[89]
Nitrite	Cured ham	IC-CD	1.0 mM $NaHCO_3$/3.2 mM Na_2CO_3 plus 0.3% of acetone (v/v) 0.7 mL/min	Metrohm IC716	Uses an advance sample processor (IC-838, Metrohm). The column was a Metrosep A Supp 5 (4.0 × 250 mm) from Metrohm, operating at room temperature. Isocratic elution	[49]
Nitrite	Spinach, lettuce	IPC-UV (220 nm)	NaH_2PO_4 5 mM (+0.5 M H_3PO_4) pH 2	Wescan Ion Analyzer model 260	Including conductivity detector Wescan 213-505. A low-capacity anion column model 269.029. Using tetraethylammonium as ion-pairing reagent	[67]
Nitrite	Baby food	IC-UV/Vis (525 nm)			Nitrate was reduced to nitrite on line by post-column reduction using vanadium(III) chloride and heat. Nitrite reacted with Griess reagent to produce a dye that was detected at 525 nm	[87]
Nitrite	15 vegetables	IC-UV				[68]
Nitrite	Honey	IC-CD	8 mM Na_2CO_3/1 mM $NaHCO_3$ 1 mL/min	Dionex ICS1000	Equipped with an IonPac AS14A (Dionex) column. Conductivity system was equipped with an ASRS-ULTRA suppression mode (Dionex)	[81]
Nitrite	Seaweeds	IC-CD	3.2 mM Na_2CO_3/1 mM $NaHCO_3$ 0.70 mL/min	Metrohm IC861	Equipped with a pump (IC-818), an eluent degasser (IC-837) and a liquid handling unit (IC-833) with a 0.45-μm filter that required a minimal volume of 10 mL for the samples. Detection was performed with a conductivity detector (IC-819). Separation was performed in a Metrosep A Supp 5–250 column (250 × 4 mm, 5-μm particle size). All measurements were carried out at 32°C (column temperature)	[59]

(Continued)

Table 16.6 (Continued)

Analyte	Sample	Detection	Eluent	Instrument	Comments	Reference
Nitrite	Fresh meat	IC-CD	(A) 2 mM NaOH (B) 21 mM NaOH 0.5 mL/min	Dionex	Composed of a GP50 quaternary gradient pump, an electrochemical detector (model ED40) set to conductivity mode IonPac AS11-HC column (250 mm × 4 mm i.d., particle size 13-μm). Gradient elution	[46]
Nitrite	Meat, dairy, fish products, leafy vegetables	IC-CD	9 mM Na_2CO_3 1.0 mL/min	Dionex	Composed of a GP50 quaternary gradient pump, an electrochemical detector (model ED40 set to conductivity mode) equipped with a temperature-compensated conductivity cell IonPac AS9-HC anion exchange column (250 mm × 4 mm i.d., particle size 13 μm) associated with a pre-column AG9-HC. Total run time, 20 min	[40]
Nitrite	Dairy, fish, fresh meat, leafy vegetables, meat products, shellfish	IC-CD	9 mM Na_2CO_3 1.0 mL/min	Dionex	Separations were accomplished using an IonPac AS9-HC anion exchange column (250 mm × 4 mm i.d., 13 μm) associated with a pre-column AG9-HC (Dionex). Dionex anion self-regenerating suppressor set to 50 mA (ASRS II, 4 mm) was used for the electrochemical suppression. Isocratic elution. Total run time, 20 min	[48]
Nitrite	Pickles	IC-UV	10 mM ammonium phosphate		The octadecyl-bonded silica (ODS) column coated with cetyltrimethylammonium bromide (CTAB) was used to separate anions	[69]
Nitrite	Baby foods	IC-CD				[86]
Nitrite	Meat products	IC-UV (210 nm)	1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$ 1.0 mL/min	Metrohm IC850	Column, Shodex IC SI-90 4E	[88]
Nitrite	Cured meat	IC-MS	NaOH		Nitrate can be separated from interfering compounds with a high-capacity anion-exchange column. The described IC/MS method is robust, sensitive to nitrate concentrations as low as 0.10 mg/kg, and can determine sugar phosphates that are useful for monitoring meat freshness	[50]
Nitrite	Vinegar	IC-CD	0.35 mM salicylic acid 0.1 mM sodium salicylate 0.8 mL/min (pH 3.5)		CarbonIC BI-01 (4.6 mm i.d. × 100 mm) column coated with cetylmethylammonium ion. Temperature, 40°C	[84]

Nitrite	Light salami Foodstuffs	IC-F (exc270–em355)	Citric acid–NaOH 0.08 M 1.5 mL/min	Shimadzu	Anionic column (Shim-pack IC-A1) connected in series with a pre-column (Shim-pack IC-GA1), both being thermostatted at 40°C. Detection was carried out with an RF-551 fluorescence detector (Shimadzu). Subsequent to the ion separation, the mobile phase merged with a 30 mM phosphate solution of tryptophan (5 μg/mL), flowing at 1 mL/min. Detects the suppression of fluorescence of the tryptophan due to the elution of the target ions	[51]
Nitrite	Sugar	HPAEC-CD	(A) 100 mM NaOH (B) Ultrapure water 1.5 mL/min	Dionex ICS3000	Separation was conducted with use of the IonPac AS11-HC 4 × 250 mm column and the ASRS-ULTRA II 4 mm conductivity suppressor produced by Dionex Time and composition program (eluents A and B expressed as percent v/v): (i) 0 min, 1% (A) and 99% (B); (ii) from 0 to 17 min, 5% (A) and 95% (B); (iii) from 17 to 25 min, 25% (A) and 75% (B); (iv) from 25 to 32 min,40% (A) and 60% (B); (v) from 32 to next run, 1% (A) and 99% (B)	[79]
Nitrite	Milk	IC				[93]
Nitrite	Food	IC-CD	2.0 mM benzoic acid/1.2 mM TAMAHP 1 mL/min (pH 4.4)		Column Carbon IC BI-01	[85]
Nitrite	Food	IC-CD	(1) 0.35 mM salicylic acid/0.l mM sodium salicylate (pH 3.5), 0.8 mL/min (2) 2.0 MM benzoic acid/1.2 mM Tris aminomethane (pH 4.4), 1.0 mL/min	Toa DKK	The ion chromatographic equipment consisted of a Model ICA-5120 pump, a Model ICA-5220 conductivity detector, a Model ICA-5410 column oven (Toa DKK). Carbon IC BI-01 (100 mm × 4.6 mm i.d.)	[41]
Nitrite	Cured meat, fresh meat, canned vegetables, fresh vegetables, sausage	IC-AWD	1.5 mM KHP 1.2 mL/min	Shimadzu IC-A6	Column: Shim-pack IC-A1 (4.6 mm i.d. × 100 mm stainless-steel) packed with an anion-exchange resin on polymethacrylate support incorporating a quaternary ammonium base. Column and detector are set at 40°C	[47]
Nitrite	Dairy products	IC-UV (225 nm)			An AS 19 column was used as the analytical column	[89]
P (H_2PO_4)	Taro	IC-CD		Showa Denko	Shodex IC NI-424 column and measured by a CD-5 conductivity detector	[77]

(Continued)

Table 16.6 (Continued)

Analyte	Sample	Detection	Eluent	Instrument	Comments	Reference
P_2O_7	Cod	IC-CD	(A) 1 g/L NaOH (B) 4 g/L NaOH 0.25 mL/min	Dionex ICS3000	Equipped with a SP-1 single gradient pump, a Dionex conductivity detector, a Dionex IonPac AS11-HC (2 × 250 mm) with a guard column and IonPac ATC-3 (4 × 35 mm) columns, and anion suppressor ASRS 300-2 mm. The 30-min gradient program was increased from (A) to (B)	[61]
P_3O_{10}	Cod	IC-CD	(A) 1 g/L NaOH (B) 4 g/L NaOH 0.25 mL/min	Dionex ICS3000	Equipped with a SP-1 single gradient pump, a Dionex conductivity detector, a Dionex IonPac AS11-HC (2 × 250 mm) with a guard column and IonPac ATC-3 (4 × 35 mm) columns, and anion suppressor ASRS 300-2 mm. The 30-min gradient program was increased from (A) to (B)	[61]
P_3O_9	Cod	IC-CD	(A) 1 g/L NaOH (B) 4 g/L NaOH 0.25 mL/min	Dionex ICS3000	Equipped with a SP-1 single gradient pump, a Dionex conductivity detector, a Dionex IonPac AS11-HC (2 × 250 mm) with a guard column and IonPac ATC-3 (4 × 35 mm) columns, and anion suppressor ASRS 300-2 mm. The 30-min gradient program was increased from (A) to (B)	[61]
Perchlorate	Milk, dairy products, leafy vegetables	IC-EIMS			Biomonitoring. Consumption of three or more servings of these foods in the 16 h before urine sample collection was associated with significantly higher levels of perchlorate in urine	[64]
Perchlorate	Milk	IC-MS	100 mM NaOH 1.00 mL/min	Dionex DX-600	IC with a GS50 gradient pump, an ASRS-Ultra 4-mm suppressor and a CD25 conductivity detector. A TAC-LP1 (4 × 35 mm) anion concentrator was used as a pre-concentration column (PCC). The wash solution (5 mM NaOH at a flow rate of 0.85 mL/min) washes off the less strongly retained anions such as chloride, nitrate and sulfate from the PCC. After 2.5 min of washing, the injector is switched to admit the main eluent, which has considerably greater eluting power, to the PCC. The analyte anions of interest are now eluted to the separation column	[35]
Perchlorate	Fresh food, canned food, wine, beer	IC-ESI-MS/MS	ACN/water 90 : 10 (v/v) 0.3 mL/min	Dionex ICS-2500	IC system coupled to either an API 2000TM or an API 3200TM mass spectrometer. ICS-2500 quaternary proportioned gradient pumping system consisting of a Dionex GS50 pump, EG50 eluent generator, AS50 autosampler	[14]

Perchlorate	Dairy milk, infant formula	IC-MS/MS	15 mM KOH 0.35 mL/min	Dionex ICS2100	A Dionex IonPac AS21 separation column (2 × 250 mm) connected to an IonPac AG21 guard column (2 × 50 mm)	[6]
Perchlorate	Milk	IC-CD	100 mM NaOH 1.0 mL/min	Dionex-320	DX-320 IC system equipped with a GP50 gradient pump, an LC-25 oven, a CD20 conductivity detector, an ASRS-Ultra electrodialytic supresor. Pre-elution samples were carried with 10 mM NaOH at 0.4 mL/min for 6.2 min and then chromatographed on AG11 (4.0 × 50 mm), AS16 (4.0 × 250 mm) columns	[32]
Perchlorate	Milk	IC-MS	100 mM NaOH 1.00 mL/min	Dionex DX-600	Biomonitorig	[83]
Perchlorate	Milk	IC-MS/MS		Dionex-DX600	AS-16 column was used prior to analysis by MS/MS.	[36]
Perchlorate	Lettuce, cantaloupe, milk	IC-MS/MS	100 mM ammonium acetate in acetonitrile/ water 50 : 50 (v/v) 0.3 mL/min	Agilent 1100	First time that an isotopically labelled internal standard was used for determination of perchlorate anion in food. Waters IC-Pak Anion HR analytical (4.6 mm i.d. × 75 mm) and Anion Guard-Pak (5 mm i.d. × 10 mm) columns	[31]
Perchlorate	Fruits, vegetables infant foods, dry products	IC-MS/MS	100 mM CH_3COONH_4 in 50 : 50 (v/v) ACN/water 0.35 mL/min	Agilent 1100	A Waters IC-Pak Anion HR analytical column (4.6 × 75 mm). Anion Guard-Pak (5 mm i.d. × 10 mm)	[5]
Perchlorate	Cantaloupe, carrots, lettuce, spinach	IC-CD	Mobile phase dynamically generated from (A) 100 mM NaOH, (B) water and (C) ACN at 40 : 55 : 05 (A:B:C) 1.0 mL/min	Dionex	An anion self-regenerating suppressor (ASRS, ULTRA II, Dionex) operated in the external water mode, and a Dionex thermostated conductivity cell (model DS3 Detection Stabilizer) with electrochemical detector (model ED40). Dionex IonPac AG16 anion-exchange guard column (4 × 50 mm) functioned as a pre-column to extract and enrich perchlorate and other anions from test solutions for injection (reverse flush) onto an AS16 anion-exchange analytical column (4 × 250 mm). During loading of the pre-column, 0.5 mL of water was pushed through to flush out the mobile phase and then 50–750 µL of test solution was delivered, followed by another 0.5 mL of water	[65]
Perchlorate	Leafy vegetables (22 especies)	IC-CD	50 mM KOH	Dionex 2500	Consisting of an IP 25 isocratic pump, an EG50 eluent generator, a continuous regenerating trap column, a CD25 conductivity detector, the 2-mm AG16/AS16 guard and separation column pair, and an AMMS III suppressor. 50 mM sulfuric acid suppression	[66]

(Continued)

Table 16.6 (Continued)

Analyte	Sample	Detection	Eluent	Instrument	Comments	Reference
Perchlorate	Milk	IC-MS/MS				[33]
Perchlorate	Vegetables, fruit	IC, IC-MS				[42]
Perchlorate	Rice, milk	IC-ESI-MS/MS		Dionex	Ion chromatography–electrospray tandem mass spectrometry with negative mode	[21]
Perchlorate	Berries, fish, vegetation	IC-CD	50 mM NaOH 1.0 mL/min	Dionex DX-500	System equipped with a GP50 gradient pump, a CD20 conductivity detector. Dionex IonPac AS16 (250 × 4.0 mm) analytical column	[44]
Perchlorate	Cheese, meat, milk, yoghurt	IC	2.5 mM Phthalic acid/ 2.4 mM Tris (hydroxymethyl) aminomethane (pH 4) 1.5 mL/min	Shimadzu C196	A Shim-pack IC-A1 (Kyoto, Japan) anion exchange column was used. Oven temperature at 40°C	[30]
Perchlorate	Cabbage, carrot, lettuce, spinach, tomato, red cabbage, lemon, orange, mandarin, grapefruit, milk, fish	IC-CD	2.5 mM Phthalic acid/ 2.4 mM Tris (hydroxymethyl) amino methane 1.5 mL/min (pH 4)	Shimadzu C196	A Shim-pack IC-A1 anion exchange column. Oven temperature of 40°C	[29]
Perchlorate	Fruits, vegetables	IC-MS/MS	30% of 100 mM CH_3COONH_4 in water and 70% of ACN	Agilent 1100	HPLC was coupled to a Waters Quattro Ultima triple quadrupole mass spectrometer with Z-spray electrospray (ESI) interface.Oven (30°C). Analytical column AS16 (2 × 250 mm, Dionex). Guard column AG16 (2 × 50 mm, Dionex). Estimated dietary exposure to perchlorate from fruits and vegetables	[43]
Perchlorate	Infant formula	ID-IC-MS/MS			Spiked with ^{18}O isotope-labelled perchlorate internal standard prior to extraction	[8]
Perchlorate	Rice, vegetables, seaweed, milk	IC-MS/MS				[22]
Perchlorate	Food	IC-CD				[82]
Phosphate	Beer	IC-CD	$Na_2CO_3/NaHCO_3$	Metrohm	Sonication for 15 min. The beer was then diluted 1 : 20 with deionized water. The modular system used for the analysis of beer comprised the Metrohm 709 IC Pump, Metrohm 732 IC Detector, Metrohm 733 IC Separation Center, Metrohm 752 Pump Unit and Metrohm 762 IC interface. Metrohm A SUPP 5 analytical column	[13]

Phosphate	Honey	IC-CD	8 mM Na_2CO_3/1 mM $NaHCO_3$ 1 mL/min	Dionex ICS1000	Equipped with an IonPac AS14A (Dionex) column. Conductivity system was equipped with an ASRS-ULTRA suppression mode (Dionex)	[81]
Phosphate	Seaweeds	IC-CD	3.2 mM Na_2CO_3/1 mM $NaHCO_3$ 0.70 mL/min	Metrohm IC861	Equipped with a pump (IC-818), an eluent degasser (IC-837) and a liquid handling unit (IC-833) with a 0.45-μm filter that required a minimal volume of 10 mL for the samples. Detection was performed with a conductivity detector (IC-819). Separation was performed in a Metrosep A Supp 5–250 column (250 × 4 mm, 5-μm particle size). All measurements were carried out at 32°C (column temperature)	[59]
Phosphate	Fishery products	IC-ECD				[60]
Phosphate	Spinach processing	IC-CD	0.65 mM $NaHCO_3$/1.5 mM Na_2CO_3	Dionex Model 2000i	Dionex AS4A column (P/N37041) anion separator column and a guard column containing the same resin (AG4A P/N 37042, for anions) with autosampler Model 035274 Dionex. The detector used was a CMD conductivity cell with 0.01 N H_2SO_4 regenerant for anions. The pump pressure was 900 psi. The results showed the anions to increase in the water during washing of raw spinach and during the freezing and canning processes	[74]
Phosphate	Cod	IC-CD	(A) 1 g/L NaOH (B) 4 g/L NaOH 0.25 mL/min	Dionex ICS3000	Equipped with an SP-1 single gradient pump, a Dionex conductivity detector, a Dionex IonPac AS11-HC (2 × 250 mm) with a guard column and IonPac ATC-3 (4 × 35 mm) columns, and anion suppressor ASRS 300-2 mm. The 30-min gradient program was increased from (A) to (B)	[61]
Phosphate	Wine	HPIC			Sample preparation required only filtration and dilution where necessary	[16]
Phosphate	Greek Kiwi wine	IC-CD		Dionex Series 4500i	The system was equipped with an anion trap column (P/N 037151), a guard column OmniPac PAX-500, P/N 042153 (4.0 mm i.d. × 50 mm) and an analytical or separation column OmniPac PAX-500, P/N 042152 (4.0 mm i.d. × 250 mm) Dionex	[15]

(Continued)

Table 16.6 (Continued)

Analyte	Sample	Detection	Eluent	Instrument	Comments	Reference
Phosphate	Sugar	HPAEC-CD	(A) 100 mM NaOH (B) Ultrapure water 1.5 mL/min	Dionex ICS3000	Separation was conducted with use of the IonPac AS11-HC 4 × 250 mm column and the ASRS-ULTRA II 4 mm conductivity suppressor produced by Dionex Time and composition program (eluents A and B expressed as percent v/v): (i) 0 min, 1% (A) and 99% (B); (ii) from 0 to 17 min, 5% (A) and 95% (B); (iii) from 17 to 25 min, 25% (A) and 75% (B); (iv) from 25 to 32 min,40% (A) and 60% (B); (v) from 32 to next run, 1% (A) and 99% (B)	[79]
Phosphate	Seafoods	IC-CD	30–80 mM KOH gradient 1.0 mL/min		Separation was carried out on an IonPac AS11-HC analytical column (250 × 4 mm) and an IonPac AG11-HC guard column (50 × 4 mm) at 35°C	[62]
Phosphate(m)	Seafoods	IC-CD	30–80 mM KOH gradient 1.0 mL/min		Separation was carried out on an IonPac AS11-HC analytical column (250 × mm) and an IonPac AG11-HC guard column (50 × 4 mm) at 35°C	[62]
Phosphate(p)	Seafoods	IC-CD	30–80 mM KOH gradient 1.0 mL/min		Separation was carried out on an IonPac AS11-HC analytical column (250 × 4 mm) and an IonPac AG11-HC guard column (50 × 4 mm) at 35°C	[62]
Phosphorus(T)	Seafoods	IC-CD	30–80 mM KOH gradient 1.0 mL/min		Separation was carried out on an IonPac AS11-HC analytical column (250 × 4 mm) and an IonPac AG11-HC guard column (50 × 4 mm) at 35°C	[62]
Polyphosphates	Fish, shrimps	IC				[90]
Se(IV), SeMet, SeCys	Milk	HPLC-ICPMS	(A) 20 mM acetic acid/ 10 mM triethylamine, pH 4.7 (B) 200 MM acetic acid/ 100 mM triethylamine, pH 4.7	Agilent 1100	The exit of the column was directly connected to the Meinhard nebulizer of the ICP-MS equipped with a collision cell (Agilent 7500c) by means of polyetheretherketone (PEEK) tubing. Hamilton PRP X-100 (150 × 4.6 mm, 5 μm) Elution steps: (i) 0–5 min, 100% (A); (ii) 5–30 min, 0–100% (B); (iii) 30–40 min, 100% (B); (iv) 40–42 min, 0–100% (A)	[34]
Se(IV), Se(VI), MeSeCys, SeCys2, SeMet	Kale	IPLC-ICPMS	0.02% HFBA 2% MeOH	Agilent 1100	Equipped with a binary HPLC pump, Column Agilent Zorbax SB-C18	[73]
Se(IV), Se(VI), SeMet	Fish: canned tuna, shark, marlin DOLT-3 BCR-627	IC-ICPMS	(A) 5 mM NH_4NO_3 (B) 50 mM NH_4NO_3, 2% (v/v) methanol, pH 8.7 1 mL/min	Metrohm	The chromatography system consisted of two 818 IC pumps, a 762 IC interface, and an 838 IC autosampler. MetrosepTM Anion Dual 3 column (100 × 4.0 mm, 6 μm) and MetrosepTM Anion Dual 3 guard column (1.7 × 3.5 mm, 0.2 μm) Gradient program: (i) 0 min, 0% (B); (ii) 2 min, 0% (B); (iii) 7min, 100% (B); (iv) 9 min, 100% (B); (v) 9.5 min, 0% (B); (vi) 12 min, 0% (B)	[57]

Se(IV), Se(VI), SeCys2, SeMet	Cereals	IC-DRC-ICPMS	(A) 0.5 mM ammonium citrate in 1% v/v methanol (pH 4.5) (B) 15 mM ammonium citrate in 1% v/v methanol (pH 8.0)	Hitachi L6000	Hamilton PRP-X100, 10 μm particles, 4.1 mm i.d. × 250 mm length Gradient elution: (i) 0–1.5 min, 100% (A); (ii) 1.5–2.5 min, 100% (A) up to 100% (B); (iii) 2.5–8 min, 100% (B)	[20]
SO_2	Food, preserved foods, dried vegetables, wines	IC-CD	1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$ 1.5 mL/min	Dionex ICS2500	GP 50 high performance pump with vacuum degas, ED 50 electrochemical detector equipped with a platinum working electrode, AS 50 autosampler, LC 30 chromatography oven and a 4-mm anion self-regenerating suppressor ASRS-Ultrall. IonPac AS9-SC (250 × 4.0 mm i.d.) analytical column and IonPac ICE AS1 (250 × 9.0 mm i.d.) guard column. SO_2 distillation	[17]
Sulfate	Beer	IC-CD	Na_2CO_3/$NaHCO_3$	Metrohm	Sonication for 15 min. The beer was then diluted 1 : 20 with deionized water. The modular system used for the analysis of beer comprised the Metrohm 709 IC Pump, Metrohm 732 IC Detector, Metrohm 733 IC Separation Center, Metrohm 752 Pump Unit and Metrohm A SUPP 5 analytical column	[13]
Sulfate	Honey	IC-CD	8 mM Na_2CO_3/1 mM $NaHCO_3$ 1 mL/min	Dionex ICS1000	Equipped with an IonPac AS14A (Dionex) column. Conductivity system was equipped with an ASRS-ULTRA suppression mode (Dionex)	[81]
Sulfate	Seaweeds	IC-CD	3.2 mM Na_2CO_3/1 mM $NaHCO_3$ 0.70 mL/min	Metrohm IC861	Equipped with a pump (IC-818), an eluent degasser (IC-837) and a liquid handling unit (IC-833) with a 0.45-μm filter that required a minimal volume of 10 mL for the samples. Detection was performed with a conductivity detector (IC-819). Separation was performed in a Metrosep A Supp 5–250 column (250 × 4 mm, 5-μm particle size). All measurements were carried out at 32°C (column temperature)	[59]
Sulfate	Spinach processing	IC-CD	0.65 mM $NaHCO_3$/1.5 mM Na_2CO_3	Dionex Model 2000i	Dionex AS4A column (P/N37041) anion separator column and a guard column containing the same resin (AG4A P/N 37042, for anions). The detector used was a CMD conductivity cell with 0.01 N H_2SO_4 regenerant for anions. The pump pressure was 900 psi. The results showed the anions to increase in the water during washing of raw spinach and during the freezing and canning processes	[74]

(Continued)

Table 16.6 (Continued)

Analyte	Sample	Detection	Eluent	Instrument	Comments	Reference
Sulfate	Wine	HPIC			Sample preparation required only filtration and dilution where necessary	[16]
Sulfate	Greek Kiwi wine	IC-CD		Dionex Series 4500i	The system was equipped with an anion trap column (P/N 037151), a guard column OmniPac PAX-500, P/N 042153 (4.0 mm i.d. × 50 mm) and an analytical or separation column OmniPac PAX-500, P/N 042152 (4.0 mm i.d. × 250 mm) Dionex	[15]
Sulfate	Sugar	HPAEC-CD	(A) 100 mM NaOH (B) Ultrapure water 1.5 mL/min	Dionex ICS3000	Separation was conducted with use of the IonPac AS11-HC 4 × 250 mm column and the ASRS-ULTRA II 4 mm conductivity suppressor produced by Dionex Time and composition program (eluents A and B expressed as percent v/v): (i) 0 min, 1% (A) and 99% (B); (ii) from 0 to 17 min, 5% (A) and 95% (B); (iii) from 17 to 25 min, 25% (A) and 75% (B); (iv) from 25 to 32 min,40% (A) and 60% (B); (v) from 32 to next run, 1% (A) and 99% (B)	[79]
Sulfate	Food	IC-CD	2.0 mM benzoic acid/1.2 mM TAMAHP 1 mL/min (pH 4.4)		Column Carbon IC BI-01	[85]
Sulfate	Food	IC-CD	(1) 0.35 mM salicylic acid/0.l mM sodium salicylate (pH 3.5), 0.8 mL/min (2) 2.0 mM benzoic acid/1.2 mM Tris aminomethane (pH 4.4), 1.0 mL/min	Toa DKK	The ion chromatographic equipment consisted of a Model ICA-5120 pump, a Model ICA-5220 conductivity detector, a Model ICA-5410 column oven (Toa DKK). Carbon IC BI-01 (100 × 4.6 mm i.d.)	[41]
Sulfite	Fresh meat	IC-CD	(A) 8 mM Na_2CO_3/2.3 mM NaOH (B) 24 mM Na_2CO_3 1.0 mL/min	Dionex	Equipped with GP50 quaternary gradient pump, an electrochemical detector (model ED40) in conductivity mode. Electrochemical suppression was by a Dionex anion self-regenerating suppressor (ASRS II, 4 mm) at an operating current of 50 mA. Anion-exchange column IonPac AS9-HC (250 × 4 mm i.d., particle size 9 μm). Isocratic elution at (A) eluent for 15 min and a step to 50% (A) and 50% (B) in 1 min and then 4 min at this eluent concentration. The system was then re-equilibrated for 20 min at the initial Na_2CO_3 and NaOH concentrations	[53]

Sulfite	Fresh meat, shrimps	IC-CD	(A) 8 mM Na_2CO_3/2.3 mM NaOH (B) 24 mM Na_2CO_3 1.0 mL/min	Dionex	Composed of a GP50 quaternary gradient pump, an electrochemical detector (model ED40) set to conductivity mode, equipped with a temperature-compensated conductivity cell. All the separations were performed using an IonPac AS9-HC column (250 × 4 mm i.d., particle size 9 μm) eluted in gradient mode. The solvent gradient program started with an isocratic step at 100% (A) for 15 min, a gradient step to 50% (A) and 50% (B) in 1 min, and then 4 min at this eluent	[52]
Sulfite	Garlic	IC	2.7 mM Na_2CO_3 0.3 mM $NaHCO_3$ 1.5 mL/min	Dionex	Analytical column IonPac AS12A (4 mm i.d. × 200 mm), guard column IonPac AG12A (4 mm i.d. × 50 mm)	[75]
Sulfite	Potato products, wine, beer	HPIC-CD			Estimate the usual daily intake of sulfites	[76]
Thiocyanate	Milk	IC-MS	100 mM NaOH 1.00 mL/min	Dionex DX-600	IC with a GS50 gradient pump, an ASRS-Ultra 4-mm suppressor and a CD25 conductivity detector. A TAC-LP1 (4 × 35 mm) anion concentrator was used as a pre-concentration column (PCC). The wash solution (5 mM NaOH at a flow rate of 0.85 mL/min) washes off the less strongly retained anions such as chloride, nitrate and sulfate from the PCC. After 2.5 min of washing, the injector is switched to admit the main eluent, which has considerably greater eluting power, to the PCC. The analyte anions of interest are now eluted to the separation column	[35]
Thiocyanate	Milk	IC-MS	100 mM NaOH 1.00 mL/min	Dionex DX-600	Biomonitoring	[83]
Thiocyanate	Powdered milk, infant formula	IC-UV Pre-concentration column	65 mM NaOH 1.0 mL/min	Thermo Finnigan	HPLC column-switching was plumbed with a Dionex IonPac 4 × 50 mm AG16 guard column (used as an enrichment pre-column) in place of the injection loop (reverse-flush mode) and a 4 × 250 mm AS16 analytical column. Columns were cleaned at 1.0 mL/min starting with a gradient from water to 20 : 80 water/ACN in 5 min, holding for 20 min, followed by a 5-min gradient to 1.5 mL/min and 100 mM NaOH, and holding for 20 min. Mobile phase was generated from 100 mM NaOH and water (65 : 35)	[7]

Table 16.7 Instrumental systems employed for IC cation determination in food samples.

Analyte	Sample	Detection	Eluent	Instrument	Comments	Reference
Al	Plant infusion	IC		Dionex		[97]
Ca	Milk	IC-CD	2 mM HNO_3 1.27 mL/min	Metrohm	IC (Metrohm ion analysis, Metrohm Ltd., Herisau, Switzerland) with injection valve, high pressure pump, peristaltic pump, and conductivity detector, all controlled by the 838 advanced sample processor (IC Net 2.3, Metrohm Ltd). Thermostat-controlled detector (30 ± 0.1°C). HPIC non-suppressed separation column (Metrosep C2 150/40 packed with 7-μm silica gel, Metrohm Ltd) was pre-equilibrated with the eluent for 45 min using 1 mL/min at 30 ± 0.1°C	[38]
Co, Cu, Fe, Mn, Ni, Zn	Pea-protein infant formulas					[91]
Co, Cu, Fe, Mn, Ni, Zn	Maize flour, sorghum white flour, sorghum red flour, infant formulas	IC-UV	1 g PDCA + 20 g sodium acetate + 8.5 g acetic acid + 0.2 g ascorbic acid in 1 L of water (pH 4.3)	Waters	IC system consisted of a Waters 626 gradient pump PEEK (polyether ether ketone) (Milford, MA), a Triathlon PEEK autoinjector, and plastic vials by Spark (Emmen, Holland). The autoinjector was customized with an air/pre-puncturing needle in titan, a vial sensor strip in PEEK, and a plastic washing chamber. Guard column CS5G and analytical column CS5A were from Dionex Corp. (Sunnyvale, CA). Post column derivatization: 240 mL of 25% ammonium (NH_3(aq)), 77 mg PAR, and 57 mL acetic acid diluted to 1 L, pH 10.2; UV/Vis (500 nm)	[27]
Li, Na, K, Mg, Ca	Buffalo milk (14) Mozzarella cheese (14)	HPIC-CD		Dionex	Multivariate statistical (join to ICP-OES and NMR data). HPIC (DX 120 EX, Dionex, Sunnyvale CA, USA, equipped with a conductometric detector)	[39]
Li, Na, K, Mg, Ca	Wine (15)	HPIC			Sample preparation required only filtration and dilution where necessary	[16]
Li, Na, NH_4, K, Mg, Ca	Feedstuff	IC-CD	8.5 mM H_2SO_4		IonPac CS12 column (250 × 4 mm i.d.). Cations were detected by suppressed conductivity detection	[96]
Na, K, NH_4, Ca, Mg	Honey	IC-CD	20 mM MSA 1 mL/min	Dionex	Determination performed by means of a CS12A (Dionex) column. For the detection, a conductivity system equipped with a CSRS-ULTRA suppression mode (Dionex) was employed	[81]
Pu, Am, Np	Bovine liver, cabbage, lamb, milk, ryegrass	IC-ICPMS	1.5 M HNO_3 and 0.01 mM dipicolinic acid		The ion column and ICP-MS were interfaced using a high-performance liquid chromatography pump, a Rheodyne model 9010 injection valve (Rheodyne, Cotati, CA, USA) and a 350-mL PEEK sample loop. A column was prepared from PLRP-S (PS–DVB) polystyrene–divinylbenzene substrate (5 mm, Polymer Laboratories, UK) packed into a 100 × 4.6 mm i.d. PEEK column. The mobile phase comprises IC for interferences. It is required to separate the small ^{239}Pu signal from the ^{238}U ion signal from which the interference $^{238}U^{1}H$ is derived	[28]

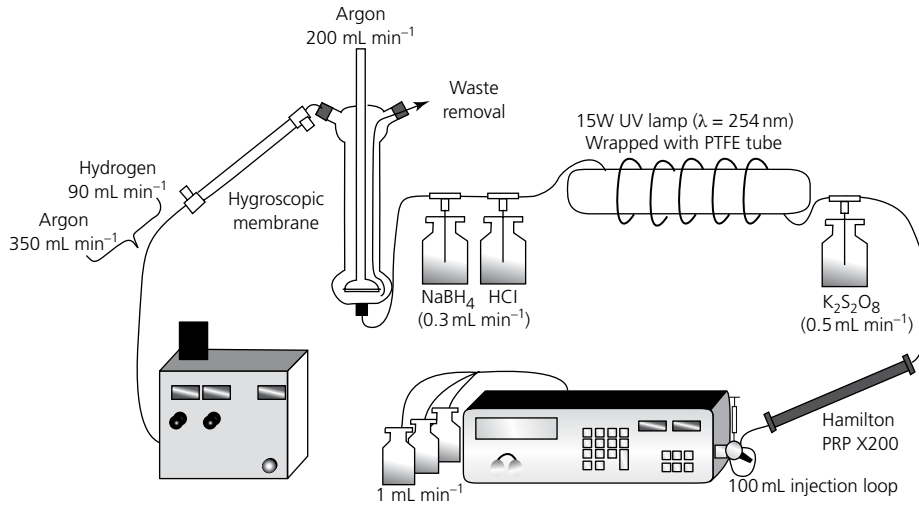


Figure 16.7 The analysis of organoarsenic species employing liquid chromatography, photo-oxidation and HGAFS. From Simon *et al*. [58]. Reproduced with permission of Elsevier.

column with the following gradient elution program: (i) 0 min, 0% (B), (ii) 2 min, 0% (B), (iii) 7 min, 100% (B), (iv) 9 min, 100% (B), (v) 9.5 min, 0% (B) and (vi) 12 min, 0% (B). The eluents, at a flow of 1 mL/min, were (A) 5 mM NH_4NO_3 and (B) 50 mM NH_4NO_3, 2% (v/v) methanol, pH 8.7.

The ion-exchange column Hamilton PRP-X100 has been employed by Chu and Jiang [78] for separation of six arsenic species (As(III), As(V), mMA, DMA, AsB, AsC) at room temperature. Gradient elution with $(NH_4)_2CO_3$ and methanol at pH 8.5 allowed the chromatographic separation of all species in less than 8 min. The column outlet was connected to the pneumatic nebulizer of the ICP-MS device through PTFE tubing.

Height species of arsenic (As(III), As(V), mMA, DMA, AsB, AsC, TMAO, $TMAs^+$) were separated using a guard column IonPac AG7 and an analytical column IonPac AS7 with the following gradient program: (i) 0–3 min, 99% (A); (ii) 3–5 min, 10% (A); and (iii) 5–12 min, 80% (A) [56]. The eluents employed were (A) 0.8 mM HNO_3, 1% MetOH, pH 3.8 and (B) 500 mM HNO_3, 1% MetOH, pH 1.4. The flow was 1.35 mL/min.

Tsai and Jiang [20] developed an IC/DRC/ICP-MS procedure for determination of four arsenic species (As(III), As(V), mMA, DMA). Separation of the species was carried out in a Hamilton PRP-X100 using the following gradient elution: (i) 0–1.5 min, 100% (A); (ii) 1.5–2.5 min, 100% (A) up to 100% (B); and (3) 2.5–8 min, 100% (B). The eluents employed were (A) 0.5 mM ammonium citrate in 1% v/v methanol at pH 4.5 and (B) 15 mM ammonium citrate in 1% v/v methanol at pH 8.0.

Seven species of arsenic (As(III), As(V), mMA, DMA, HAMPA, HACPA, Rox) were determined using IC/ICP-MS [54]. Separation of the arsenic species was achieved on the column Dionex IonPac AS18 with guard AG18 using the following elution gradient: (i) 0–17 min of 45% (A); (ii)17.1–42 min of 70% (A); and (iii) 42.1–50 min of 45% (A). The eluents employed at a flow of 1 mL/min were (A) 100 mM TMAH with 1% (v/v) methanol and (B) 1% (v/v) methanol in water.

Four species of arsenic (As(III), As(V), mMA, DMA) were determined by IC/ICP-MS [11]. Separation of the arsenic species was performed with an anion-exchange column Hamilton PRP-X100 using a Dionex ICS-3000 ion chromatograph. For detection, the outlet of the column was connected to ICP-MS. The arsenic species were identified by comparison of the retention times with those of the standards. The stability of the instrumental signal during the analytical run was controlled every 10 samples by analysing standard solutions and certified reference materials.

Bromine species (bromate, bromide)

Eluents employed for bromate separation have included isocratic 30 mM $(NH_4)_2CO_3$ at 1.0 mL/min flow rate through a 40°C thermostatized column GL-IC-A15G/

GL-IC-A15 [24], isocratic 20 mM KOH at 0.8 mL/min thermostatized at 25°C [23] or NaOH gradient elution [25]. Determinations were carried out using ICP-MS [24] and UV [23, 25] detectors, respectively. KOH in a linear gradient through analytical column Dionex IonPac AS19 at 30°C was also employed. At first, the KOH eluent was maintained at 8 mM for 10 min, then changed to 35 mM within 10 min in a linear mode, maintained at 35 mM for 10 min, and finally changed to 8 mM at 30.1 min to equilibrate the system. Using this linear gradient eluent, bromate could be separated efficiently from fluoride and chloride and detected by CD [26].

For bromide separation, different ratios of the Na_2CO_3/$NaHCO_3$ eluents were employed. Isocratic separation, at room temperature, using the eluent 1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$ at a flow of 2 mL/min through the analytical column Dionex AS4A, was employed by Di Narda *et al*. [71], isocratic separation at 32°C employing the eluent 3.2 mm Na_2CO_3/1.0 mM $NaHCO_3$ at a flow of 0.7 mL/min through the analytical column Metrosep A Supp 5–250 was suggested by Gómez-Ordoñez *et al*. [59], and isocratic separation using the eluent 8 mM Na_2CO_3/1 mM $NaHCO_3$ at a flow of 1 mL/min through the analytical column IonPac AS14A was proposed by Fermo *et al*. [81]. On all occasions conductimetric detection was employed.

Chlorine species (perchlorate, chlorate, chlorite, hypochlorite, chloride)

Perchlorate disrupts thyroid function by competitively inhibiting iodide transport and can be detected unambiguously by IC-suppressed conductivity (IC-CD) [29, 32, 44, 65, 66, 82] and/or IC-MS [5, 6, 8, 14, 21, 22, 30, 31, 33, 35, 42, 43, 64, 83]. To improve IC detection limits for perchlorate determination in foods, a low dilution of the material to be analysed is needed. However, the use of less-diluted samples complicates IC analysis because of the presence of high concentrations of matrix components that co-elute with perchlorate, which may interfere in the determination or increase the background noise. It is usually most appropriate to dilute the samples to avoid the high concentration of such matrix components. The application of MS for perchlorate determination can improve method selectivity significantly. Compared with suppressed conductivity detection, MS detection can minimize the impact of matrix components and therefore reduces the possibility of false-positive or false-negative results in perchlorate determination.

Conductimetric detection of perchlorate has been carried out after separation through a Dionex IonPac AS16 analytical column, using 50–100 mM NaOH eluent at a 1 mL/min flow, and the Dionex AG11 [32, 44] and Dionex AG16 [66] guard columns.

Niemann *et al*. [65] employed an ASRS ULTRA II anion self-regenerating suppressor (Dionex) and a DS3 Dionex 40°C thermostated conductivity cell with ED40 electrochemical detector for determination of perchlorate. They employed an IonPac AG16 anion-exchange guard column as a pre-column to extract and enrich perchlorate and other anions onto an AS16 anion-exchange analytical column. During loading of the pre-column, 0.5 mL of water was pushed through to flush out the mobile phase and then 50–750 µL of test solution were delivered, followed by additional 0.5 mL of water. During injection, the coupled columns were eluted at 1.0 mL/min with a mobile phase dynamically generated from (A) 100 mM NaOH, (B) water and (C) ACN at 40 : 55 : 5 (A : B : C). Figure 16.8 shows the manifold employed in the aforementioned study.

Sungur and Sangün [29] determined perchlorate by IC-CD using a Shim-pack IC-A1 anion-exchange column thermostatized at 40°C. The eluent employed for separation, at a flow rate of 1.5 mL/min, was a mixture of 2.5 mM phthalic acid and 2.4 mM tris(hydroxymethyl) aminomethane solutions at pH 4.

Isotopically labelled internal standard perchlorate determination in foods by IC tandem MS/MS was carried out, for the first time, by Krynitsky *et al*. [5, 31] using 100 mM CH_3COONH_4 in 50 : 50 (v/v) ACN/water eluent at a 0.35 mL/min flow, and a Waters IC-Pak Anion HR analytical (4.6 mM i.d. × 75 mM) and an Anion Guard-Pak (5 mM i.d. × 10 mM) columns. The $^{18}O_4$-labelled perchlorate internal standard corrected efficiently the matrix interferences on measured signals and greatly simplified the extract preparation and clean-up before IC-MS/MS analysis.

El Aribi *et al*. [14] employed an acetonitrile/water 90 : 10 (v/v) eluent at a flow of 0.3 mL/min for IC-ESI-MS/MS determination of perchlorate. The use of ^{18}O labelled perchlorate as internal standard and matrix diversion technique with a gradient step to a high concentration of KOH provided accurate quantification of perchlorate. The use of MS/MS to monitor the $^{35}Cl/^{37}Cl$ ratios improved the specificity of this method and data reliability in perchlorate determination in food and beverages.

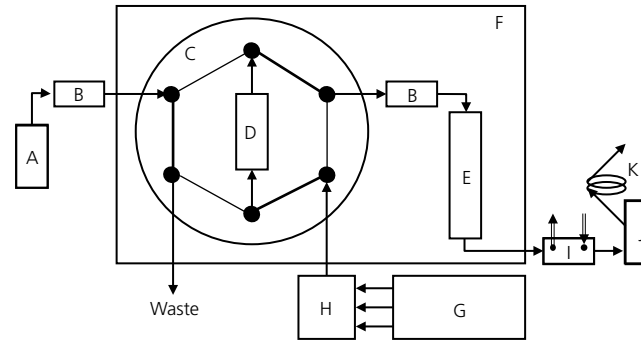


Figure 16.8 Column-switching ion chromatograph assembled from HPLC. **(a)** Syringe and adapter; **(b)** in-line filters; **(c)** injection valve shown in the inject position; **(d)** pre-column; **(e)** analytical column; **(f)** convective oven; **(g)** solvent degasser; **(h)** multisolvent high-pressure pump; **(i)** anion self-regenerating suppressor; **(j)** conductivity cell; **(k)** cell back-pressure tubing. Adapted from Niemann *et al.* [65].

Wang *et al.* [43] used IC-MS/MS for sample analysis. The Agilent HPLC 1100 was coupled to a Waters Quattro Ultima triple quadrupole mass spectrometer with Z-spray electrospray interface. The mobile phase contained 30% of 100 mM ammonium acetate in water and 70% of acetonitrile. A guard Dionex AG16 and analytical Dionex AS16 columns were employed for chromatographic runs in 17 min. At the first and the last 6 min of one chromatograph run the waste was diverted out the MS detector by switching a directional valve. The presence of perchlorate in samples was quantified by isotope dilution MS.

Her *et al.* [6] used for separation a Dionex IonPac AS21 analytical column connected to an IonPac AG21 guard column, employing a 15 mM KOH solution as eluent at a flow rate of 0.35 mL/min. The detection was carried out using an Agilent 6410 triple quadrupole mass spectrometer in the ESI mode.

A 100 mM NaOH eluent at 1.0 mL/min flow was employed by Dyke *et al.* [35] and Kirk *et al.* [83]. Kirk *et al.* [83] used a Dionex DX600 IC instrument for anion determination in foods and Dyke *et al.* [35] used a TAC-LP1 as a pre-concentration column. The less strongly retained anions, such as chloride, nitrate and sulfate, were washed off the pre-concentration column by passing a washing solution (5 mM NaOH at a flow rate of 0.85 mL/min) for 2.5 min. After that, the injector was switched to admit the main eluent and the analyte anions of interest were eluted to the separation column.

Trinetta *et al.* [45] determined the inorganic by-products (ClO_3^-, ClO_2^- and Cl^-) of chlorine dioxide, applied on foods, by IC-UV using a Dionex IonPac column. The method involved elution with a 2 mM Na_2CO_3/0.75 mM $NaHCO_3$ solution at a flow rate of 2 mL/min. Standard solutions were prepared concurrently at four concentration levels (0.1, 0.5, 1.0 and 5 mg/L) for each analyte and blank. The calibration curve was built for each chemical species by plotting UV detector response against standard concentration.

Jackson *et al.* [12] determined chlorate and chloride using a conductimetric detector. They employed a Dionex IonPac AG9-HC guard and Dionex AS9-HC analytical columns with a 9 mM Na_2CO_3 solution eluent at a flow of 1.3 mL/min. Chlorite was determined by Kawasaki *et al.* [63] employing an analytical column IonPac AS 9-HC (4 × 250 mM), a guard column AG9-HC (4 × 50 mM) and conductimetric detection.

Chloride has been determined using conductimetric detecton by a number of different authors [13, 15, 16, 41, 59, 74, 79, 81, 84, 85]. Mixtures of Na_2CO_3 and $NaHCO_3$ solutions are the habitual eluent employed for chloride separation. Soufleros *et al.* [15] employed a Dionex system equipped with an anion trap column, a guard column OmniPac PAX-500 (4.0 mM i.d. × 50 mM) and an analytical column OmniPac PAX-500 (4.0 mM i.d. × 250 mM). Bruce [13] used a modular Metrohm system comprising the 709 IC Pump, 732 IC Detector, 733 IC Separation Center, 752 Pump Unit, 762 IC interface and Metrohm A SUPP 5 analytical column. Martínez-Tomé and Vera [74] used a Dionex AS4A column anion separator column and a guard column containing the same resin (AG4A for anions) with 0.65 mM $NaHCO_3$/1.5 mM Na_2CO_3 as eluent. Gómez-Ordoñez *et al.* [59] employed a Metrohm IC861 equipped with a pump (IC-818), an eluent degasser (IC-837), a liquid handling unit (IC-833) and a conductivity detector (IC-819). The separation was performed in a Metrosep A Supp 5-250 column (250 × 4 mM, 5-μm particle size). The eluent was a mixture of 3.2 mM Na_2CO_3 and 1 mM $NaHCO_3$ at a flow rate of 0.7 mL/min. All measurements were carried out at 32°C (column temperature).

Wojtczak *et al*. [79] employed the IonPac AS11-HC column and the ASRS-ULTRA II 4-mm conductivity suppressor for chloride determination. Eluents employed were mixed according to the following time and composition program (in v/v percentage): (i) 0 min, 1% (A); (ii) from 0 to 17 min, 5% (A); (iii) from 17 to 25 min, 25% (A); (iv) from 25 to 32 min, 40% (A); (v) from 32 to next run, 1% (A). The eluents employed were (A) 100 mm NaOH and (B) ultrapure water at a flow rate of 1.5 mL/min.

By using the Carbon IC BI-01 (4.6 mM i.d. × 100 mM) column, thermostatized at 40°C, eluents employed for chloride determination by conductimetric detection included 0.35 mM salicylic acid/0.1 mM sodium salicylate (pH 3.5) at a flow of 0.8 mL/min [84]; 2.0 mM benzoic acid/1.2 mM TAMAHP (pH 4.4) at a flow of 1 mL/min [85]; and a mixture of eluents (i) 0.35 mM salicylic acid/0.l mM sodium salicylate (pH 3.5) at a flow of 0.8 mL/min and (ii) 2.0 mM benzoic acid/1.2 mM Tris aminomethane (pH 4.4) at a flow of 1.0 mL/min [41].

Fluoride

Fluoride was separated on a Dionex IonPac AS9-HC anion-exchange column with a 9 mM Na_2CO_3 eluent at a flow of 1 mL/min [37] or by isocratic separation at 32°C by employing the eluent 3.2 mM Na_2CO_3/1.0 mM $NaHCO_3$ at a flow of 0.7 mL/min through the analytical column Metrosep A Supp 5-250 [59]. On both occasions conductimetric detection was employed.

Iodide

Iodide separation was carried out at 1 mL/min flow rate using 65 mM [7] or 100 mM [35, 83] NaOH eluents. Niemann and Anderson [7] used a Dionex IonPac AG16 guard column as enrichment pre-column in place of the injection loop (reverse-flush mode) and an AS16 analytical column. Columns were cleaned at 1.0 mL/min starting with a gradient from water to 20 : 80 water/ACN in 5 min, holding for 20 min, followed by a 5 min gradient to 1.5 mL/min and 100 mM NaOH, and holding for 20 min. Mobile phase was generated from 100 mM NaOH and water (65 : 35). After the on-line enrichment the iodide was determined with photodiode array detection. Dyke *et al*. [35] employed a TAC-LP1 (4 × 35 mM) column as anion concentrator. The analytes of interest were eluted to the separation column and determined by MS.

Nitrogen species (nitrite, nitrate)

Anion-exchange columns, filled with a suitable exchanger, have been employed for separation and determination of nitrate and nitrite ions by IC. The eluents that achieve proper separation are usually sodium carbonate and/or sodium bicarbonate solutions. Nitrogen species detection by IC most often has been made through conductimetric or UV detectors. In the case of a complex matrix, UV detection after separation is sometimes preferred for nitrate and nitrite determinations. The main problems are related to proper separation of NO_2^- from Cl^- ions. Irrespective of the column used, the retention times of the ions (related to their structure, ionic radius, and selectivity against the exchanger) are close. In food samples with chloride concentrations usually several times higher than those of nitrite ions, the peak assigned to nitrite can be masked by the large peak assigned to chloride [3].

In the vast majority of the studies reviewed the joint determination of nitrate and nitrite was made with conductimetric detection [40, 41, 46, 48, 49, 59, 79, 81, 84–86] and UV/Vis detection [67–69, 87–89], although fluorescence [51], acoustic wave [47] and MS [50] detectors have also been employed. The most employed eluents for nitrate and nitrite determinations include sodium carbonate or bicarbonate solutions and mixtures of them [13, 40, 49, 59, 74, 88], NaOH solutions [46, 48, 50, 79, 81] and phosphate salt solutions [47, 50].

Bruce [13] determined nitrate by using a Metrohm 709 IC pump, a Metrohm 732 IC conductimetric detector, a Metrohm 733 IC Separation Center, with the Metrohm A SUPP 5 analytical column, the eluent employed being the Na_2CO_3/$NaHCO_3$ mixture. Martínez-Tomé and Vera [74] determined nitrate by using the eluent mixture 0.65 mM $NaHCO_3$/1.5 mM Na_2CO_3 and the Dionex AS4A and the guard AG4A columns.

Gómez-Ordoñez *et al*. [59] employed a Metrohm IC861 modular chromatograph, equipped with a pump (IC-818), an eluent degasser (IC-837), a liquid handling unit (IC-833) and conductivity detector (IC-819). Separation was performed in a Metrosep A Supp 5–250 column (250 × 4 mM, 5-μm particle size) at 32°C with the eluent 3.2 mM Na_2CO_3/1 mM $NaHCO_3$ at a flow rate of 0.70 mL/min.

Armenteros *et al*. [49] achieved the separation of nitrate and nitrite by means of a Metrosep A Supp 5 (4.0 × 250 mM) column from Metrohm, operating at

room temperature, and isocratic elution with the mixture 1.0 mM $NaHCO_3$/3.2 mM Na_2CO_3 plus 0.3% of acetone (v/v) at a flow rate of 0.7 mL/min. The total run time was 35 min. Conductimetric detection was employed.

Iammarino *et al*. [40] carried out nitrate and nitrite separation in a 20-min run time. Chromatographic determinations were performed on a Dionex system composed of a GP50 quaternary gradient pump, an electrochemical detector (model ED40) equipped with a temperature-compensated conductivity cell. The chromatographic separations were accomplished using an IonPac AS9-HC anion-exchange column (4 mM i.d. × 250 mm) associated to a pre-column AG9-HC eluted in isocratic mode with a 9 mM sodium carbonate solution at a flow rate of 1.0 mL/min

For nitrite and nitrate separation, Iammarino and di Taranto [46] employed a Dionex instrument composed of a GP50 quaternary gradient pump, an electrochemical detector (model ED40) set to conductivity mode and an IonPac AS11-HC column (4 mM i.d. × 250 mM, particle size 13 μm), and a gradient elution between eluent (A) 2 mM NaOH and (B) 21 mM NaOH at a flow rate of 0.5 mL/min.

Wojtczak *et al*. [79] separated nitrate and nitrite with the use of the IonPac AS11-HC 4 × 250 mM column and the ASRS-ULTRA II 4 mM conductivity suppressor produced by Dionex employing the eluents 100 mM NaOH (A) and ultrapure water (B), at a flow rate of 1.5 mL/min, according to the following time and gradient program steps: (i) 0 min, 1% (A); (ii) from 0 to 17 min, 5% (A); (iii) from 17 to 25 min, 25% (A); (iv) from 25 to 32 min, 40% (A); and (v) from 32 to next run, 1% (A).

Yoshikawa *et al*. [41, 85] employed a Carbon IC BI-01 (4.6 mM i.d. × 100 mM) column thermostatized at 40°C, with conductimetric detection, for separation of nitrate and nitrite. The eluents employed were 2.0 mM benzoic acid/1.2 mM TAMAHP (pH 4.4) at a flow of 1 mL/min [85] and a mixture of eluents (i) 0.35 mM salicylic acid/0.l mM sodium salicylate (pH 3.5) at a flow of 0.8 mL/min and (ii) 2.0 mM benzoic acid/1.2 mM Tris aminomethane (pH 4.4) at a flow of 1.0 mL/min [41].

By using the Carbon IC BI-01 (4.6 mM i.d. × 100 mM) column thermostatized at 40°C, Sakuragawa and Nakane [84] employed the eluent 0.35 mM salicylic acid/0.1 mM sodium salicylate (pH 3.5) at a flow of 0.8 mL/min for nitrate and nitrite conductimetric determination. Butt *et al*. [67] determined nitrate and nitrite at 220 nm by coupling a spectrophotometer to the chromatograph. The eluent employed a pH 2 buffer of 5 mM NaH_2PO_4/0.5 M H_3PO_4 which did not interfere at the detection wavelength. Ji *et al*. [69] employed UV detection of nitrite and nitrate ions after their chromatographic separation through an octadecyl-bonded silica (ODS) column coated with cetyltrimethylammonium bromide (CTAB). A 10 mM ammonium phosphate solution was used as eluent.

Nakashima *et al*. [88] employed an in-line dialysis system combined with IC for the simultaneous determination of nitrate and nitrite (Figure 16.9). A Shodex IC SI90 4E column was used for separation of anions with 1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$ eluent at a flow of 1.0 mL/min. The detection was carried out at 210 nm. Detection at 225 nm was employed by Zhong *et al*. [89] after separation of nitrate and nitrite on an AS19 column. After separation of the nitrogen species, Casanova *et al*. [87] employed the Griess reaction to produce a dye that was detected at 525 nm.

Stalikas *et al*. [51] developed an indirect fluorimetric procedure for detection of nitrite and nitrate ions. The separation of nitrite and nitrate was carried out on the anionic column Shim-pack IC-A1 connected in series with the pre-column Shim-pack IC-GA1, both being thermostated at 40°C using the citric acid–NaOH 0.08 M eluent at 1.5 mL/min flow rate. Subsequent to the ion separation, the mobile phase was merged with a 30 mM phosphate solution of tryptophan (5 μg/mL), flowing at 1 mL/min. Detection was carried out with an RF-551 fluorescence detector (Shimadzu) using 220 nm as excitation wavelength and 355 nm as emission wavelength. The fluorescence suppression of the tryptophan, due to the elution of the target ions, is proportional to their concentration.

Yu *et al*. [47] employed 1.5 mM potassium hydrogenphthalate at a flow of 1.2 mL/min as eluent for separation of nitrite and nitrate on a Shim-pack IC-A1 (4.6 mM i.d. × 100 mM) packed with an anion-exchange resin on polymethacrylate support incorporating a quaternary ammonium base. The column and the acoustic wave detector were set at 40°C.

Phosphorus species

In all reviewed studies the detection system employed for the determination of phosphorus species in food samples was conductimetric [13, 15, 16, 59–62, 74, 77,

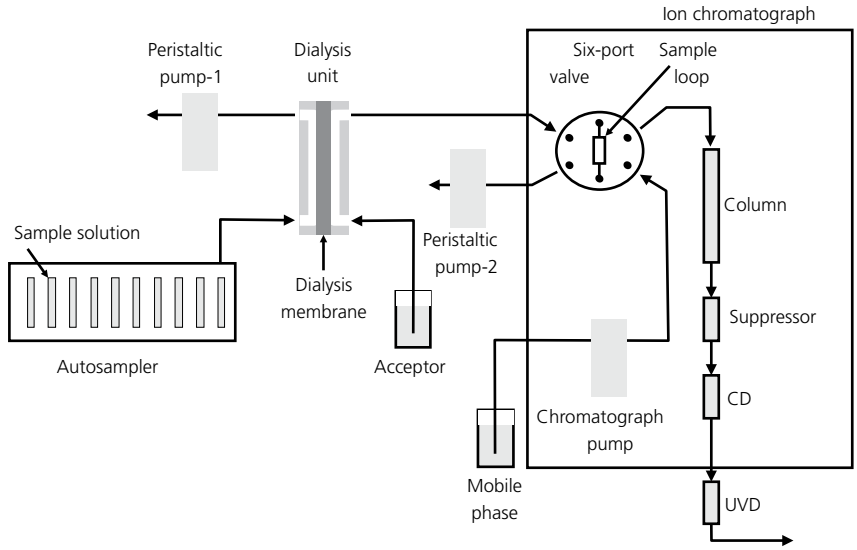


Figure 16.9 Flow diagram of the in-line dialysis/ion chromatograph system. Adapted from Nakashima *et al.* [88].

79, 90]. As in chloride determinations, mixtures of Na_2CO_3 and $NaHCO_3$ solutions are the habitual eluent mixture for phosphate separation [13, 15, 59, 74].

Wojtczak *et al.* [79] employed a gradient eluent program via an IonPac AS11-HC column and the ASRS-ULTRA II 4-mm conductivity suppressor for phosphate determination. Eluents employed at 1.5 mL/min flow, i.e. (A) 100 mM NaOH and (B) ultrapure water, were mixed according to the following steps with concentrations in percentage [v/v]): (i) 0 min, 1% (A); (ii) from 0 to 17 min, 5% (A); (iii) from 17 to 25 min, 25% (A); (iv) from 25 to 32 min, 40% (A); and (v) from 32 to next run, 1% (A).

Zhong and Li [62] carried out the separation of orthophosphate, metaphosphate and pyrophosphate on an IonPac AS11-HC analytical column (250 × 4 mM) and an IonPac AG11-HC guard column (50 × 4 mM) at 35°C by employing a 30–80 mM KOH gradient at a flow of 1.0 mL/min.

Nguyen *et al.* [61] employed a 30-min gradient program for separation of orthophosphate, pyrophosphate and triphosphate. The gradient was increased from 1 g/L NaOH (eluent A) to 4 g/L NaOH (eluent B) with a constant flow rate of 0.25 mL/min. Dionex chromatographic system comprised Dionex IonPac AS11-HC (2 × 250 mM) and a IonPac ATC-3 (4 × 35 mM) columns.

Selenium species

Bierla *et al.* [34] developed a procedure for the simultaneous determination of SeMet, Se(IV) and SeCys by IC/ICP-MS. The device used a Hamilton PRP X-100 (150 × 4.6 mM, 5 μm) column and several elution steps with eluents (A) 20 mM acetic acid/10 mM triethylamine, pH 4.7 and (B) 200 mM acetic acid/100 mM triethylamine, pH 4.7. Elution steps were (i) 0–5 min, 100% (A); (ii) 5–30 min, 0–100% (B); (iii) 30–40 min, 100% (B); and (iv) 40–42 min, 0–100% (A). The exit of the column was directly connected to the Meinhard nebulizer of the ICP-MS equipped with a collision cell by means of PEEK tubing. For quantification the ^{80}Se isotope was used. SeCys and SeMet were quantified using a calibration graph made by standard additions. An internal standard (^{77}Se-labelled SeMet) served to correct for the SeMet derivatization yield and recovery.

Three selenium species, Se(IV), Se(VI) and SeMet, were determined with the procedure developed by Reyes *et al.* [57]. The chromatography system consisted of two pumps (IC-818), an interface (IC-762), a Metrosep Anion Dual 3 column (100 × 4.0 mM, 6 μm) and a Metrosep Anion Dual 3 guard column (1.7 × 3.5 mM, 0.2 μm) and were employed for separation of the species using a gradient elution program at a flow of 1 mL/min: (i) 0 min, 0% (B); (ii) 2 min, 0% (B); (iii) 7 min, 100% (B); (iv) 9 min, 100% (B); (v) 9.5 min, 0% (B);and (vi)

12 min, 0% (B). The eluents employed were (A) 5 mM NH_4NO_3 solution and (B) 50 mM NH_4NO_3 in 2% (v/v) methanol solution at pH 8.7.

Five selenium species – Se(IV), Se(VI), MeSeCys, SeCys2 and SeMet – were determined by IPLC/ICP-MS using 0.02 % FBA 2% MetOH as eluent and the analytical column Agilent Zorbax SB-C18 [73]. Tsai and Jiang [20] developed an IC/DRC/ICP-MS procedure for determination of four selenium species, Se(IV), Se(VI), SeCys2 and SeMet. Separation of the species was carried out in a Hamilton PRP-X100 using gradient elution as follows: (i) 0–1.5 min, 100% (A); (ii) 1.5–2.5 min, 100% (A) up to 100% (B); and (iii) 2.5–8 min, 100% (B). The eluents employed were (A) 0.5 mM ammonium citrate in 1% v/v methanol at pH 4.5 and (B) 15 mM ammonium citrate in 1% v/v methanol at pH 8.0. The ionization of Se in the plasma was improved by adding 2% (v/v) methanol to the mobile phase. All separations were performed at room temperature. The column outlet was connected to a pneumatic nebulizer of the ICP-MS device through PTFE tubing.

Sulfur species (sulfate, sulfite, SO_2, thiocyanate). Conductimetric detection has been employed in all applications reviewed for sulfate [13, 15, 16, 41, 59, 74, 79, 85] and sulfite [52, 53, 75, 76] determinations via IC. As in chloride and phosphate determinations, the mixtures of Na_2CO_3/$NaHCO_3$ solutions are the habitual eluent mixture for sulfur species separation [13, 15, 59, 74].

Wojtczak *et al*. [79] employed the IonPac AS11-HC column and the ASRS-ULTRA II 4-mm conductivity suppressor for sulfate determination. Eluents employed were mixed according to the following time and composition program (in v/v percentage): (i) 0 min, 1% (A); (2) from 0 to 17 min, 5% (A); (iii) from 17 to 25 min, 25% (A); (iv) from 25 to 32 min, 40% (A); and (v) from 32 to next run, 1% (A). The eluents employed were (A) 100 mM NaOH and (B) ultrapure water at 1.5 mL/min flow.

Using the Carbon IC BI-01 (4.6 mM i.d. × 100 mM) column thermostatized at 40°C, eluents employed for sulfate determination, with conductimetric detection, were 2.0 mM benzoic acid/1.2 mM TAMAHP (pH 4.4) at a flow of 1 mL/min [85] and a mixture of eluents (i) 0.35 mM salicylic acid/0.l mM sodium salicylate (pH 3.5) at a flow of 0.8 mL/min and (ii) 2.0 mM benzoic acid/ 1.2 mM Tris aminomethane (pH 4.4) at a flow of 1.0 mL/min [41].

The determination of sulfite anion is not easy because of its low level in foods and its rapid oxidation to sulfate in aqueous solution. Shimoi *et al*. [75] have determined sulfite by employing the 2.7 mM Na_2CO_3/0.3 mM $NaHCO_3$ eluent at a flow of 1.5 mL/min, with the analytical column IonPac AS12A (4 mM i.d. × 200 mM) and guard column IonPac AG 12A (4 mM i.d. × 50 mM).

Iammarino *et al*. [52, 53] developed a procedure for chromatographic separation of sulfite performed on a Dionex system All the separations were made using an IonPac AS9-HC column (250 × 4 mM i.d.) working in gradient mode at a flow rate of 1.0 mL/min. The mobile phase consisted of 8 mM Na_2CO_3 and 2.3 mM NaOH (A) and 24 mM Na_2CO_3 (B). The solvent gradient program started with an isocratic step at100% (A) for 15 min, a gradient step to 50% (A) and 50% (B) in 1 min, and then 4 min at this eluent concentration. Finally, the system was re-equilibrated for 20 min at 100% (A).

Zhong *et al*. [17] employed an eluent mixture comprising 1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$ at 1.5 mL/min flow for SO_2 determination by IC-CD using an IonPac AS9-SC (250 × 4.0 mM i.d.) analytical column and IonPac ICE AS1 (250 × 9.0 mM i.d.) guard column.

Thiocyanate determination has been carried out using MS [35, 83] and UV detectors, in the latter case after a previous concentration [7], but in all cases a 100 mM NaOH solution was employed as eluent. Dyke *et al*. [35] employed a TAC-LP1 (4 × 35 mM) column as anion concentrator. The analytes of interest were eluted with the 100 mM NaOH eluent at a flow of 1.00 mL/min and directed towards the separation column; finally the thiocyanate was determined by MS.

Niemann and Anderson [7] employed a Dionex IonPac 4 × 50 mM AG16 guard column as enrichment pre-column, in place of the injection loop (in reverse-flush mode), and a 4 × 250 mM AS16 analytical column. Columns were cleaned at 1.0 mL/min, starting with a gradient from water to 20 : 80 water/ACN in 5 min, holding for 20 min, followed by a 5 min gradient to 1.5 mL/min and 100 mM NaOH, and holding again for 20 min. Mobile phase was generated from 100 mm NaOH and water (65 : 35). The output of the chromatograph was coupled to a photodiode array detector (UV6000LP). Full-scan UV spectra (200–300 nm) were acquired and real-time quantitation used peak height response at 225 nm (primary) and 210 nm (secondary).

16.4.2 Cation determination

In the period under review we have not found many applications of cation determination by IC in food analysis. Fredrikson *et al*. [91] determined Co, Cu, Fe, Mn, Ni and Zn spectrophotometrically at 500 nm after separation on a Dionex guard CS5G and analytical CS5A columns and post-column derivatization with ammonium/PAR/acetic acid reagent at pH 10.2.

Evans *et al*. [28] developed a procedure for Pu, Am and Np determination by IC/ICP-MS. The ion column and ICP-MS were interfaced using an HPLC pump and a Rheodyne model 9010 injection valve. The column was prepared from polystyrene–divinylylbenzene substrate (PS–DVB) packed into a 100 × 4.6 mM i.d. PEEK column. It was required to separate the small ^{239}Pu signal from the ^{238}U ion signal from which the interference $^{238}U^{1}H$ is derived.

Li, Na, K, Ca and Mg [16, 39] and Na, K, Ca, Mg and NH_4 [81] have been determined by using conductimetric detectors coupled to the ionic chromatograph. Rahimi-Yazdi *et al*. [38] developed a procedure for determination of Ca using a Metrohm instrument with a thermostatized conductimetric detector (30°C). The HPIC non-suppressed separation column Metrosep C2 150/40 (packed with 7 μm silica gel) was pre-equilibrated with the eluent for 45 min using 1 mL/min of 2 mM HNO_3 eluent at 30°C. After that, calcium was separated at a flow of 1.27 mL/min.

16.5 Advantages and drawbacks of ion chromatography

From thise literature update it can be concluded that IC is an instrumental technique useful for the separation and determination of organic and inorganic anions or cations and other substances after conversion to their ionic form, and can be used for the simultaneous analysis of around 12 common ions in approximately 15 min.

The advantages of this technique include quick analysis, high efficiency, high sensitivity and very good repeatability of results. Furthermore, the short measurement time, usually less than 15 min, and the small volume of samples required for analysis (20–100 μL) make it possible to detect and quantify many analytes at below milligram per litre levels. Using a single analytical technique, it is possible to obtain complete information on the anion composition of the analysed sample [79], thus providing important information about the mineral composition of foods.

Determination of sulfites in fresh meats and shrimps by gradient elution IC suppressed conductivity detection requires minimum sample pretreatment and represents an improvement in the determination of sulfites by the official techniques. Compared with the classical Monier–Williams method, it offers interesting advantages, such as improved accuracy, precision, speed, and automation capability, without the need for laborious pretreatment of samples [52].

The possibility of using different detectors coupled to the effluent of the chromatograph, from the most popular conductimetric to UV/Vis, fluorescence and MS, makes IC particularly recommended for speciation analysis. These kinds of analyses have been performed for the separation and determination of nitrate and nitrite ions in a simple run, or sulfate and sulfite ions, perchlorate and chlorate ions, and different arsenic or selenium species can also be determined in the same sample, or all together, as presented in previous sections of this chapter.

The most common detector employed in IC measures the conductivity of the column eluate by the mobile phase. However, the high concentration of the electrolyte employed in the mobile phase usually creates problems, because mobile-phase ions dominate the conductivity signal. In these cases, an ion-suppressor column can be placed between the analytical column and the detector. This column selectively removes mobile-phase electrolyte ions without removing the ions of interest.

Conductivity detection has been widely used for the determination of trace levels of perchlorate ion in water, but chromatographic retention times are not considered to be a unique identifier and often cannot be used in legal proceedings without another confirmatory test. Because of this and the lack of selectivity of conductivity detectors, false-positive results could be found by IC. However, the use of MS detection overcomes the aforementioned problem and it is clear that IC-MS has become a powerful tool in anion determination and element speciation in foods [14].

In summary, the advantages and drawbacks of IC in mineral profile determination of foods, the so-called SWOTS (strengths, weakness, opportunities and threats) of this technique, are indicated in Figure 16.10. It can be seen that the multi-element and multispecies capability of IC is its main strength, together with the

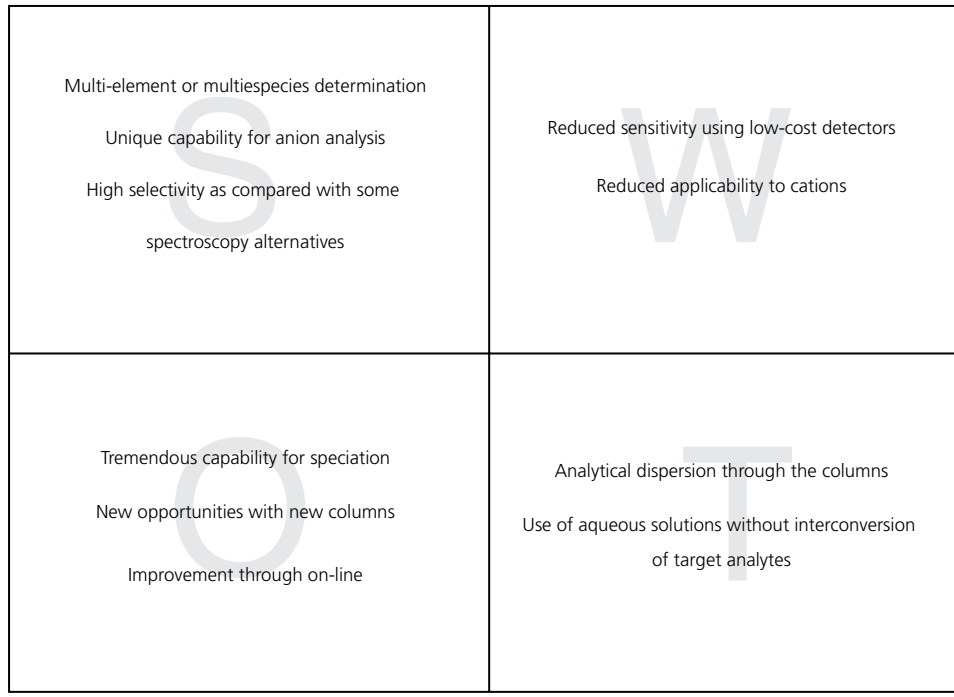


Figure 16.10 SWOT diagram of the use of ion chromatography in mineral profile determination of food.

increase in selectivity provided by the use of the chromatographic signal. However, the sensitivity obtained in many cases is not sufficient for trace level determinations and there is a lack of development of IC in cation analysis.

On considering the future use of IC in food analysis, we hope that new developments in column preparation together with improvements in on-line pre-concentration strategies can improve the sensitivity of IC determinations, despite the fact that the need for sample dissolution and ion formation strongly limits the applicability of IC in the analysis of liposoluble foods. It is likely that the main field where IC will be used in the near future is in speciation studies for both organic and inorganic anions of many elements present in foods in different oxidation states.

16.6 Future perspectives

From the point of view of instrumental developments, the coupling of IC with high-sensitivity and/or high-selectivity detectors opens a new field of research. This coupling will improve the separation potential of IC, with the greatest sensitivity and selectivity when using MS detectors in all their variants. In other cases it will be necessary for on-line post-column derivatization to transform the species of interest to chemical forms that can be detected using spectrophotometric, fluorimetric and atomic fluorescence detectors. In previous sections of this chapter we have presented applications with some of the aforementioned couplings, and have shown that they offer a promising future.

Other than complete food characterization, biomonitoring is another field of application that has gained interest in recent years. In addition to knowing the concentration of chemical species in a sample, it is also necessary to have a broader view of the interrelationship between the different elements considered. Such an in-depth knowledge of mineral inputs and outputs will make an important contribution to understanding the origin of pollution and its interaction with the environment, air, water, soil, vegetation and fauna that ultimately affects human nutrition. Throughout this chapter, we have presented some examples of this contribution.

- The consumption of American long-grain rice results in significant increase in urinary DMA excretion, which supports previous findings that rice contains high levels of arsenic [19].
- The evidence that the main source of perchlorate in milk may be forage and water fed to cattle, and that even trace levels of perchlorate in source waters and soils will result in high concentrations of perchlorate in crops and which are ultimately absorbed by humans [21].
- Estimation of the dietary exposure to perchlorate from fruits and vegetables [43].
- The correlations between perchlorate concentrations in soil and plants and between concentrations in irrigation water and plants [29].

In short, the future of food analysis by IC will involve interdisciplinary studies and in-depth speciation studies suitable for evaluating the natural cycle of all the elements in the periodic table.

Abbreviations

ACN: Acetonitrile
AsB: Arsenobetaine
AsB2: Trimethylarsoniopropionate
AsC: Arsenocholine
As(III): Arsenite
As(V): Arsenate
BDSA: Benzene-1,3-disulfonic acid
CD: Conductimetric detector
CTAB: Cetyltrimethylammonium bromide
DMA: Dimethylarsinic acid
DRC: Dynamic reaction cell
EtOH: Ethanol
ESI: Electrospray ionization
FAAS: Flame atomic absorption spectrometry
HACPA: (4-Hydroxy-3-acetaminophenyl) arsonic acid
HAMPA: (4-Hydroxy-3-aminophenyl) arsonic acid
HFBA: Heptafluorobutyric acid
HGAFS: Hydride generation atomic fluorescence spectrometry
HPAEC-CD: High-performance anion exchange chromatography conductimetric detector
HPIC: High performance ion chromatography
HPIC-CD: High performance ion chromatography conductimetric detector
HPLC: High performance liquid chromatography
IC: Ion chromatography
IC-AWD: Ion chromatography acoustic wave detector
IC-CD: Ion chromatography conductimetric detector
IC-DA: Ion chromatography direct amperometric detector
IC-DRC: Ion chromatography dynamic reaction cell
IC-ECD: Ion chromatography electrochemical detector
IC-EIM: Ion chromatography electrospray mass spectrometry
IC-ESI-MS/MS: Ion chromatography electrospray ionization tandem mass spectrometry
IC-F: Ion chromatography fluorescence
IC/ICP-MS: Ion chromatography inductively coupled plasma mass spectrometry
IC-MS/MS: Ion chromatography tandem mass spectrometry
IC-PED: Ion chromatography pulse electrochemical detector
IC-UV: Ion chromatography ultraviolet/visible detector
IC-UVox-HGAFS: Ion chromatography ultraviolet oxidation hydride generation atomic fluorescence spectrometry
ICP: Inductively coupled plasma
ICP-MS: Inductively coupled plasma mass spectrometry
ID-IC-MS/MS: Isotope dilution ion chromatography tandem mass spectrometry
IPLC: Ion pairing liquid chromatography
IPRP-HPLC: Ion pairing reversed-phase high performance liquid chromatography
KHP: Potassium hydrogenphthalate
LC: Liquid chromatography
MeSeCys: Methylselenocysteine
MetOH: Methanol
MMA: Monomethylarsonic acid
MS: Mass spectrometry
MS/MS: Tandem mass spectrometry
***o*-APAA:** *o*-Aminophenylarsonic acid
ODS: Octadecyl bonded silica
***p*-APAA:** *p*-Aminophenylarsonic acid
PAA: Phenylarsonic acid
PAO: Phenylarsine oxide
PDCA: Pyridine-2,6-dicarboxylic acid
PEEK: Polyether ether ketone
Phosphate(p): Pyrophosphate
Phosphate(m): Metaphosphate
Phosphorus(T): Total phosphorus
PTFE: Polytetrafluoroethylene
PVDF: Polyvinylidene fluoride
Rox: Roxazone (3-nitro-4-hydroxyphenylarsonic acid)
SDME: Single drop microextraction
SDS: Sodium dodecyl sulfate

Se(IV): Selenite
Se(VI): Selenate
SeMet: Selenomethionine
SeCys: Selenocysteine
SeCys2: Selenocystine
SPE: Solid-phase extraction
SWOT: Strengths, weakness, opportunities, threats
TAMAHP: Tris aminomethane(2-amino-hydroxymethyl-1,3-propanediol)
TMAH: Tetramethylammonium hydroxide
TMAO: Trimethylarsine oxide
TMAs$^+$: Tetramethylarsonium ion
UV/Vis: Ultraviolet/visible

References

1 Haddad PR. Ion exchange. Overview. In: Worsfold P, Townshend A, Poole C (eds) *Encyclopedia of Analytical Science*, 2nd edn. Elsevier, Amsterdam, 2005, pp. 440–446.
2 Yashin YI, Yashin AY. Analysis of food products and beverages using high-performance liquid chromatography and ion chromatography with electrochemical detectors. *J Anal Chem Engl Tr* 2004;**59**:1121–1127.
3 Michalski R, Kurzyca I. Determination of nitrogen species (nitrate, nitrite and ammonia ions) in environmental samples by ion chromatography. *Pol J Environ Stud* 2006;**15**:5–18.
4 Michalski R, Jablonska M, Szopa S, Łyko A. Application of ion chromatography with ICP-MS or MS detection to the determination of selected halides and metal/metalloids species. *Crit Rev Anal Chem* 2011;**41**:133–150.
5 Krynitsky AJ, Niemann RA, Williams AD, Hopper ML. Streamlined sample preparation procedure for determination of perchlorate anion in foods by ion chromatography-tandem mass spectrometry. *Anal Chim Acta* 2006;**567**:94–99.
6 Her N, Kim J, Yoon Y. Perchlorate in dairy milk and milk-based powdered infant formula in South Korea. *Chemosphere* 2010;**81**:732–737.
7 Niemann RA, Anderson DL. Determination of iodide and thiocyanate in powdered milk and infant formula by on-line enrichment ion chromatography with photodiode array detection. *J Chromatogr A* 2008;**1200**:193–197.
8 Wang Z, Lau BPY, Tague B, Sparling M, Orsyth D. Determination of perchlorate in infant formula by isotope dilution ion chromatography/tandem mass spectrometry. *Food Addit Contam A* 2011;**28**:799–806.
9 Chai CW, Liu KN, Mou SF. Determination of micro amounts of iodide in dairy products by ion chromatography with direct amperometric detector. *Chin J Chromatogr* 2001;**19**:94–96.
10 Vela NP, Heitkemper DT. Total arsenic determination and speciation in infant food products by ion chromatography-inductively coupled plasma-mass spectrometry. *J AOAC Int* 2004;**87**:244–252.
11 Llorente-Mirandes T, Calderón J, Centrich F, Rubio R, López-Sánchez JF. A need for determination of arsenic species at low levels in cereal-based food and infant cereals. Validation of a method by IC–ICPMS. *Food Chem* 2014;**147**:377–385.
12 Jackson DS, Crockett DF, Wolnik KA. The indirect detection of bleach (sodium hypochlorite) in beverages as evidence of product tampering. *J Forensic Sci* 2006;**51**:827–831.
13 Bruce J. Analysis of anions in beer using ion chromatography. *J Autom Method Manag Chem* 2002;**24**:127–130.
14 El Aribi H, Le Blanc YJC, Antonsen S, Sakuma T. Analysis of perchlorate in foods and beverages by ion chromatography coupled with tandem mass spectrometry (IC-ESI-MS/MS). *Anal Chim Acta* 2006;**567**:39–47.
15 Soufleros EH, Pissa I, Petridis D *et al*. Instrumental analysis of volatile and other compounds of Greek kiwi wine: sensory evaluation and optimisation of its composition. *Food Chem* 2001;**75**:487–500.
16 Prusisz B, Mulica K, Pohl P. Ion exchange and ion exclusion chromatographic characterization of wines using conductivity detection. *J Food Drug Anal* 2008;**16**:95–103.
17 Zhong Z, Li G, Zhu B, Luo Z, Huang L, Wu X. A rapid distillation method coupled with ion chromatography for the determination of total sulphur dioxide in foods. *Food Chem* 2012;**131**:1044–1050.
18 Dufailly V, Nicolas M, Richoz-Payot J, Poitevin E. Validation of a method for arsenic speciation in food by ion chromatography-inductively coupled plasma/mass spectrometry after ultrasonic-assisted enzymatic extraction. *J AOAC Int* 2011;**94**:947–958.
19 Pearson GF, Greenway GM, Brima EI, Haris PI. Rapid arsenic speciation using ion pair LC-ICPMS with a monolithic silica column reveals increased urinary DMA excretion after ingestion of rice. *J Anal Atom Spectrom* 2007;**22**:361–369.
20 Tsai CY, Jiang SJ. Microwave-assisted extraction and ion chromatography dynamic reaction cell inductively coupled plasma mass spectrometry for the speciation analysis of arsenic and selenium in cereals. *Anal Sci* 2011;**27**:271–276.
21 Shi Y, Zhang P, Wang Y *et al*. Perchlorate in sewage sludge, rice, bottled water and milk collected from different areas in China. *Environ Int* 2007;**33**:955–962.
22 Yang M, Kim J, Han J, Her N. Development of enhanced analytical method for perchlorate in food by IC-MS/MS with an internal standard. *Korean J Food Sci Technol* 2011;**43**:532–536.
23 Wang N, He S, Zhu Y. Low-level bromate analysis by ion chromatography on a polymethacrylate-based monolithic column followed by a post-column reaction. *Eur Food Res Technol* 2012;**235**:685–692.
24 Akiyama T, Yamanaka M, Date Y *et al*. Specific determination of bromate in bread by ion chromatography with ICP-MS. *J Food Hyg Soc Japan* 2002;**43**:348–351.
25 Guo J, Wang F, Qin Y, He G. Estimation of bromate in flour and flour products by ion chromatography using post column derivatization method with triiodide. *J Liq Chromatogr Rel Technol* 2013;**36**:1–11.

26 Shi Y, Liang L, Cai Y, Mou S. Determination of trace levels of bromate in flour and related foods by ion chromatography. *J Agric Food Chem* 2006;**54**:5217–5219.

27 Fredrikson M, Carlsson N-G, Almgren A, Sandberg AS. Simultaneous and sensitive analysis of Cu, Ni, Zn, Co, Mn, and Fe in food and biological samples by ion chromatography. *J Agric Food Chem* 2002;**50**:59–65.

28 Evans P, Elahi S, Lee K, Fairman B. A rapid and accurate method for the determination of plutonium in food using magnetic sector ICP-MS with an ultra-sonic nebuliser and ion chromatography. *J Environ Monit* 2003;**5**:175–179.

29 Sungur A, Sangün MK. Ion chromatographic determination of perchlorate in foods consumed in Hatay region. *Food Chem* 2011;**126**:326–331.

30 Sungur S, Atan MM. Determination of nitrate, nitrite and perchlorate anions in meat, milk and their products consumed in Hatay region in Turkey. *Food Addit Contam B* 2013;**6**:6–10.

31 Krynitsky AJ, Niemann RA, Nortrup DA. Determination of perchlorate anion in foods by ion chromatography-tandem mass spectrometry. *Anal Chem* 2004;**76**:5518–5522.

32 Kirk AB, Smith EE, Tian K, Anderson TA, Dasgupta PK. Perchlorate in milk. *Environ Sci Technol* 2003;**37**:4979–4981.

33 Sanchez CA, Blount BC, Valentin-Blasini L, Lesch SM, Krieger RI. Perchlorate in the feed-dairy continuum of the Southwestern United States. *J Agric Food Chem* 2008;**56**: 5443–5450.

34 Bierla K, Szpunar J, Lobinski R. Specific determination of selenoaminoacids in whole milk by 2D size-exclusion-ion-paring reversed phase high-performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP MS). *Anal Chim Acta* 2008;**624**:195–202.

35 Dyke JV, Kirk AB, Kalyani Martinelango P, Dasgupta PK. Sample processing method for the determination of perchlorate in milk. *Anal Chim Acta* 2006;**567**:73–78.

36 Kirk AB, Dyke JV, Ohira ShI, Dasgupta PK. Relative source contributions for perchlorate exposures in a lactating human cohort. *Sci Total Envir* 2013;**443**:939–943.

37 Yiping H, Caiyun W. Ion chromatography for rapid and sensitive determination of fluoride in milk after headspace single-drop microextraction with in situ generation of volatile hydrogen fluoride. *Anal Chim Acta* 2010;**661**:161–166.

38 Rahimi-Yazdi S, Ferrer MA, Corredig M. Nonsuppressed ion chromatographic determination of total calcium in milk. *J Dairy Sci* 2010;**93**:1788–1793.

39 Brescia MA, Monfreda M, Buccolieri A, Carrino C. Characterisation of the geographical origin of buffalo milk and mozzarella cheese by means of analytical and spectroscopic determinations. *Food Chem* 2005;**89**:139–147.

40 Iammarino M, di Taranto A, Cristino M. Endogenous levels of nitrites and nitrates in wide consumption foodstuffs: results of five years of official controls and monitoring. *Food Chem* 2013;**140**:763–771.

41 Yoshikawa K, Okamura M, Inokuchi M, Sakuragawa A. Ion chromatographic determination of organic acids in food samples using a permanent coating graphite carbon column. *Talanta* 2007;**72**:305–309.

42 Sanchez CA, Barraj LM, Blount BC *et al*. Perchlorate exposure from food crops produced in the lower Colorado River region. *J Expo Sci Environ Epidemiol* 2009;**19**:359–368.

43 Wang Z, Forsyth D, Lau BPY, Pelletier L, Bronson R, Gaertner D. Estimated dietary exposure of Canadians to perchlorate through the consumption of fruits and vegetables available in Ottawa markets. *J Agric Food Chem* 2009;**57**:9250–9255.

44 Smith PN, Utley SJ, Cox SB, Anderson TA, McMurry ST. Monitoring perchlorate exposure and thyroid hormone status among raccoons inhabiting a perchlorate-contaminated site. *Environ Monit Assess* 2005;**102**:337–347.

45 Trinetta V, Vaidya N, Linton R, Morgan M. Evaluation of chlorine dioxide gas residues on selected food produce. *J Food Sci* 2011;**76**:T11–T15.

46 Iammarino M, di Taranto A. Nitrite and nitrate in fresh meats: a contribution to the estimation of admissible maximum limits to introduce in Directive 95/2/EC. *Int J Food Sci Technol* 2012;**47**:1852–1858.

47 Yu BS, Chen P, Nie LH, Yao SZ. Simultaneous determination of nitrate and nitrite in saliva and foodstuffs by non-suppressed ion chromatography with bulk acoustic wave detector. *Anal Sci* 2001;**17**:495–498.

48 Iammarino M, di Taranto A, Cristino M. Endogenous levels of nitrites and nitrates in wide consumption foodstuffs: results of five years of official controls and monitoring. *Food Chem* 2013;**140**:763–771.

49 Armenteros M, Aristoy MC, Toldrá F. Evolution of nitrate and nitrite during the processing of dry-cured ham with partial replacement of NaCl by other chloride salts. *Meat Sci* 2012;**91**:378–381.

50 Saccani G, Tanzi E, Cavalli S, Rohrer J. Determination of nitrite, nitrate, and glucose-6-phosphate in muscle tissues and cured meat by IC/MS. *J AOAC Int* 2006;**89**:712–719.

51 Stalikas CD, Konidari CN, Nanos CG. Ion chromatographic method for the simultaneous determination of nitrite and nitrate by post-column indirect fluorescence detection. *J Chromatogr A* 2003;**1002**:237–241.

52 Iammarino M, di Taranto A, Muscarella M, Nardiello D, Palermo C, Centonze D. Development of a new analytical method for the determination of sulfites in fresh meats and shrimps by ion-exchange chromatography with conductivity detection. *Anal Chim Acta* 2010;**672**:61–65.

53 Iammarino M, di Taranto A, Muscarella M. Investigation on the presence of sulphites in fresh meat preparations: estimation of an allowable maximum limit. *Meat Sci* 2012;**90**:304–308.

54 Conklin SD, Shockey N, Kubachka K, Howard KD, Carson MC. Development of an ion chromatography-inductively coupled plasma-mass spectrometry method to determine inorganic arsenic in liver from chickens treated with roxarsone. *J Agric Food Chem* 2012;**60**:9394–9404.

55 Kohlmeyer U, Jakubik S, Kuballa J, Jantzen E. Determination of arsenic species in fish oil after acid digestion. *Microchim Acta* 2005;**151**:249–255.

56 Leufroy A., Noël L, Dufailly V, Beauchemin D, Guérin T. Determination of seven arsenic species in seafood by ion exchange chromatography coupled to inductively coupled

plasma-mass spectrometry following microwave assisted extraction: method validation and occurrence data. *Talanta* 2011;**83**:770–779.

57 Reyes LH, Mar JLG, Rahman GMM, Seybert B, Fahrenholz T, Kingston HMS. Simultaneous determination of arsenic and selenium species in fish tissues using microwave-assisted enzymatic extraction and ion chromatography-inductively coupled plasma mass spectrometry. *Talanta* 2009;**78**:983–990.

58 Simon S, Lobos G, Pannier F, De Gregori I, Pinochet H, Potin-Gautier M. Speciation analysis of organoarsenical compounds in biological matrices by coupling ion chromatography to atomic fluorescence spectrometry with on-line photooxidation and hydride generation. *Anal Chim Acta* 2004;**521**: 99–108.

59 Gómez-Ordóñez E, Alonso E, Rupérez P. A simple ion chromatography method for inorganic anion analysis in edible seaweeds. *Talanta* 2010;**82**:1313–1317.

60 Kruse R, Bartelt E. Phosphates in fishery products: quantified analysis of polyphosphates in fishery products by thermodifferential photometry. *Fleischwirtschaft* 2009;**89**:144–148.

61 Nguyen MV, Jonsson JO, Thorkelsson G, Arason S, Gudmundsdottir A, Thorarinsdottir KA. Quantitative and qualitative changes in added phosphates in cod (Gadus morhua) during salting, storage and rehydration. *LWT Food Sci Technol* 2012;**47**:126–132.

62 Zhong Z, Li G. Determination of phosphate, pyrophosphate, metaphosphate and total phosphorus in seafoods by ion chromatography, *Chin J Chromatogr* 2009;**27**:499–504.

63 Kawasaki Y, Kubota H, Yomota C, Tanamoto K. Determination of sodium chlorite in processed herring roe by ion chromatography with a conductivity detector. *J Food Hyg Soc Japan* 2005;**46**:161–164.

64 Blount BC, Valentín-Blasini L, Ashley DL. Assessing human exposure to perchlorate using biomonitoring. *J ASTM Int* 2006;3(7).

65 Niemann RA, Krynitsky AJ, Nortrup DA. Ion chromatographic determination of perchlorate in foods by on-line enrichment and suppressed conductivity detection. *J Agric Food Chem* 2006;**54**:1137–1143.

66 Sanchez CA, Crump KS, Krieger RI, Khandaker NR, Gibbs JP. Perchlorate and nitrate in leafy vegetables of North America. *Environ Sci Technol* 2005;**39**:9391–9397.

67 Butt SB, Riaz M, Iqbal MZ. Simultaneous determination of nitrite and nitrate by normal phase ion-pair liquid chromatography. *Talanta* 2001;**55**:789–797.

68 Chung SY, Kim JS, Kim M *et al*. Survey of nitrate and nitrite contents of vegetables grown in Korea. *Food Addit Contam A* 2003;**20**:621–628.

69 Ji FD, Ji BP, Li B. Column coated with cetyltrimethylammonium for simultaneous determination of nitrate and nitrite in pickles by ion chromatography. *J Food Sci Technol* 2007;**44**:491–494.

70 Schmidt AC, Kutschera K, Mattusch J, Otto M. Analysis of accumulation, extractability, and metabolization of five different phenylarsenic compounds in plants by ion chromatography with mass spectrometric detection and by atomic emission spectroscopy. *Chemosphere* 2008;**73**:1781–1787.

71 Di Narda F, Toniolo R, Susmel S, Pizzariello A, Bontempelli G. A comparison among different instrumental approaches for bromide analysis in foodstuffs digested by a suitably modified microwave procedure. *Talanta* 2003;**60**:653–662.

72 Huang HC, Lai SF, Liu YC, Chen HYI, Tu WC. Determination of bromide ion residues in broccoli after fumigation with methyl bromide by nonsuppressed ion-chromatography. *J AOAC Int* 2005;**88**:1784–1787.

73 Chan Q, Afton SE, Caruso JA. Investigation of selenium metabolites in Se-enriched kale, Brassica oleracea A, via HPLC-ICPMS and nanoESI-ITMS. *J Anal Atom Spectrom* 2010;**25**:186–192.

74 Martínez-Tomé M, Vera A. Healthy improvement of ionic balance with industrial processing of vegetables. *Nutr Bull* 2004;**29**:26–30.

75 Shimoi T, Ibe A, Tabata S *et al*. The problem and improvement of colorimetric method for determination of sulphite in foods containing sulphur compounds. *J Food Hyg Soc Japan* 2004;**45**:332–338.

76 Vandevijvere S, Temme E, Andjelkovic M *et al*. Estimate of intake of sulfites in the Belgian adult population. *Food Addit Contam A* 2010;**27**:1072–1083.

77 Ohmae Y, Kobayashi NI, Tanoi K *et al*. Mineral composition of frozen taro for determination of geographic origin. *J Radioanal Nucl Chem* 2012;**291**:241–244.

78 Chu YL, Jiang SJ. Speciation analysis of arsenic compounds in edible oil by ion chromatography-inductively coupled plasma mass spectrometry. *J Chromatogr A* 2011;**1218**: 5175–5179.

79 Wojtczak M, Antczak A, Lisik K. Contamination of commercial cane sugars by some organic acids and some inorganic anions. *Food Chem* 2012;**136**:193–198.

80 Milstein LS, Essader A, Murrell C *et al*. Sample preparation, extraction efficiency, and determination of six arsenic species present in food composites. *J Agric Food Chem* 2003;**51**:4180–4184.

81 Fermo P, Beretta G, Maffei Facino R, Gelmini F, Piazzalunga A. Ionic profile of honey as a potential indicator of botanical origin and global environmental pollution. *Environ Pollut* 2013;**178**:173–181.

82 Yu L, Cheng Q, Cañas J, Valentin-Blasini L, Blount BC, Anderson T. Challenges in determining perchlorate in biological tissues and fluids: implications for characterizing perchlorate exposure. *Anal Chim Acta* 2006;**567**:66–72.

83 Kirk AB, Dyke JV, Martin CF, Dasgupta PK. Temporal patterns in perchlorate, thiocyanate, and iodide excretion in human milk. *Environ Health Perspect* 2007;**115**:182–186.

84 Sakuragawa A, Nakane M. Ion chromatographic determination of organic acids in vinegar samples using cetyltrimethylammonium ion coated graphite carbon column. *Bunseki Kagaku* 2004;**53**:851–856.

85 Yoshikawa K, Okamura M, Inokuchi M, Sakuragawa A. Ion chromatographic determination of organic acids in food samples using permanent coating graphite carbon column

and mobile phase with benzoate. *Bunseki Kagaku* 2005;**54**: 1215–1218.

86 McMullen SE, Casanova JA, Gross LK, Schenck FJ. Ion chromatographic determination of nitrate and nitrite in vegetable and fruit baby foods. *J AOAC Int* 2005;**88**: 1793–1796.

87 Casanova JA, Gross LK, McMullen SE, Schenck FJ. Use of griess reagent containing vanadium(III) for post-column derivatization and simultaneous determination of nitrite and nitrate in baby food. *J AOAC Int* 2006;**89**:447–451.

88 Nakashima Y, Suzuki S, Yamazaki M, Inoue Y, Fukatsu Y, Yamamoto A. Determination of nitrite and nitrate in meet products by inline dialysis ion chromatography. *Bunseki Kagaku* 2010;**59**:679–683.

89 Zhong Y, Chen P, Yu X, Xu D, Fang K, Peng J. Determination of nitrite and nitrate in dairy products by improved ion chromatography. *Chin J Chromatogr* 2012;**30**:635–640.

90 Kaufmann A, Maden K, Leisser W, Matera M, Gude T. Analysis of polyphosphates in fish and shrimps tissues by two different ion chromatography methods: implications on false-negative and -positive findings. *Food Addit Contam A* 2005;**22**:1073–1082.

91 Fredrikson M. Pea-protein products for food applications: methods for improving iron and zinc availability. *Doktorsavhandlingar vid Chalmers Tekniska Hogskola* 2001; **17**(43):1–38.

92 Caglar E, Lussi A, Kargul B, Ugur K. Fruit yogurt: any erosive potential regarding teeth? *Quintessence Int* 2006;**37**:647–651.

93 Yeh TS, Liao SF, Kuo CY, Hwang WI. Investigation of the nitrate and nitrite contents in milk and milk powder in Taiwan. *J Food Drug Anal* 2013;**21**:73–79.

94 Perring L, Bourqui B. An alternative method for fluoride determination by ion chromatography using post-column reaction. *Am Lab* 2002;**34**:28–30.

95 Shinoda T, Miyamoto N, Kuromoto T *et al*. Pyrohydrolysis coupled to ion chromatography for sensitive determination of iodine in food-related materials. *Anal Lett* 2012;**45**:862–871.

96 Ding Y, Mou S. Determination of choline chloride and trimethylamine in feedstuff by ion chromatography. *Chin J Chromatogr* 2004;**22**:174–176.

97 Malik J, Frankova A, Drabek O, Szakova J, Ash C, Kokoska L. Aluminium and other elements in selected herbal tea plant species and their infusions. *Food Chem* 2013;**139**: 728–734.

CHAPTER 17
Neutron activation analysis of solid foods

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Abstract: The detailed methodologies of neutron activation analysis specifically for elemental determination of solid foods are presented. A brief overview of essential, non-essential and potentially toxic elements in the human body is also given. The basics of neutron activation analysis, including neutron–nuclei reactions, calculation of concentrations, detection systems and procedures, are presented. A detailed description of specific reactions and possible nuclear and spectral interferences for neutron activation analysis of food is also evaluated. Quality control measures are also deliberated.

Keywords: neutron activation analysis, multi-element determination, quality control

17.1 Introduction

The study of the elemental composition of food has been of great importance for many decades. The three primary reasons are (i) to identify any potential contamination of the food from elements such as arsenic, cadmium, lead and mercury; (ii) evaluate the essential major elements such as calcium, chlorine, magnesium, potassium and sodium; and (iii) assess the importance of trace and ultratrace essential elements in food consumption. A synopsis of the categories of these elements found in the human body is shown in Table 17.1 [1].

A perusal of Table 17.1 shows that ultratrace essential and non-essential elements, typically present at levels less than 1 μg/g, can also be toxic at higher concentrations. Some examples of elements that overlap these categories include antimony, arsenic, chromium, copper and selenium. In recent years, other trace elements notably rare earths (known as lanthanides) have been studied for their application in agricultural fertilizers [2–5] and their potential environmental contamination of soil and food. Kastori *et al*. [6] reviewed the effects of yttrium and rare-earth elements on higher plants, while the consequences on germination and growth of selected crop and native plant species of lanthanum, cerium and yttrium have also been studied [7]. One of the first comprehensive assessments of trace elements in the human body was undertaken by Underwood [8] in 1956. Now, many country organizations list daily intakes of trace elements required to maintain the body's needs. Therefore, knowledge of the elemental content of foods is of prime importance when making recommendations for the consumption of various foods.

There are many analytical techniques capable of determining an array of major, minor, trace and ultratrace elements in food. These methodologies include the variations of atomic absorption spectrometry (AA), atomic emission spectrometry, electronanalytical methods, inductively coupled plasma optical emission spectroscopy (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS) and atomic and nuclear methods such as X-ray fluorescence (XRF), particle induced X-ray emission (PIXE) and neutron activation analysis (NAA). Of the above-mentioned techniques ICP-OES, ICP-MS, XRF, PIXE and NAA are the ones best known for their multi-element determination capabilities for solid and

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Table 17.1 Overview of essential, non-essential and potentially toxic elements in the human body.

Essential elements (major)
Calcium
Carbon
Chlorine
Hydrogen
Magnesium
Nitrogen
Oxygen
Phosphorus
Potassium
Sodium
Sulfur
Essential elements (trace)
Fluorine
Iodine
Iron
Silicon
Zinc
Essential elements (ultratrace)
Chromium
Cobalt
Copper
Manganese
Molybdenum
Nickel
Selenium
Vanadium
Non-essential trace elements
Stimulatory
Boron
Titanium
Unknown role
Antimony
Arsenic
No role
Barium
Cesium
Germanium
Rubidium
Strontium
Potentially toxic trace elements
Aluminium
Arsenic
Antimony
Cadmium
Chromium
Copper
Lead
Mercury
Thorium
Tin
Uranium

liquid samples. NAA remains a classic methodology for non-destructively determining a large number of elements with low detection limits. In particular, NAA can be used for sample sizes from a few milligrams to several grams of material, which is not the case for any other method except XRF. The focus of this chapter is to elucidate the strengths and weaknesses of NAA and its applications in solid food samples. An in-depth theory of NAA technique, details of gamma-ray spectrometry and comparison of detection limits with other methods is beyond the range of this review. Speciation of the elements in the food once consumed is also not discussed, while radioactivity in food is outside the scope of this chapter.

17.2 Current investigation of trace elements in food by neutron activation analysis

Below is a sampling of the many articles that have been recently published using NAA in a variety of foods. This is by no means an exhaustive literature search, but rather one to give the reader an appreciation of the applications of NAA in various international studies. The trace elements bromine, calcium, cesium, potassium, sodium, rubidium and zinc were determined by NAA in bovine milk from two Brazilian dairy regions to determine their presence for nutritional and possible toxicological relevance [9]. Biomonitoring using mussels transplanted in the coast of the state of Sao Paulo, Brazil was done to evaluate discharge releases of vanadium by expulsions from industries as well as shipping vessels and boats [10]. Selenium is an essential micronutrient for humans and is deficient in at least 1 billion people worldwide. A study to determine selenium in bread-wheat samples grown under a supplementation regime in actual field conditions was undertaken [11]. A series of 15 elements in white rice was determined in China, Indonesia, Japan, Korea, Malaysia, Philippines and Thailand to ascertain the presence of arsenic, heavy metals and essential trace elements [12]. Exposure to arsenic, even at low levels, can have deleterious effects on the human body. Determination of arsenic in food and dietary supplement standard reference materials by NAA has been done for two certified reference materials [13]. Estimation of thorium intake due to consumption of vegetables by inhabitants of high background radiation areas in India has been researched using NAA [14].

17.3 Basics of neutron activation analysis

Instrumental NAA is a well-accepted technique capable of determining 30–40 elements including many environmentally crucial ones (e.g. aluminium, vanadium, zinc, copper, arsenic, antimony, chromium, selenium, iron, nickel). NAA is also capable of determining major elements such as sodium, chlorine and potassium, as well as many rare earth elements. The determination of element concentrations is based on the measurement of induced radioactivity. The radioactive decay of each induced isotope emits a characteristic gamma-ray energy spectrum. Hence, an individual nuclide 'fingerprint' can be measured and quantified. NAA measures the total amount of an element in a material without regard to chemical or physical form. It has the following advantages:

1. samples for NAA can be liquids, solids or powders;
2. NAA is non-destructive and since no pre-chemistry is required, reagent-introduced contaminants are completely avoided;
3. NAA is a multi-element analytical technique in which many elements can be readily analysed simultaneously;
4. NAA is sensitive to trace elements.

These factors have pushed the detection limits of most elements of interest to very low levels, not readily achievable by other analytical techniques. NAA is also totally unaffected by the presence of organic material in the sample, a significant problem in many types of conventional chemical methods. NAA is particularly well suited for analysing biological, environmental and geological samples. The relative ease and precision (detection limits typically range from nanogram per gram to microgram per gram) also make NAA a useful method for determining metal content in a variety matrices. NAA measures a gamma spectrum produced by an irradiated sample and compares it with the spectra of standard reference materials to yield multi-element concentration information.

17.3.1 Neutron–nuclei reactions

A variety of reactions may occur when a neutron collides with a nucleus. The reaction cross-sections are a function of neutron energy and vary between target nuclei. The most common reactions are elastic scatter (n,n), inelastic scatter (n,n'), radiative capture (n,γ), charged particle (n,p) or (n,α), neutron multiplying (n,2n), and fission (n,f). Radiative capture reactions are most often used by NAA. The target nucleus ^{A}Z absorbs a neutron n and forms a compound nucleus ^{A+1}Z in an excited state. A prompt gamma ray (γ) is emitted when the compound nucleus de-excites as shown in equation 17.1:

$$n + {}^{A}Z \rightarrow {}^{A+1}Z^{*} \rightarrow {}^{A+1}Z + \gamma \qquad (17.1)$$

To utilize the prompt gamma-ray a monoenergetic neutron source is required, a feature available with several research reactor facilities. NAA measures the decay energy of one or more gamma-rays from radioactive product nuclei ^{A+1}Z. These radioactive decay photons emitted at specific energies for each radionuclide are detected with a high-resolution gamma-ray detector and stored as counts in a multichannel analyser (MCA) to produce a gamma-ray spectrum. The peak energies in the spectrum identify the radioisotopes in ^{A+1}Z that arise from the target nuclei ^{A}Z. The intensities of the peaks are used to determine the concentrations of the target nuclei. If a product nucleus is stable, NAA cannot be used to determine the concentration of the target element. A schematic of the neutron interaction and subsequent radioactive products is shown in Figure 17.1.

Different stable target elements can undergo different reactions to produce the same product nucleus ^{A+1}Z. For example, ^{28}Al can be produced by the following three reactions:

$$n + {}^{27}Al \rightarrow {}^{28}Al + \gamma \qquad (17.2)$$

$$n + {}^{28}Si \rightarrow {}^{28}Al + p \qquad (17.3)$$

$$n + {}^{31}P \rightarrow {}^{28}Al + \alpha \qquad (17.4)$$

Reaction 17.2 is the expected reaction while 17.3 and 17.4 are called interference reactions. Interference reactions increase the intensity of a gamma-ray peak. The concentration of the desired target atom can still be determined by subtracting the contribution of the interference reactions from that of the isotope of interest.

17.3.2 Reaction rates

The rate of neutron interactions R_i of type i is obtained by integrating the product of the interaction cross-section $\sigma_i(E)$, the neutron flux $\varphi(E)$ and the number of target nuclei N over the energy range of the incident neutrons:

$$R = N \int_{0}^{\infty} \sigma_i(E)\varphi(E)\,dE \qquad (17.5)$$

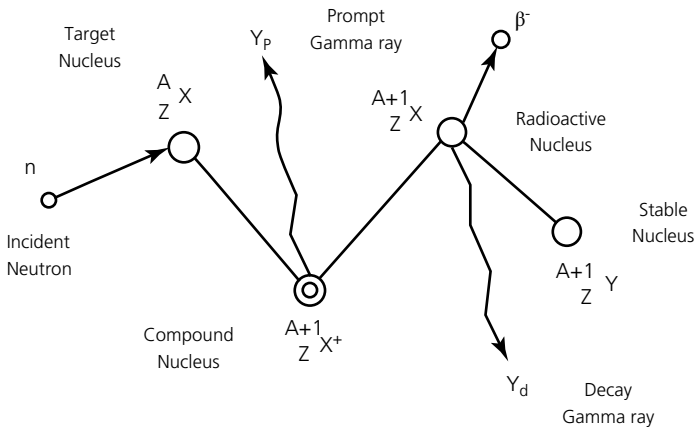


Figure 17.1 Diagram illustrating the sequence of events for a typical (n,γ) reaction.

Concentrating on only radiative capture reactions, the reaction rate is generally broken into three terms that depend on the energy ranges (thermal, epithermal and fast) of the incident neutrons:

$$R = R_{th} + R_{epi} + R_{fast} \tag{17.6}$$

Many nuclides, especially those with low atomic number, have neutron capture cross-sections that are proportional to $1/v$ at energies below 0.5 eV, where v is the speed of the neutron. Generally, the thermal cross-section σ_{th} corresponding to the thermal neutron energy (0.025 eV) is used to determine the thermal reaction rate R_{th} due to the thermal flux φ_{th}:

$$R_{th} = N\int_{th}\sigma(E)\varphi(E)dE = N\sigma_{th}\varphi_{th} \tag{17.7}$$

Resonance peaks dominate the neutron capture cross-sections at energies in the epithermal range between 0.5 eV and 0.5 MeV. The epithermal neutron flux φ_{epi} in a water-moderated reactor like the TRIGA follows a $1/E$ distribution. Since the epithermal capture cross-section φ_{epi} varies rapidly, the radiative capture integral I_0 is used to refer to the cross-section. The epithermal reaction rate R_{epi} can be calculated:

$$\begin{aligned} R_{epi} &= N\int_{epi}\sigma(E)\phi(E)dE \\ &= N\int_{epi}\sigma(E)\Phi_{epi}(E)dE/E \\ &= N\Phi_{epi}\int_{epi}\sigma(E)dE/E \\ &= N\Phi_{epi}I_0 \end{aligned} \tag{17.8}$$

where the epithermal flux Φ_{epi} is in units n/(s-cm^2-ln(E)). The fast reaction rate R_{fast} is negligible because the capture cross-section at high energies is very small. The radiative capture reaction rate can then be written as:

$$R = N\sigma_{th}\Phi_{th} + NI_0\Phi_{epi} \tag{17.9}$$

In reactor-based thermal irradiations, both terms in equation 17.9 are important. The thermal reaction rate term is eliminated for epithermal irradiations. By employing a cadmium filter, neutrons below energy 0.4 eV are removed from the flux. Irradiations executed in this manner are called epithermal, although epi-cadmium is more correct. In the epithermal region, neutron cross-sections do not strictly follow the $1/v$ rule. Some elements possess large resonance peaks superimposed on the $1/v$ continuum in the epithermal range. Such an element is said to have a large resonance integral I_0. Elements with large resonance integral to thermal neutron cross-section ratios, I_0/σ_{th}, are very sensitive to epithermal NAA.

17.3.3 Calculation of concentrations by neutron activation analysis

The expressions for reaction rates developed in the preceding section can be used to calculate the concentration of an element in an unknown sample from knowledge of the concentration of the element in a reference standard. The reaction rate for a specific target can be written:

$$R = N\sigma_{th}\Phi_{th} + NI_0\Phi_{epi} = N\sigma_{act}\Phi \tag{17.10}$$

where $\sigma_{act}\Phi$ is the product of the activation cross-section and the neutron flux integrated over the appropriate energy range. The rate of change in the number of radioactive nuclei N^* is given by equation 17.11, where λ is the radioactive decay constant.

$$\frac{dN^*}{dt} = \sigma_{act}\Phi N - \lambda N^* \qquad (17.11)$$

Integrating equation 17.11 to obtain $N^* = N^*(t)$ yields:

$$N^*(t) = \frac{\sigma_{act}\Phi N}{\lambda}(1 - e^{-\lambda t_i}) \qquad (17.12)$$

In practical applications a sample is irradiated for a time t_i allowed to cool during a decay time t_d and counted for a time t_c. These three time variables are chosen to optimize the analytical sensitivity of the radionuclides of interest. The number of radioactive nuclei that decay during a counting time t_c is given by:

$$N^* = \frac{\sigma_{act}\Phi N}{\lambda}(1 - e^{-\lambda t_i})e^{-\lambda t_d}(1 - e^{-\lambda t_c}) \qquad (17.13)$$

The count measured depends on the intensity of the emitted gamma-rays at a given energy I_γ and the efficiency of the detector for this gamma-ray ε_γ. Combining these factors with the previous expression yields the following result for counts measured:

$$N^* = \frac{\sigma_{act}\Phi N}{\lambda}(1 - e^{-\lambda t_i})e^{-\lambda t_d}(1 - e^{-\lambda t_c})I_\gamma \varepsilon_\gamma \qquad (17.14)$$

By using the comparative methods, standards and samples are introduced together and are both counted using identical geometric configurations with the same detector. Then the ratio of unknown (sample) counts to known (standard) counts measured is:

$$\frac{N_u^*}{N_k^*} = \frac{N_k}{N_u}\frac{e^{-\lambda t_{dk}}}{e^{-\lambda t_{du}}}\frac{(1 - e^{-\lambda t_{ck}})}{(1 - e^{-\lambda t_{cu}})} \qquad (17.15)$$

The number of target nuclei N is a product of concentration C and mass M. If samples and standards are counted for the same length of time, equation 17.15 can then be rewritten solving for the concentration of the unknown sample:

$$C_u = C_k \frac{M_k}{M_u}\frac{N_u^*}{N_k^*}\frac{e^{-\lambda t_{dk}}}{e^{-\lambda t_{du}}} \qquad (17.16)$$

where u and k denote unknown sample and known standard respectively. If the sample and standards are irradiated at different times, then flux monitors are employed to account for any variations in neutron fluence.

17.3.4 Detection system

Gamma-ray spectra of the radioactive samples are collected with high purity germanium (HPGe) detectors. Photons entering the HPGe detector can undergo three different interactions: photoelectric effect, Compton effect, or pair production. Low-energy gamma-rays most often interact by the photoelectric effect. The incident photon transfers all its energy to an inner orbital electron in the detector. The ejected electron and the X-rays or auger electrons produced as outer electrons fill the hole it left have short ranges, usually depositing all their energy in the HPGe detector. If all the energy is deposited in the detector, a photopeak of the initial gamma-ray is produced.

The Compton effect is an inelastic scattering interaction between the incident photon and an orbital electron in which the initial photon energy is split between the scattered photon and recoil electron. The partitioning of energy depends on the angle θ at which the photon is scattered. All scattering angles are possible. The wavelength of the scattered photon λ_s in terms of the wavelength of the incident photon λ_i and angle between their paths is:

$$\lambda_s = \lambda_i + \frac{hc}{m_e c^2}(1 - \cos\theta) \qquad (17.17)$$

where h is Planck's constant, c is the speed of light, and m_e is the rest mass of an electron. The recoil electron has a short range and generally deposits all its energy in the HPGe detector. The scattered gamma-ray may interact via the photoelectric effect, a second Compton event, or escape from the detector. A photoelectric interaction produces a full energy photopeak. A second Compton scatter produces a repeat of these three possibilities. Escape by the scattered photon produces the elevated background of the Compton continuum. Compton scattering produces a significant background making low levels of elements with gamma-ray energies between 150 keV and 2 MeV difficult to quantify precisely. Pair production is an interaction between a high-energy photon and the field of the target nucleus. When the photon energy is greater than $2m_e c^2 = 1.022$ MeV, the photon can disappear with the creation of an electron–positron pair.

Any energy above 1.022 MeV is transferred to the kinetic energy of the pair. The electron–positron pair often stops in the HPGe detector, but one or both of the 0.511-MeV photons released by the annihilation of the positron with an electron often escape the detector. Single or double escape peaks, 0.511 and 1.022 MeV below the photopeak, are frequently present in the spectrum, sometimes interfering with the photopeaks created by lower energy photons. Pair production can also occur between orbital electrons and a photon of energy greater than $4m_ec^2 = 2.044$ MeV, but the interaction cross-section is much smaller than with the nucleus.

A block diagram of a typical activation analysis installation is shown in Figure 17.2. The gamma-rays emitted in the sample are detected by a solid-state Ge detector. Ideally, each gamma-ray deposits its full energy proportional to the energy deposited. These pulses are amplified and sorted on a multichannel analyser. Because of Compton scattering in which only part of the full gamma-energy is deposited in the detector, a broad continuum is produced below the full gamma peak. For gamma energies over 1.022 MeV, single and double escape peaks may also be present at energies 0.511 and 1.022 MeV below the full gamma energy. These peaks occur because a gamma-ray with energy greater than 1.022 MeV may produce a positron–electron pair. The positron annihilates essentially instantaneously, and one or both of the 0.511-MeV annihilation photons may escape from the detector, so that the detector 'sees' the full energy of the peak minus 0.511 (SE) or 1.022 MeV (DE). Backscatter peaks may also be present in a spectrum.

A typical spectrum is shown in Figure 17.3 for cobalt from the reaction $^{59}Co(n,\gamma)^{60}Co$. ^{60}Co ($t_{½} = 5.27$ years) has strong peaks at 1.173 and 1.332 MeV as well as a strong Compton continuum. The spectra of actual samples may be very complex consisting of many gamma peaks with the possibility that several may overlap. A complete assessment of gamma-ray spectroscopy has been published by Gilmore [15].

17.4 Procedures in neutron activation analysis

Unlike almost all the other analytical methods for elemental determination, NAA is unique in that there are many parameters that can be adjusted which ultimately affect detection limits and precision. There are currently 245 research reactors worldwide of which 160 are in developed countries and 86 in developing countries [16]. There are another 11 under construction or being planned. However, the majority of research reactors that perform NAA have power levels between 20 kW and 10 MW. NAA analysts can adjust the power of the research reactor up to the maximum of their facility. Other parameters such as irradiation, decay and counting times as well as detector sizes and resolution and choice of neutron energy (thermal or epithermal) can all optimize the procedures employed. In-house built fast pneumatic facilities can also be judiciously used to determine very short-lived isotopes belonging to fluorine, selenium and silver. Commercially bought software such as the popular GENIE 2000 [17] or the many programs developed in-house to deconvolute the gamma-ray spectra with overlapping peaks or for correctly measuring small photopeaks are very important tools for achieving high accuracy. These aspects are not covered in this chapter and the reader should search out the latest software developments. The usual main problems in NAA in analysing environmental, geological and

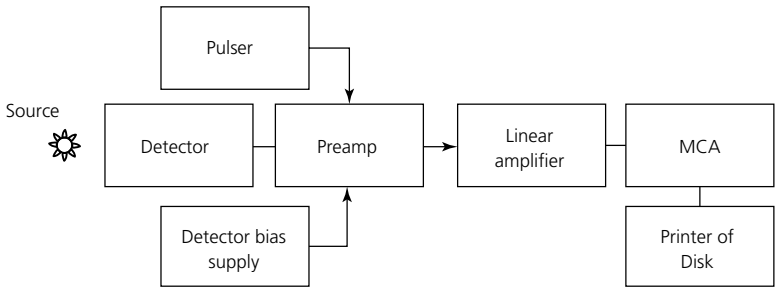


Figure 17.2 Block diagram of neutron activation analysis set-up.

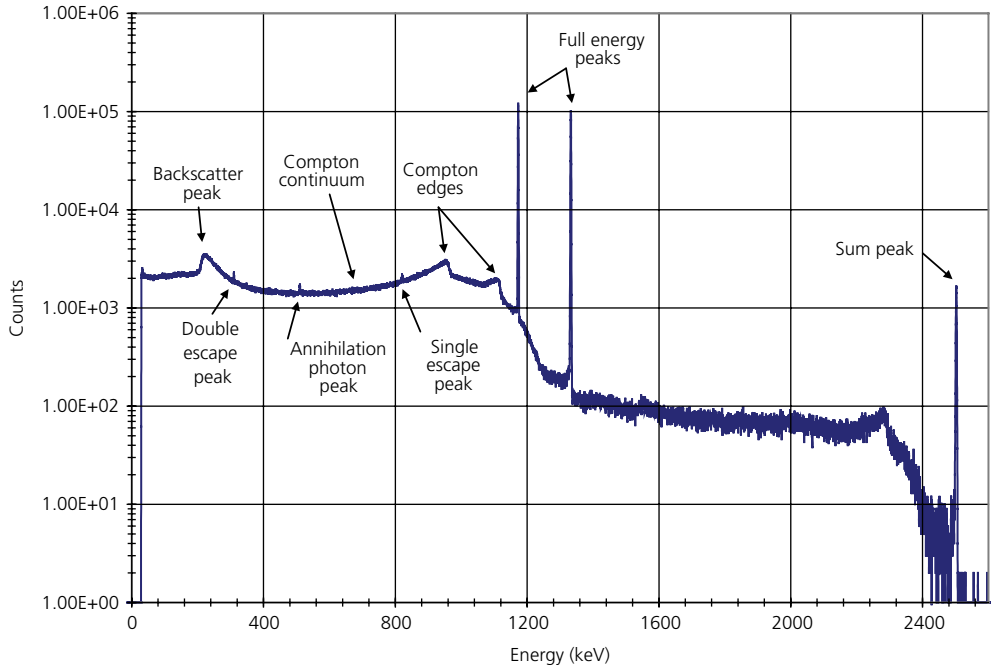


Figure 17.3 Decay spectrum for ^{60}Co.

biological specimens are the product isotopes belonging to ^{23}Na(n,γ)^{24}Na, ^{37}Cl(n,γ)^{38}Cl, ^{81}Br(n,γ)^{82}Br, ^{27}Al(n,γ)^{28}Al and ^{55}Mn(n,γ)^{56}Mn. These elements, especially sodium, chlorine and bromine, can be in large concentrations in these specimens and this, coupled with their relatively large neutron cross-sections, give rise to high backgrounds due to Compton scattering. While aluminium is usually a small or negligible component in food, the presence of phosphorus and the ^{31}P(n,α)^{28}Al reaction can give equally high backgrounds for short-lived NAA. Thus the short-lived NAA (^{24}Na, ^{38}Cl, ^{27}Al) and the medium-lived NAA (^{24}Na and ^{82}Br) are the main isotopes that give rise to high backgrounds. Typically long-lived NAA products for biological specimens do not give high backgrounds. However, in certain samples such as fish the presence of phosphorus gives rise to the ^{31}P(n,β)^{32}P reaction with a high bremsstrahlung background up to 400 keV. However, in the recent years the use of epithermal NAA with and without Compton suppression has greatly improved the procedures to determine elements whose radionuclide products sit on high backgrounds with poor statistics. These methods have been employed for several studies in elemental analysis of food samples with excellent results. A typical Compton suppression system is depicted in Figure 17.4.

To demonstrate the usefulness of a Compton suppression system, a comparison of four spectra for a coal sample irradiated for short-lived NAA is shown in Figure 17.5. These spectra are for a thermal normal, thermal Compton, epithermal and epithermal Compton irradiation and counting modes. As can be seen, the lowest detection background can be seen in the epithermal Compton irradiation and counting mode. However, it should be noted that epithermal NAA is best for elements that have high resonance integral cross-sections and Compton suppression is ideal for radionuclides that decay by only one or by a major gamma-ray [18]. A complete evaluation of thermal and epithermal NAA and Compton suppression is described in two papers investigating trace elements in a variety of foods and fruits [19, 20]. Tables 17.2, 17.3 and 17.4 list detailed but not exhaustive data for short-, medium- and long-lived radionuclides commonly used in elemental analyses of food. For completeness, rare-earth elements are also included.



Figure 17.4 Typical Compton suppression system with a cutaway showing large Na(I) detector surrounding the hyperpure germanium detector.

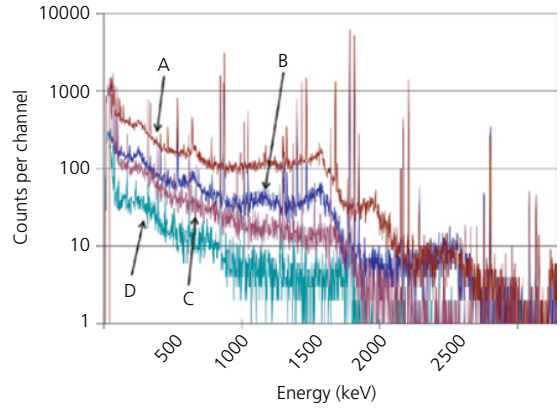


Figure 17.5 Comparison of four spectra for a coal sample irradiated for short-lived NAA for four different irradiation and counting modes. A, thermal normal; B, thermal Compton; C, epithermal normal; D, epithermal Compton. (See insert for colour representation of the figure.)

For short-lived NAA the procedures for irradiation and counting times do not vary significantly. Depending on the neutron flux, neutron energy (thermal or epithermal) and weight of samples, irradiation times can vary from 10–30 s to 1–5 min. Decay times usually vary from 5 to 15 min depending on the deadtimes of the radioactive samples and the half-lives of interest. In special cases, like the determination of selenium using the ^{77m}Se radionuclide and its 17.4-s half-life, automated pneumatic facilities are required.

For medium-lived NAA the procedures can be followed in one of two ways. The first approach is to irradiate the sample for 1–5 hours and then use a decay time of 2–3 days to count the medium-lived radionuclides. This is then followed by a decay time of 2–3 weeks for the long-lived radionuclides. However, a better approach is to irradiate the sample for 30–60 min in order to decrease the contribution of the ^{23}Na(n,γ)^{24}Na and ^{81}Br(n,γ)^{82}Br reactions, which dominate the spectrum making it difficult to determine other elements such as arsenic and antimony through the ^{75}As(n,γ)^{76}As and ^{121}Sb(n,γ)^{122}Sb reactions. Even potassium is difficult to determine through the ^{41}K(n,γ)^{42}K reaction with its 12.4-hour half-life. The disadvantage of this procedure is that an additional irradiation is needed for long-lived NAA.

In general, for long-lived NAA several hours of irradiation and counting times are needed to achieve the required statistics. Since no high-activity radionuclides are usually present, the background continuum is much lower than in the measurement of medium-lived radionuclides following long activation times. For the measurement of long-lived radionuclides, shielding of the detector with cadmium- and copper-lined lead brick is a highly advantageous means to reduce the effect of background radiation from the counting room that can otherwise deteriorate the detection limits.

In general, there is little nuclear or spectral interference in the analysis of foods, as with geological samples, and those few can easily be taken into account. In fact, the vast

Table 17.2 Properties of reactions producing short-lived radionuclides.

Element	Isotope	Half-life	Gamma-ray energies (keV)
Ag	^{110}Ag	24.6 s	657.8
Al	^{28}Al	2.24 min	1778.9
Ba	^{139}Ba	83.2 min	165.9
Br	^{80}Br	17.7 min	616.2
Br	^{80m}Br	4.42 h	37.1
Ca	^{49}Ca	8.7 min	3084.4
Cl	^{38}Cl	37.3 min	1642.4, 2167.5
Co	^{60m}Co	10.48 min	58.6
Cu	^{66}Cu	5.1 min	1039.4
Dy	^{165}Dy	2.33 h	94.7
F	^{20}F	11.0 s	1633.8
I	^{128}I	25.0 min	442.3
K	^{42}K	12.36 h	1524.7
Mg	^{27}Mg	9.45 min	843.8, 1014.4
Mn	^{56}Mn	2.58 h	846.7, 1810.7
Na	^{24}Na	15.0 h	1368.6, 2754.1
P	^{28}Al*	2.24 min	1778.9
Sb	^{122m}Sb	4.15 min	61.5
Se	^{77m}Se	17.4 s	161.7
Si	^{29}Al†	6.6 min	1273.0
Sn	^{125}Sn	9.2 min	331.9
Sr	^{87m}Sr	2.81 h	368.4
Ti	^{51}Ti	5.8 min	320.1
U	^{239}U	23.5 min	74.6
V	^{52}V	3.76 min	1434.1

*P is determined by the ^{31}P(n,α)^{28}Al reaction.
†Si is detected by the ^{29}Si(n,p)^{29}Al reaction.

majority of the photopeaks can be evaluated manually by taking backgrounds on both sides of the peak. Simple computer programs can be written to do this procedure automatically and then used in the analysis.

A list of the salient features of the elements that can be determined, including their spectral and nuclear interferences, is presented in the following sections.

17.4.1 Short-lived activation products

Ag

A fast pneumatic facility is typically required to detect ^{110}Ag, which has a 24.3 s half-life. Typical irradiation times are between 10 and 20 s. Sensitivities are significantly enhanced using epithermal neutrons. Because of the rapidly decaying short-lived products, good control of the varying deadtime is needed (e.g. pulser, deadtime stabilizer or loss-free counter).

Table 17.3 Properties of reactions producing medium-lived radionuclides.

Element	Isotope	Half-life	Gamma-ray energies (keV)
As	^{76}As	26.3 h	559.1
Br	^{82}Br	35.3 h	554.3, 776.5
Cd	^{115m}In*	53.5 h	336.3
Hg	^{197}Hg	64.1 h	77.4
Ho	^{166}Ho	26.8 h	80.6
K	^{42}K	12.36 h	1524.7
La	^{140}La	40.23 h	1596.2, 328.8, 487.0
Mo	^{99}Mo	66.02 h	140.5
Na	^{24}Na	15.02 h	1368.6, 2754.1
Sb	^{122}Sb	2.72 days	564.0
Sm	^{153}Sm	46.7 h	103.2
U	^{239}Np†	2.35 days	277.7
Zn	^{69m}Zn	13.8 h	438.6

*Cd is determined from the ^{114}Cd(n,$\gamma\beta$)^{115}Cd$\rightarrow$^{115}In reaction.
†U is determined from the ^{238}U(n,$\gamma\beta$)^{239}U$\rightarrow$^{239}Np reaction.

Table 17.4 Properties of reactions producing long-lived radionuclides.

Element	Isotope	Half-life	Gamma-ray energies (keV)
Ag	^{110m}Ag	249.8 days	657.8
Ce	^{141}Ce	32.5 days	145.4
Cr	^{51}Cr	27.72 days	320.0
Co	^{60}Co	5.27 years	1173.2, 1332.4
Eu	^{132}Eu	13.4 years	1408.0
Fe	^{59}Fe	44.5 days	1099.2, 1291.6
Hg	^{203}Hg	46.6 days	279.2
Lu	^{177m}Lu	160 days	378.5
Nd	^{147}Nd	10.99 days	91.1
Ni	^{58}Co*	70.9 days	810.8
Rb	^{86}Rb	18.7 days	1076.6
Sb	^{124}Sb	60.2 days	1691.0
Sc	^{46}Sc	83.8 days	889.3, 1120.5
Se	^{72}Se	119.8 days	136.0, 264.7, 400.7
Sn	^{113}Sn	114.4 days	391.7
Sr	^{85}Sr	64.84 days	514.0
Tb	^{160}Tb	72.1 days	879.4
Th	^{233}Pa†	27.0 days	311.9
Tm	^{170}Tm	129 days	84.3
Yb	^{175}Yb	4.19 days	396.3
Zn	^{65}Zn	243.8 days	1115.5

*Ni is determined using the ^{58}Ni(n,p)^{58}Co reaction.
†Th is determined using the ^{232}Th(n,$\gamma\beta$)^{233}Th$\rightarrow$^{233}Pa$\rightarrow$^{233}U reactions.

Al

The detection of aluminium is very sensitive. However, the interfering $^{28}Si(n,p)^{28}Al$ reaction must also be taken into account when using the $^{27}Al(n,\gamma)^{28}Al$ reaction. Typically, there are lower concentrations of silicon in food. However, in food samples the presence of phosphorus can lead to the $^{31}P(n,\alpha)^{28}Al$ reaction which can severely limit the ability to determine aluminium. Only when the reactor has a very small or no epithermal neutron component is detection of aluminium possible without the use of complicated irradiation schemes.

Ba

There are normally no spectral or nuclear problems associated with the determination of ^{139}Ba using the 165.9-keV gamma-ray. Sensitivity may be increased by employing longer decay times and counting times and/or epithermal neutrons.

Br

The analytical accuracy and sensitivity of ^{80}Br using the 616.2-keV photopeak is dependent on several factors. Good spectral resolution is needed from the 620.4-keV gamma-ray arising from the double escape peak belonging to the 1642.4-keV photopeak of ^{38}Cl and the possible 619.0-keV photopeak coming from the medium-lived radionuclide ^{82}Br (see section 17.4.2). The 616.2-keV photopeak is also situated on a high background with possible poor detection limits. There is also an internal transition of ^{80m}Br which has a half-life 4.42 hours and 'feeds' the 616.2-keV photopeak. If identical irradiation and counting times are not applied to the samples and standards, then incorrect calculations may occur. The very sensitive 37.1-keV photopeak of the internal transition may be used but care must be taken for overlapping peals and self- attenuation of the low-energy gamma-ray.

Ca

No interferences exist for ^{49}Ca, although a high-efficiency detector is usually needed for the isotope which is activated from the low abundant (0.187%) ^{48}Ca isotope with its 3084-keV photopeak.

Cl

Normally no interferences exist. The use of the 2167.8-keV photopeak usually results in a better detection limit since it is usually situated on a lower background.

Co

The 58.6-keV photopeak of the metastable ^{60m}Co can be used with no known interferences. Sensitivities are not as good as for ^{60}Co following a long irradiation.

Cu

Analytical sensitivities for ^{66}Cu are only moderately good and very low levels cannot usually be detected due to the high background under the 1039.0-keV photopeak.

Dy

There is a possible interference from a 94.7-keV gamma-ray belonging to ^{233}Th isotope (half-life 23.3 min). Contribution of this gamma-ray should be done by taking its ratio to the 359.9-keV gamma-ray of ^{233}Th. Analytical sensitivities for the detection of ^{165}Dy can be improved by using both epithermal NAA and/or longer decay and counting times.

F

Fluorine is best determined by epithermal NAA. As with silver, a fast pneumatic system delivering the sample to the detector within a few seconds is essential. The detection of ^{20}F is subject to interference from the $^{23}Na(n,\alpha)^{20}F$ reaction. A careful evaluation of the interference factor must be taken into account.

I

Iodine is determined with good results using the ^{128}I isotope. Excellent sensitivities are achieved using epithermal neutrons. Some potential may exist for loss of iodine during irradiation due to its volatility.

K

No spectral or nuclear interferences are associated with the detection of ^{42}K. This isotope can also be detected following a medium- or long-lived irradiation with a decay on only 1 day since it has a half-life of 12.3 hours.

Mg

Sensitivities are usually adequate for the detection of the 843.8-keV photopeak of ^{27}Mg. Rather serious errors can be introduced due either to the competing $^{27}Al(n,p)^{27}Mg$ nuclear interference reaction (for reactors which do not have well-thermalized neutrons) or to the possible strong overlapping peak of 846.7 keV from the $^{55}Mn(n,\gamma)^{56}Mn$ reaction. If good resolution is not achieved between

these two photopeaks, the less sensitive 1014.4-keV photopeak, also belonging to ^{27}Mg, must be used. However, aluminium concentrations are usually very low in food samples and the interference is very small or negligible.

Mn

Manganese can be determined using the 846.7-keV or 1810.7-keV photopeak. As previously stated a good spectral resolution is required to remove the overlapping 843.7-keV gamma-ray of ^{27}Mg. If time permits, one can wait 1–2 hours to let the ^{27}Mg gamma-ray decay with its 9.45-min half-life.

Na

The measurement of sodium is excellently suited for neutron activation measurements. In general, there are no spectral or nuclear interferences. Then both photopeaks of ^{24}Na, 1368.4 keV and 2754.1 keV, can be used.

P

Phosphorus can be determined by the ^{31}P(n,α)^{28}Al reaction if there is an epithermal neutron flux component, but it may have substantial interference with the ^{27}Al(n,γ)^{28}Al reaction. However, aluminium concentrations are usually very low in food samples and the interference is very small or negligible.

Sb

Antimony can be determined using epithermal neutron and the 61.6-keV gamma-ray although this method of analysis is not often used. Care must be taken to account for any overlapping X- or gamma-rays in the vicinity, and possible self-absorption effects.

Se

This element can be detected using the ^{77m}Se metastable isotope. As with silver and fluorine, a fast delivery system is required. If the phosphorus concentration is high in the sample, this may result in a high deadtime in the counting system.

Si

The determination of silicon is very difficult in biological samples using conventional NAA methods. Silicon is best detected by the ^{29}Si(n,p)^{29}Al reaction and the analytical sensitivity for the determination of ^{29}Al is greatly improved by using epithermal neutrons.

Sn

The determination of tin is much better utilizing epithermal neutrons. There are no nuclear or spectral interferences.

Sr

No nuclear or spectral interferences are normally associated with the detection of ^{87m}Sr. The use of epithermal NAA and/or longer decay and counting times may result in improved detection limits.

Ti

No nuclear or spectral interferences are normally associated with the detection of ^{51}Ti.

U

Uranium can be detected using the 74.6-keV peak belonging to ^{239}U. However, excitation of lead bricks (used for shielding) by gamma-rays interacting as a result of the photoelectric effect will produce photopeaks corresponding to the lead K X-rays at 72.8 keV and 74.9 keV. Even the use of cadmium- and copper-lined lead brick may not totally eliminate the problem. Sensitivity is enhanced using epithermal neutrons. Another preferred method of analysis is based on the ^{238}U(n,γβ)^{239}U → ^{239}Np reaction followed by measurement of the 277.1-keV photopeak (see section 17.4.2).

V

The sensitivity of vanadium is excellent with no known spectral or nuclear interferences.

17.4.2 Medium-lived activation products

As

Arsenic is detected with good precision using the 559.1-keV gamma-ray. Spectral resolution of the 554.3-keV and 564.0-keV gamma-rays belonging to ^{82}Br and ^{122}Sb, respectively, is essential. Otherwise, the less sensitive 657.0-keV photopeak, also belonging to ^{76}As, can be used. Increased sensitivity can be achieved using epithermal neutrons.

Br

The detection of bromine is very sensitive using the 554.3-keV photopeak of ^{82}Br. Typically, good spectral resolution is needed to resolve the 554.3-keV, 559.1-keV and 564.0-keV peaks of ^{82}Br, ^{76}As and ^{122}Sb, respectively. Sensitivities are also improved using epithermal

neutrons. Bromine can also be determined using the 776.5-keV gamma-ray.

Cd

Cadmium is best determined using the $^{114}Cd(n,\gamma)$ $^{115}Cd \rightarrow {}^{115m}In$ reaction where the 336.3-keV gamma-ray characteristic of ^{115m}In is used. A decay time of at least 35 hours is required so that equilibrium can be achieved between ^{115}Cd and ^{115m}In; also so that the ^{115m}In (half-life 4.49 hours), which may have come originally from any indium in the sample, will have decayed to negligible amounts. The 336.3-keV gamma-ray is then representative of the 53.5-hour half-life of ^{115}Cd.

Hg

Mercury can be detected using the low-energy 77.4-keV photopeak. It is well known that mercury can volatilize under normal reactor irradiation conditions. Sealing of the samples in high-purity quartz tubing is essential if accurate quantitative results are to be obtained. Spectral interferences may also arise from the many other low-energy gamma-rays or high-energy X-rays in the vicinity of the 77.4-keV photopeak. Therefore, mercury is usually determined using a long-lived radionuclide (see Table 17.4).

Ho

Holmium can be determined with its 80.6-keV gamma-ray with no spectral or nuclear interferences. However, the photopeak usually lies on a high background with poor statistics. Epithermal NAA may be beneficial.

K

Potassium is easily detected using the 1524.7-keV photopeak. There are normally no spectral or nuclear interferences associated with this photopeak.

La

Lanthanum is easily detected using the 1595.2-keV photopeak with very good detection limits. Other usable photopeaks include the 328.8-keV and 487.0-keV photopeaks. The nuclear reaction arising from the fission of uranium, $^{238}U(n,f)$, may result in the potential interference of ^{140}La.

Mo

Molybdenum is determined using the 140.5-keV photopeak. Usually, a decay time of 4–6 days is needed to optimize the sensitivity for measurement of ^{99}Mo. The $^{238}U(n,f)$ uranium fission reaction strongly interferes with this determination and a very careful evaluation of uranium in the food sample must be done. There is a significant decrease in fission interference and improvement in detection limits using epithermal neutrons.

Na

The measurement of sodium is excellently suited for neutron activation measurements. In general there are no spectral or nuclear interferences. However, ^{124}Sb also decays with a characteristic gamma-ray at 1368.2 keV. If there are high concentrations of antimony, this could potentially interfere with the sodium analysis. Then both photopeaks of ^{24}Na, 1368.4 keV and 2754.1 keV, should be analysed as an internal check of the potential antimony interference.

Sb

Antimony is usually determined by the 564.0-keV gamma-ray of ^{122}Sb. However, there is a small but significant spectral interference arising from ^{76}As. This gamma-ray of energy 562.8 keV is characteristic of the ^{76}As decay scheme. It comprises approximately 2.5% of the abundance of the dominant 559.1-keV photopeak. If the arsenic concentration is much higher than that of antimony, the interference may be very important. The interference factor should be calculated using single elemental standards. Since ^{122}Sb has a half-life almost three times longer than that of ^{76}As, one can also wait sufficiently long for the ^{76}As to decay to negligible amounts. Detection of antimony can also be improved using epithermal neutrons. Spectral resolution from the 559.1-keV gamma-ray of ^{76}As is important.

Sm

The determination of samarium is extremely sensitive by neutron activation methods. Several potential interferences exist from uranium and thorium reaction products.

U

Uranium is best determined using the $^{238}U(n,\gamma\beta)$ $^{239}U \rightarrow {}^{239}Np$ reaction. There are normally no spectral or nuclear interferences using the 277.7-keV gamma-ray. Analytical sensitivities are significantly improved using epithermal neutrons.

Zn

Zinc is usually determined by means of the long-lived ^{65}Zn isotope, but can also be determined by the ^{69m}Zn isotope. The sensitivity is usually not sufficiently adequate

in thermal NAA. Improvement can be achieved using epithermal NAA.

17.4.3 Long-lived activation products

Ag

Silver can be determined using the 658.8-keV photopeak of ^{110m}Ag. No spectral or nuclear interferences are normally present.

Ce

Cerium is detected with the 145.4-keV photopeak of ^{141}Ce. Nuclear spectral interference from uranium fission must be taken into account. There is also a potential spectral interference from the l42.6-keV photopeak belonging to ^{59}Fe.

Cr

Chromium is detected with good sensitivity using the 320.0-keV gamma-ray. The interference from the ^{54}Fe(n,α)^{51}Cr is usually negligible.

Co

NAA is well suited to determine cobalt. There are normally no nuclear or spectral interferences.

Eu

Europium can be readily determined using the 1408.0-keV gamma-ray characteristic of ^{152}Eu. The 121.8-keV gamma-ray should not be used since there are other spectral interferences at that energy.

Fe

Iron is detected with good precision using both the 1098.2-keV and 1291.6-keV photopeaks.

Hg

For the reliable determination of mercury, the samples must be encapsulated and heat sealed in quartz. There is a serious spectral interference from the 279.6-keV gamma-ray of ^{75}Se, which completely overlaps the 279.2-keV photopeak of ^{203}Hg. Subtraction of the interferences can be made by normalizing to the 400.7-keV photopeak of ^{75}Se. Typically, normalization to the 136.0-keV or 264.7-keV gamma-rays should not be done since special interferences already exist from other gamma-rays (see Se), but the interfering elements of tantalum and hafnium are negligible in food samples.

Lu

Lutetium is best determined using the 378.5-keV gamma-ray of ^{177m}Lu. However, there is a possibility of a uranium spectral interference.

Nd

Neodymium is determined using the 91.1-keV gamma-ray belonging to ^{147}Nd. Uranium fission has to be taken into account.

Ni

Nickel is determined using the ^{58}Ni(n,p)^{58}Co reaction using the 810.8-keV gamma-ray line. The analytical detection limit for nickel is improved using epithermal NAA. However, the long-lived background continuum is usually low enough so that thermal activation is suitable. There is a potential spectral interference from ^{152}Eu which also decays with a 810.8-keV gamma-ray.

Rb

Rubidium is determined using the 1076.6-keV gamma-ray of ^{86}Rb. There are no nuclear or spectral interferences.

Sb

Antimony can also be determined using the 1691.0-keV gamma-ray line belonging to ^{124}Sb. Although the activation cross-section is not as high for ^{122}Sb, the 1691.0-keV peak is situated on a much lower background than the 564.0-keV photopeak of ^{122}Sb.

Sc

The sensitivity for scandium is excellent using NAA. The 889.3-keV photopeak from ^{46}Sc should be used instead of the 1120.3-keV photopeak since there is a 1120.0-keV background gamma-ray from the ^{226}Ra series.

Se

Selenium is detected using both the 136.0-keV and 264.7-keV photopeaks of ^{75}Se. The 264.7-keV gamma-ray is preferred. The 400.7-keV photopeak can also be used.

Sn

Tin is detected using the 391.7-keV gamma-ray. There is a possible spectral interference from ^{160}Tb which also decays with a 392.5-keV gamma-ray. Normalization is done with the 897.4-keV gamma-ray of ^{160}Tb.

Sr

Strontium can also be detected using the 514.0-keV gamma-ray belonging to ^{85}Sr. Normally there are no nuclear interferences. However, the 511.0-keV annihilation photopeak must be spectrally resolved.

Tb

The 879.9-keV gamma-ray of ^{160}Tb is preferred since it is interference free.

Th

Thorium is determined via the ^{232}Th(n.γβ)^{233}Th → ^{233}Pa → ^{233}U reaction using the 311.9-keV gamma-ray line. There are no spectral or nuclear interferences.

Tm

Thulium is determined by the 84.3-keV photopeak of ^{170}Tm. There are normally no spectral or nuclear interferences. Care should be taken that there are no background photopeaks in the energy region from any shielding material.

Yb

Ytterbium is best determined using the 396.3-keV gamma-ray of ^{175}Yb. There are normally no spectral or nuclear interferences but the 398.2-keV photopeak from ^{233}Pa must be spectrally resolved.

Zn

Zinc is determined using the 1115.5-keV gamma-ray of ^{63}Zn. There are two overlapping peaks at 1120.3 keV from ^{46}Sc and at 1112.3 keV from ^{152}Eu. Good spectral resolution is required. The potential spectral interference from the 1115.3-keV gamma-ray from ^{160}Tb is usually negligible.

17.5 Sample and sample preparation

Since NAA of solid material is non-destructive, sample preparation is minimal. However, as in other chemical techniques homogeneity of the sample is important in order to properly evaluate the individual elemental concentrations. Typically, food samples are dried because of the significant water content, especially in fruits and vegetables. Freeze-drying techniques are well known and are usually used in most types of food analyses. However, less expensive methods such as air-drying are also available. Dried samples having weights between tens of milligrams and a couple of grams are placed in polyethylene vials for neutron irradiation. One concern is the inherent trace content of contaminants that may be in the vials. Elements such as iodine, selenium and bromine may be part of the chemical composition of the vials. Thus, analysis of blank vials is often needed to ascertain any possible contamination. Alternatively, after irradiation of the samples it may be essential to transfer to unirradiated vials.

17.6 Quality control and reference materials

Methodologies for quality control measurements now exist for all techniques in analytical chemistry and the same holds true for NAA. In NAA, sample positioning is crucial and every effort should be made to retain a nearly identical geometry for the samples and standards. The vertical germanium detectors are usually more susceptible to effects of small changes in the counting position than are the horizontal ones. Automatic turning of the sample in front of the detector can also be used to ensure better reproducibility of the counting detector. A quality control check can be performed by turning the sample 180° and seeing if the new calculated concentrations are significantly different. With the use of pelletized samples either vertical or horizontal oriented detectors can be used. Perhaps the most comprehensive description of quality control is the review article by Greenberg *et al.* [21], in which NAA as a primary method of measurement is expertly elucidated with many relevant references. A very good overview of a quality control and quality assurance programme is depicted in Figure 17.6 from the NAA group at the Reactor Institute of Delft at the Technical University of Delft, Netherlands (P. Bode, personal communication, 2012). There are now several international organizations that produce many high-quality certified reference materials in a variety of foods [22–25] with a wide range of elements at the micro and trace level concentrations to help ensure quality control.

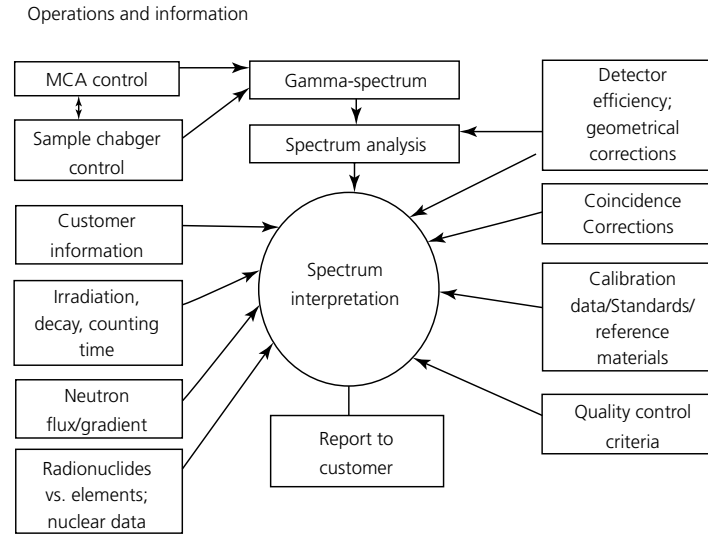


Figure 17.6 Overview of quality control and quality flow chart from the Reactor Institute of Delft at the Technical University of Delft, Netherlands. From P. Bode, personal communication, 2012. Reproduced with permission.

17.7 Conclusions

NAA is a method that is very well suited to determine a variety of macro, micro and trace elements including rare earths in food for nutritional, environmental or toxicological studies. The gamma-ray spectra generated by the activation products are far less complex than those spectra observed in geological samples, with far fewer overlapping peaks and spectral interferences. However, care still needs to be taken when determining rare earths since they are subjected to fission interferences from any uranium in the food itself [26]. With the use of epithermal NAA with and without a Compton suppression system, much lower detection limits are achievable, enabling very good accuracy and precision.

Abbreviations

AA: Absorption spectrometry
HPGe: High purity germanium
ICP-MS: Inductively coupled plasma mass spectrometry
ICO-OES: Inductively coupled plasma optical emission spectroscopy
keV: Kilo electronvolt
MCA: Multichannel analyser
MeV: Million electronvolt
NAA: Neutron activation analysis
PIXE: Particle-induced X-ray emission
XRF: X-ray fluorescence

References

1 Gilbert RT, Kriss RV, Foster N, Davies G. *Chemistry*. W.W. Norton & Company, New York, 2012.
2 Pang X, Li D, Peng A. Application of rare-earth elements in the agriculture of China and its environmental behavior in soil. *J Soil Sediments* 2001;**1**:124–129.
3 Yan Li Y, Yang J-L, Jiang Y. Trace rare earth element detection in food and agricultural products based on flow injection walnut shell packed microcolumn preconcentration coupled with inductively coupled plasma mass spectrometry. *J Agric Food Chem* 2012;**60**:3033–3041.
4 Fuad MM, Shazili NAM, Faridah M. Trace metals and rare earth elements in Rock Oyster Saccostrea cucullata along the east coast of Peninsular Malaysia. *Aquat Ecosyst Health Manage* 2013;**16**:78–87.
5 Jiang DG, Yang J, Zhang S, Yang DJ. A survey of 16 rare earth elements in major foods in China. *Biomed Environ Sci* 2012;**25**:267–271.
6 Kastori RR, Masksimovic IV, Zeremski-Škoic TM, Putnik-Delic MI. Rare earth elements yttrium and higher plants: a review. *Proc Nat Sci Matica Srpska Novi Sad* 2010;**118**:87–98.
7 Thomas PJ, Carpenter D, Boutin C, Allison JE. Rare earth elements (REEs): effects on germination and growth of selected crop and native plant species. *Chemosphere* 2014;**96**: 57–66.

8 Underwood EJ. *Trace Elements in Human and Animal Nutrition*. Academic Press, New York, 1956.
9 Dos Santos LGC, de Nadai Fernandes EA, da Silva Cofani dos Santos SSN, Bacchi MA, Sarriés GA, Junior FB. Macro, minor and trace elements in bovine milk from two Brazilian dairy regions. *J Radioanal Nucl Chem* 2012;**291**:207–211.
10 Seo D, Vasconcellos MBA, Saiki M *et al*. Vanadium biomonitoring by using *Perna perna* (Linnaeus, 1758) mussels transplanted in the coast of the State of Sao Paulo, Brazil. *J Radioanal Nucl Chem* 2102;**291**:101–105.
11 Galinha C, Freitas MC, Pacheco AMG, Coutinho J, Maçãs B, Almeida AS. Determination of selenium in bread-wheat samples grown under a Se-supplementation regime in actual field conditions. *J Radioanal Nucl Chem* 2012; **291**: 231–235.
12 Moon JH, Ebihara M, Ni BF *et al*. A NAA collaborative study in white rice performed in seven Asian countries. *J Radioanal Nucl Chem* 2012;**291**:217–221.
13 Paul RL. Determination of arsenic in food and dietary supplement standard reference materials by neutron activation analysis. *J Radioanal Nucl Chem* 2013;**297**:365–370.
14 Sathyapriya RS, Nair S, Kamesh V *et al*. Estimation of thorium intake due to consumption of vegetables by inhabitants of high background radiation area by INAA. *J Radioanal Nucl Chem* 2012;**294**:387–390.
15 Gilmore G. *Practical Gamma-ray Spectroscopy*, 2nd edn. John Wiley & Sons, Chichester, 2008.
16 International Atomic Energy Authority. Research reactors. Available at http://nucleus.iaea.org/RRDB/RR/Reactor Search.aspx.
17 Canberra Industries Inc. Genie 2000 software family. Available athttp://www.canberra.com/products/radiochemistry_lab/genie-2000-software.asp.
18 Landsberger S, Wu D. A comprehensive study for the determination of 48 elements in the certification of a hazardous standard reference material using thermal, epithermal and Compton suppression neutron activation analysis. *J Radioanal Nucl Chem* 1995;**193**:49–59.
19 Ahmed YA, Landsberger S, O'Kelly DJ *et al*. Compton suppression method and epithermal NAA in the determination of nutrients and heavy metals in Nigerian food and beverages. *Appl Radiat Isotopes* 2010;**68**:1909–1914.
20 Michenaud-Rague A, Robinson S, Landsberger S. Trace elements in 11 fruits widely-consumed in the USA as determined by neutron activation analysis. *J Radioanal Nucl Chem* 2012; **291**:237–240.
21 Greenberg RR, Bode P, de Nadai Fernades EA. Neutron activation analysis: a primary method of measurement. *Spectrochim Acta B* 2011;**66**:193–241.
22 National Research Council Canada. http://www.nrc-cnrc.gc.ca.
23 National Institue of Standards and Technology. http://www.nist.gov.
24 European Commission Joint Research Centre. http://irmm.jrc.ec.europa.eu/.
25 National Metrology Institute of Japan. https://www.nmij.jp/english/service/C.
26 Landsberger S. Update of uranium fission interferences in neutron activation analysis. *Chem Geol* 1989;**77**:65–70.

CHAPTER 18

Methodological aspects of speciation analysis in food products

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Abstract: Ensuring the efficient uptake of micronutrients and protection against toxicity of non-essential elements are the two important issues in trace element research for human nutrition. Since the uptake, *in vivo* utilization, specific biological functions or toxic effects of a given element are subject to its actual physicochemical form, food speciation analysis has received much attention over the past few decades. Different analytical schemes have been developed, depending on elements/species of interest, their physicochemical properties, total concentrations, chemical composition of the sample and the specific goal of the analysis. In this chapter, the methodological aspects of food speciation analysis are presented, including the description of different fractionation schemes, non-chromatographic approaches and hyphenated techniques. Studies evaluating the effect of food elaboration and gastrointestinal ingestion have also been considered. The role of atomic spectrometry techniques as the primary detection tools in analysis of known species/forms is highlighted and the applications of structure-characterizing techniques for species confirmation/identification in innovating speciation studies are also mentioned. Representative examples from the recent literature are reviewed in order to present the trends in sample pretreatment, species separation and quatification. New challenges due to the expanding knowledge of trace elements in human nutrition, the production of selenium-fortified prducts, the use of transgenic food and the presence of metal/metalloid-based nanomaterials have been featured. Finally, an outlook on the approaches to quantification and quality control is briefly presented.

Keywords: analytical speciation, foodstuff, fractionation, gastrointestinal bioaccessibility and bioavailability, non-chromatographic speciation, hyphenated techniques, liquid chromatography, gas chromatography, atomic spectrometry, mass spectrometry, sample pretreatment, arsenic, mercury, selenium

18.1 Challenges of element speciation in food

The term 'speciation' was introduced to analytical chemistry about 40 years ago and has gained a leading position in the area of trace element research. According to the IUPAC Gold Book, 'chemical species of an element' refers to a specific element form defined as to isotopic composition, electronic or oxidation state and/or complex or molecular structure. In this same IUPAC source, 'chemical speciation' is described as distribution of an element among defined chemical species in a given system and 'speciation analysis' as analytical activities for identifying and/or measuring the quantities of one or more individual chemical species in a sample. The particular importance of food speciation analysis is due to the fact that the uptake, specific biological functions or toxicity of a given element depend on its actual physicochemical form. Speciation results help to evaluate the delivery of essential micronutrients from food to the bloodstream, and forewarn of health risks due to the intake of potentially toxic elements/species and also contribute to the promotion of more specific food regulations.

In general, speciation analysis involves three procedural steps: (i) pretreatment necessary to convert the original sample to a form suitable for further processing, (ii) separation of element species/forms and (iii) species detection and quantification. Ideally, the first two operations should

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enable selective and efficient extraction/pre-concentration of target element forms present in the sample, without affecting their identity and quantity. In some applications, for example while using selective extraction or chemical vapour generation, these two phases can be combined in a single step; otherwise separation is achieved by powerful chromatographic or electrophoretic techniques. Atomic spectrometry techniques are the most convenient detectors in speciation schemes; however they can be used only for the analysis of known species whose authentic standards are available. Confirmation and identification of new or unexpected compounds is achieved by molecular mass spectrometry (MS) or other structure-characterizing techniques such as nuclear magnetic resonance, X-ray absorption or fluorescence spectrometry.

The scientific community is aware of difficulties accompanying analytical speciation, which include low concentrations, possible similar physicochemical properties exhibited by the species of one element, transformation of species or their interconversion, presence of unknown species, chemically complex sample matrix, and still too few certified reference materials available. In food analysis, additional aspects have to be considered, such as difficulty in obtaining representative samples, varying composition of raw material taken for preparation of this same food product, and inevitable changes in native speciation during food elaboration and during its gastrointestinal digestion. As often commented in the literature, common issues include lability of metal complexes, changes of oxidation state, hydrolysis or species transformation by microorganisms [1–4].

Based on the above considerations, speciation procedures in food analysis need to be carefully designed in order to provide answers to the specific questions and, accordingly, the challenges can be grouped as follows:

- routine quality control, in which the determination of known element species is required in commercial products;
- speciation analysis of foods enriched with micronutrients;
- studies aimed at the effectiveness of mineral nutrient delivery from food to the bloodstream;
- speciation analysis in food products elaborated with the aim of achieving controlled bioavailability of mineral elements;
- development of analytical procedures for full characterization of all element species present in the sample.
- analysis of transgenic food to evaluate possible effects of genetic engineering on species identity and distribution in relation to element bioavailability and specific biological function;
- evaluation of food products containing metal-based nanostructures as additives or contaminants in relevance to their possible transformations and bioavailability.

In the early stage of trace element analysis in food, the main emphasis was on the determination of total elements and their oral bioaccessibility [7]. Within the domain of speciation analysis, the most studied products include those based on vegetables, fish, algae, wheat, rice, beverages and milk, whereas other items such as cheese, meat or sweets have rarely been considered. It should also be noted that primarily raw food ingredients have been analysed, while speciation analysis of processed food is a relatively new addition to the field. Several topics have received major attention, among them selenium speciation in dietary supplements and biofortified yeast, vegetables or mushrooms [4–6], arsenic and mercury in edible marine organisms [7, 8] and arsenic in wheat and rice [9]. Several reports are available that describe current trends and analytical tools for element speciation in foods [1, 4, 9–16]. The purpose of this chapter is to present a systematic description of speciation schemes in use, depending on element/species, physicochemical properties, chemical composition of the sample and specific goal of analysis. Representative examples from the literature published since 2010 will be given to provide information on recent progress with an emphasis on the applications of atomic spectrometry tecniques.

18.2 Diversity of speciation schemes in relation to the specific objectives of food analysis

The variety and the amount of chemical species in food depend in the first place on their natural content in the raw materials; however this initial speciation can be modified as a result of the enrichment procedure, the application of food additives, environmental contamination, the extensive use of agrochemical products and also to possible changes during food elaboration. According to mineral requirements in human nutrition, food content of iron, cobalt, chromium, copper, iodine, manganese and molybdenum is of primary importance [17]; however in the area of analytical speciation, selenium has been the

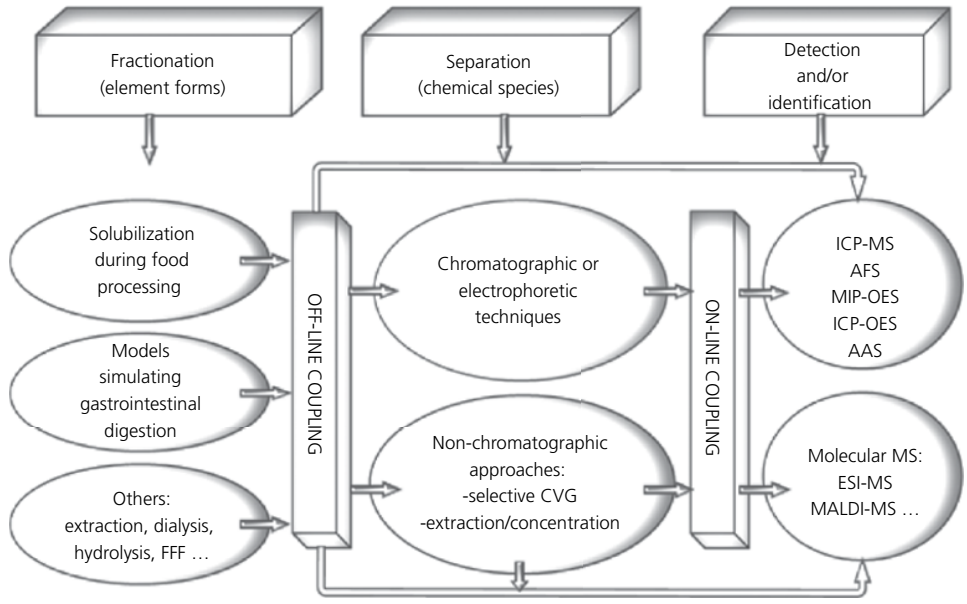


Figure 18.1 General scheme of fractionation and speciation approaches used in food analysis.

most studied essential element [4–6]. In regard to food safety, potentially contaminating and toxic elements have received much attention in food speciation analysis, among them mercury, arsenic, tin, hexavalent chromium, aluminium and nickel [12, 18]. A schematic presentation of different analytical fractionation/speciation approaches used in food analysis is shown in Figure 18.1.

From a nutritional point of view, the main goal of element analysis in food is to evaluate the true bioavailability of elements. It is worth noting that bioaccessibility and bioavailability are two different terms, the first describing the maximum fraction of element released from food in the gastrointestinal tract and the second the element fraction that passes from the gastrointestinal tract to the bloodstream [5, 12]. To this end, different operationally defined fractionation schemes have been proposed that aim to simulate the food elaboration process and/or gastrointestinal digestion. A particularly important issue in studies of element uptake from food is to characterize possible transformation of species and species-specific bioaccessibility/bioavailability; for such purposes, initial fractionation has to be combined with chemical speciation. Some representative examples of sample fractionation procedures used prior to total element determination or speciation analysis are summarized in Table 18.1 and discussed in the section 18.3.1.

Another goal of element speciation is to assess the quality and/or safety of food. There has been continuous development of analytical procedures and their applications for the determination of well-known element species in diverse solid and liquid food products. For essential elements, mainly naturally rich or fortified products have been analysed, whereas for potentially toxic elements almost all food types have been included. As presented in Figure 18.1 and summarized in Tables 18.2, 18.3 and 18.4, speciation procedures can be classified into three groups: (i) those based on non-chromatographic fractionation, (ii) those based on liquid chromatography or electrophoresis and (iii) those based on gas chromatography separations. The choice of detection system is usually dictated by the requirements of high detection power, sensitivity, selectivity and relatively easy coupling with separation techniques. It is not surprising therefore that inductively coupled plasma mass spectrometry (ICP-MS) and atomic fluorescence spectrometry (AFS) have been preferentially used. Particular features of ICP-MS include high sensitivity and multi-element and isotopic capabilities, whereas AFS is specifically well suited for elements determined

Table 18.1 Fractionation schemes simulating food processing or gastrointestinal (GI) digestion.

Sample type	Elements	T/S/SD	Highlights of the procedure	Relevant results	Reference
Vegetables, rice, nuts, seafood	As, Se, Hg	T/S	*In vivo* and *in vitro* GI extraction	Comprehensive review covering studies on bioaccesibility and bioavailability performed before 2011	[12]
Seafood	Se	T	Fresh fish and seaweed samples were homogenized and freeze-dried, commercial dehydrated seaweeds were pulverized and canned fish was freezed-dried. Sample aliquots were *in-vitro* digested with gastric and intestinal juices. During intestinal digestion, dialysis membranes (10 kDa) filled with 0.15 N PIPES solution were placed inside the flasks, and the obtained dialysates and residual fraction were collected for MW acid digestion and ICP-MS determination of Se. Fat content, protein, carbohydrate and dietary fibre were also assessed	Low dialysability percentages of Se were found for fish and mollusc samples (6.7 ± 3.4 and 5.5 ± 2.4%, respectively), whereas the results obtained for seaweed indicated higher bioavailability (66.7 ± 31.1% range). Correlations between Se bioavailability and food nutrient content have been established; a positive correlation was found with carbohydrate and dietary fibre content; correlation with protein content was also significant, but inverse. Fat had no effect on Se bioavailable fraction	[36]
Se-enriched food supplements (FS)	Se	T/S	Three commercials products (yeast-FS, Se(VI)-FS, Se(IV)-FS) were submitted to batch digestion with gastric and intestinal juices, followed by absorption through Caco-2 monolayer; total Se and HPLC/ICP-MS speciation was carried out in the original FS, in bioaccessible and bioavailable fractions. For Se speciation in original FS samples and in Caco-2 cells, proteolysis with protease XIV was performed. Ion-pair reversed-phase separation was achieved on C8 Altima column (250 × 4.6 mm, 5 μm) in a gradient elution with 0.2% HFBA and CH_3OH, at a total flow rate of 1 mL/min	Se(IV), Se(VI), SeMet, MeSeCys were separated within 5 min, polyatomic interferences were eliminated in CRC (90 mL/min H_2); QLs were 0.12 μg/L for total Se and 0.5 μg/L for Se species (as referred to the final solution). Se bioaccessibility in Se(VI)-FS, Se(IV)-FS and yeast-FS was 100%, 80% and 57%, respectively; almost total bioaccessible Se was released at gastric stage of the *in vitro* digestion. The bioavailability was strongly dependent on Se speciation: for Se(IV)-FS ~ 1% of the original Se(IV) content was absorbed through Caco-2 monolayer, for Se(VI) it corresponded to 14% of the original Se(VI) and in yeast 35% of the bioaccessible SeMet was bioavailable	[40]
Se-biofortified edible mushrooms	Se	T/S	Fruiting bodies of *Pleurotus florida* cultivated on Se-rich wheat straw were dried (40°C) and powdered. Typical GI enzymolysis was carried out and supernatant was collected for total Se determination and Se speciation. Size-exclusion fractionation (Superdex 75 10/300 GL column with CH_3COONH_4 mobile phase at pH 7.5) and anion-exchange separation (PRP-X100 column, 250 × 4.1 mm, 5 μm, gradient elution with CH_3COOH 20–200 mM + 100 mM triethylamine) were coupled to ICP-MS detection; DRC pressurized with CH_4, 0.7 mL/min, enabled elimination of polyatomic interferences on Se	Total Se found in biofortified mushrooms (141 μg/g) was about 800 times higher as compared to control cultures; 75% of the Se taken up by the mushroom was solubilized during GI extraction. High molecular mass Se-containing compounds (>5 kDa) were not detected in extracts by SEC HPLC/ICP-MS, whereas small Se species separated on anion-exchange column were: SeMet (73% of all Se species), traces of SeOMet, Se(IV) and about 25% of Se eluted from the column corresponded to unidentified species	[51]

Se-biofortified wheat flour and bread	Se	T/S	Wheat cultived in Se(IV)-fertilized soil (0–100 g/ha) was processed to wholemeal, white flour and bread by standard industrial procedures (flour improver, soya flour, yeast, salt, water added to bread); for Se speciation the samples were digested with protease and lipase and analysed by HPLC/ICP-MS. Anion-exchange PRP-X100 column (250 × 4 mm) was used with 5 mM ammonium citrate in 2% methanol mobile phase and pH step gradient elution (pH 3.65–8.0); CRC was pressurized with H_2 at 3.5 mL/min. Total Se was ICP-MS determined after MW acid digestion	Dose-dependent fortification of wheat was observed. Se species found in grain, flour and bread were SeMet (primary species), SeCys, MeSeCys, Se(IV), Se(VI); unknown species were also detected. No significant loss of Se occurred in grain processing to bread. The predominant extractable selenium species in flour and bread was SeMet, corresponding to >65% of total Se in flour and bread. It was concluded that biofortification of wheat yielded more efficient retention of Se from grain to the product consumed, as compared with broccoli, peas or mushrooms, where substantial loss of Se has been reported	[29]
Chicken eggs, iodized kitchen salt	I	T/S	Boiled eggs were separated into yolk and whites, pooled, freeze-dried and homogenized; commercial powdered yolk and white products were also included. GI digestion of egg and kitchen salt samples was carried out in two steps and both gastric and GI extracts were analysed for total I and for iodide/iodate speciation (SEC column Superdex 75, 10 × 300 mm, 10 μm, isocratic elution with 30 mM Tris-HCl, pH 7.5). Iodide/iodate standards were processed equally. In parallel, I species were extracted from the original samples with Tris-HCl (pH 7.5), defatted with CH_2Cl_2 and analysed by SEC-HPLC. Total I was ICP-MS determined after MW digestion with HNO_3/H_2O_2	SEC/ICP-MS enabled quantification of iodide and iodate in the extracts; ICP-MS detection limits were in the range 0.14–0.70 ng/mL. The iodine content in yolk was up to 37 times higher than in white; bioaccessibilty was established as 33% from white and 10% from yolk, whereas 100% iodide was bioaccessible in kitchen salts. The concentration of I in GI extracts decreased with longer time of egg boiling	[45]
Raw and cooked edible seaweeds	I, Br	T	Simulated gastric and intestinal digestion/dialysis: powdered food was suspended in water, acidified to pH 2 and incubated in pepsin (37 °C, 2 h), cooled in ice-water bath, then pancreatin and bile salts in sodium bicarbonate added. During intestinal digestion, dialysis was incorporated using membranes (10-kDa cut-off) filled with PIPES buffer, pH 7.5, 37 °C, 2 h. Both dialysates and residual fractions were analysed for Br and I by ICP-MS	For total element determination, MW-assisted digestion with TMAH was applied. DLs based on the 3σ criterion were 24.6 and 19.9 ng/g for iodine and bromine, respectively. Accuracy was tested by analysing CRM NIES-09 and by evaluating mass balance. Different distributions of the two elements between dialysable and non-dialysable fractions were observed, depending on the food matrix	[10]

(Continued)

Table 18.1 (Continued)

Sample type	Elements	T/S/SD	Highlights of the procedure	Relevant results	Reference
Commercial seaweeds: dehydrated, canned and agar-agar	I, Br	T/S	A portion of dried and pulverized sample was submitted to GI digestion/dialysis (as above) and dialysate was analysed by HPLC/ICP-MS. Anion exchange (IonPac AS7, gradient elution with 175 mM NH_4NO_3 + 15% CH_3OH) enabled the separaton of iodide, iodate, monoiodotyrosine (MIT), diiodotyrosine (DIT) or bromide and bromate within 20 min; separation of organic species was achieved on reversed-phase column (Zorbax Eclipse XDB-C8, 150 × 4.6 mm) using acetate buffer : CH_3OH. For ICP-MS detection, CRC was pressurized with 4.5 mL/min H_2 for elimination of polyatomic interferences on Br ($^{38}Ar^{40}Ar^1H^+$, *m/z* 79)	The DLs for I species were in the range 2.4–3.4 ng/g and for Br species 112 and 123 ng/g; iodide, MIT and bromide were the most abundant species in food dialysates for iodine and bromine, respectively. HPLC-ESI-Orbitrap MS measurements (after target separation on Zorbax Eclipse column) was applied for confirmation of MIT and DIT structures; however unknown species observed in food dialysates remained unidentified	[47]
Edible seaweeds	As	T/S	Simulated gastric and intestinal digestion/dialysis (as above) was followed by two-dimensional HPLC with ICP-MS and ESI-MS/MS. Size-exlusion separation was performed on TSK gel G 3000 PWxL column (300 × 7.8 mm, 6 μm) with CH_3COONH_4 mobile phase at pH 8.5; for anion-exchange separation, PRP-X100 column (250 × 4.1 mm, 10 μm) was used with NH_4HCO_3 and 1% CH_3OH mobile phase at pH 9.0. Alternatively, ultrasound extraction with CH_3COONH_4 was carried out for 1 h	In the analysis of different seaweeds, 40–61% of total As was found in the aqueous acetate extracts, of which 86–110% accounted for arsenosugars (four compounds identified by ESI-MS/MS were glycerol, phosphate and two sulfonate sugars with molecular mass 328, 482, 392, 408, respectively). These results were compared with arsenosugars content in dialysable and non-dialysable fraction obtained in GI extraction: 12–16% of seaweed tAs was bioavailable and 86–110% of the dialysable As corresponded to arsenosugars. Different results were obtained for sea lettuce compared with Komby, Wakame and Nori	[44]
Parboiled versus non-parboiled rice cooked in As-containing water	As	T	Rice was cooked in water spiked with As(V) up to 450 μg/L and, after elimination of water excess, the obtained gruel was dried and milled to a fine powder. An aliquot was resuspended in water for dynamic *in vitro* digestion with pepsin at pH 2 and 37 °C. At different times of incubation, small portions were withdrawn, centrifuged at 4 °C and supernatants were analysed by ICP-MS	Accuracy was tested by analysing NIES CRM rice flour no. 10-b. Parboiled rice showed higher As bioaccessibility compared with non-paraboiled; As bioaccessibility in cooked rice was much higher when cooked in water with high As concentration (≥47 μg/L), whereas rice cooked with water containing up to 10 μg/L As showed lower As concentrations than the uncooked rice	[41]
Arsenosugar metabolites	As	S	Caco-2 intestinal barrier model	It was suggested that presystemic metabolism of arsenosugars and, in particular, formation of thio-DMA(V) and thio-DMAE(V) (thio- dimethylarsinoyl ethanol) might enhance the intestinal bioavailability of As from arsenosugar-containing foods	[49]

Different marine fish species	Hg	T/S	Fish from local market and those previously fed with $^{203}Hg(II)$ or $Me^{203}Hg$ radiotracers were analysed. Tissue samples and their subcellular fractions were submitted to the three-stage GI digestion model. After fractionation, MeHg was determined by CV-AFS (EPA method 1630). When radiotracers were used, the total species concentrations and subcellular distributions were evaluated by direct gamma counting. Effect of different cooking procedures (steaming, grilling, frying) on *in vitro* bioavailability of MeHg was also evaluated	Influence of exposure route, subcellular distribution, speciation and coexisting phytochemicals on MeHg bioaccessibility in fish was studied for different cooking procedures. The obtained results were discussed in terms of possible reduction of MeHg bioaccessibility from food	[24]
Seafood	As	T/S	A portion of dried and powdered sample was packed into a stainless steel column (5 cm, 4.8 mm diameter) and three digestive reagents (saliva, gastric and intestinal juices) were successively passed at 37 °C; the effluent was on-line diluted and transported to ICP-MS. Alternatively, column fractions were collected and analysed for As(III), MMA(V), DMA(V), As(V), AsB species on anion-exchange column (IonPac AS7, 250 × 4 mm, 10 μm) coupled to ICP-MS. Total As in the raw material and in residual fraction were assessed by MW-assisted digestion followed by ICP-MS	Maximum bioavailability of total As and its species was evaluated in several reference materials (DORM-3, DOLT-4, BCR-627, SRM-1566) and in the real seafood products. The DLs for saliva and gastric juice extracts were 0.010 and 0.040 μg/g (dry sample mass). Saliva was shown to play an important role in As mobilization; the bioaccessibility of the most toxic iAs forms was in the range 7–66% in shellfish (except in scallop) and 31–68% in fish samples. Major species (AsB) and MMA(V), DMA(V) were highly bioaccessible	[39]
Crude, washed and cooked rice (brown and white)	As; Cu, Fe, V, Zn	T/S S	Continuous leaching fractionation similar to above was used; rice was packed in PTFE tube (10 cm, 4.7 mm outer diameter); the effluent was introduced to ICP-MS for total element determination. Three fractions were collected for As speciation (separation of four As species and Cl^- achieved within 5.5 min, using IonPac AG7 column and gradient elution with diluted HNO_3/CH_3OH); non-bioavailable fraction was determined after nitric acid digestion of spent rice from the column. Batch GI extraction was also performed	Saliva accounted for the largest percentage of total Cu, Fe, V, Zn and As leached in comparison to gastric and intestinal juices. Washing rice a few minutes prior to cooking removed ~90% of bioaccessible elements; cooking had negligible effect on As bioaccessibility but appeared to increase relative As(III) content. HPLC procedure enabled baseline separation of As(III), MMA(V), DMA(V) and As(V) in both saliva and gastric juice leachates. On-line leaching presented advantages in terms of shorter time, simplicity and easy coupling with ICP-MS compared with the batch procedure	[30]

(*Continued*)

Table 18.1 (Continued)

Sample type	Elements	T/S/SD	Highlights of the procedure	Relevant results	Reference
Rye and oat flakes and oat porridge	P, S, Mn, Fe, Ni, Cu, Zn, Mo As, Co, Cd, Pb	T/S T	Commercial flakes and porridge prepared with defatted milk were fractionated by two-stage batch GI enzymolysis and alternatively by extraction with Tris-HCl at pH 7.5. The supernatants were analysed by SEC HPLC/ICP-MS (Superdex 75 HR 10/30 300 × 10 mm column with 0.02 m Tris-HCl at pH 7.5 as a mobile phase). Total elements were also quantified by FAAS, ICP-MS and P by UV/Vis spectrophotometry using a methyl blue method	P and S in column effluent were monitored as oxides ($^{47}PO^+$, $^{48}SO^+$). Multi-element calibration was performed by injecting standard solutions in FIA mode before the elution of column void volume (though a second injector). For P and Cu almost complete column recovery was obtained, but for Zn and Mn the recovery was much lower. GI extract contained higher concentrations of P and other elements compared with Tris-HCl extract; the distribution of elements in molecular mas fractions determined by SEC/ICP-MS indicated partial release of elements from higher molecular compounds to the region of low or medium mass	[31]
Pumpkin seeds	Zn	T/S	In two-stage *in vitro* GI digestion, two fractions corresponding to gastric and intestinal filtrates (0.45 μm) were analysed for total Zn by ICP-MS; GI and ultrasound water extracts were fractionated by SEC/ICP-MS using Superdex peptide 10/30 column and 30 mM Tris-HCl mobile phase; spatial distribution of Zn in seeds was assessed by laser ablation ICP-MS and total Zn in seeds was determined after MW acid digestion	In water extract, ~30% of element was associated with LMM fraction (0.5–2 kDa). The results obtained in gastric extracts indicated that at this stage Zn was completely released to Zn(II); however under intestinal conditions it again became less bioaccessible, which was ascribed to Zn(II) complexation with phytic acid at neutral pH. Whole seeds contained 91.2 μg/g Zn; however the element was mainly present in the endosperm envelope, as demonstrated by LA/ICP-MS	[52]
Porridges of five sorghum varieties	Fe, Zn	T	Flour of each variety was suspended in water and several traditional recipes of fermentation or cooking were examined. In the obtained supernatants, elements were determined by FAAS and, additionally, Fe-binding phenolic groups and phytates were determined	The lowest chelating factors and the highest trace minerals bioavailability were observed in the fermented Senegal landrace variety. It was concluded that the selection of traditional varieties and appropriate processing methods can improve nutritional value of sorghum porridge in terms of efficient uptake of Zn and Fe	[32]
Edible plant soups	Fe, Mn, Zn	T	GI digestion of soups was simulated with gastric and duodenal juices; egg lecithin-derived liposome was prepared and used as a model of biomembrane between GI tract and blood vessels. Element bioavailable fraction was measured in liposomes after separation from aqueous phase by filtration (0.22-μm membrane). Elements in each fraction were determined by ICP-MS after digestion with HNO_3	Based on the obtained results, vegetable composition of soup was proposed for enhanced nutritional value in terms of bioavailability of the three mineral micronutrients	[26]

Conventional versus transgenic soybean and maize	V, Cr, Mn, Fe, Co, Cu, Zn, Cd, Pb	T	Bionic GI tract included sample incubation with saliva, gastric and duodenal juices, bile, exposure to intestinal biota and affinity-liposome metal as biomembrane absorption model. The extracts analysed for total element content by ICP-MS were (i) gastric digest, (ii) GI digest and (iii) GI digest after microbiota metabolism	Significant differences in metal bioavailability between conventional and transgenic crops were associated with different content of phytic acid, variation in L/D amino acids and changes in micronutrien composition (e.g. different Fe/Zn ratio)	[37]
Chicken meat spiked with AgNPs	Ag	T/SD	Homogenization in ammonium carbonate buffer (pH 9) or enzymolysis with Proteinase K and asymmetric flow field fractionation (AF^4). Detailed description of AF^4 given in the article	Enzymolysis followed by AF^4/ICP-MS allowed evaluation of AgNPs size distribution with the recovery ~80%; however residual sample matrix affected elution behaviour. TEM and single particle ICP-MS in combination with fraction collection were evaluated as complementary size-characterizing tools. In the latter approach, non-nano Ag fraction was detected and suggested as ionic Ag bound to organic constituents of the enzymatic meat digest	[43]
Wheat, cookies, pasta	Ti Fe, Cu, Zn	T/SD T	Extraction with Tris-HCl (1 h, 90 °C) was followed by enzymolysis with α-amylase (12 h, 40 °C), filtration on Isopore track-etched polycarbonate and drying	A combination of ICP-MS and SEM enabled quantitative determination and morphological characterization of metal particles. The results indicated a decrease in the concentration of metal particles from wheat to flour and from durum wheat to semolina samples, thus indicating external contamination of grains by metal particles	[42]

T, total element determination; S, speciation analysis; SD, size distribution.

Table 18.2 Recent advances in non-chromatographic procedures for element speciation in food.

Sample type	Elements/species	Analytical procedure	Relevant results	Reference
Seafood, fruit juices, milk	As, Hg, Se	Separation of element forms/species by extraction or derivatization procedures were described and applications to food analysis presented	Comprehensive review	[57]
Different foods	As(III), As(V), DMA(V), MMA(V); Se(IV), Se(IV); Sb(III), Sb(V); Hg(II)/ MeHg	Off- and on-line procedures based on controlled conditions during chemical vapour generation and AFS element detection were described. For Hg speciation, special attention was given to on-line UV decomposition of MeHg in the presence of different chemical agents followed by CV-AFS	Comprehensive review	[19]
Fish	Hg(II), MeHg*	*CV-AAS* MW-assisted alkaline extraction of Hg species (5 M NaOH) was carried out from freeze-dried and homogenized samples; the extracts were analysed by CV-AAS commercial system, using Sn(II)/$NaBH_4$ for Hg(II) and determining total Hg after pre-oxidation with $K_2S_2O_8$. Alternatively, total Hg was also determined by MW digestion with HNO_3/H_2O_2; quantification was accomplished by AAS	For accuracy checking, analysis of DORM-2 certified reference material and the recovery experiments were carried out; the DLs for MeHg and Hg(II) were 0.117 and 0.133 µg/kg, respectively. The concentration found in 10 fish species were in the range 28.4–56.3 µg/kg for MeHg and 3.01–8.11 µg/kg for Hg(II)	[60]
Fish	Hg(II), MeHg*	*FIA catalytic CV-AAS* For Hg speciation, an aliquot of homogenized muscle or liver was extracted with 2 M HCl containing thiourea (ultrasonic bath, 5 min, 100% power). The suspension was centrifuged, antifoam solution was added and cold vapour was generated with 0.0001% $NaBH_4$ solution for assessing Hg(II); then an aliquot of Fe(III) was added (final concentration 100 mg/L) followed by 1% $NaBH_4$ for catalytic conversion of MeHg into Hg^0 and its determination by CV-AAS. Experimental and FIA conditions were thoroughly selected, enabling sample throughput of 180 per hour	In the presence of Fe(III), the sensitivity for MeHg was practically the same as for Hg(II) allowing quantification of both species with a sole Hg(II) calibration. Effect of Fe(III) on CV of other mono- and di-alkylated Hg species was examined confirming catalytic effect of this metal ion. Other metal cations were also tested but catalytic effect was obtained only for Fe(III). The DL was evaluated as 0.2 µg/L. Based on experimental data, mechanism of catalytic Fe(III) action was proposed	[61]
Fish	MeHg, Hg(II)	*FIA/SPE/CV/ICP-MS* 5-min ultrasound extraction with 5 M HCl was carried out and after centrifugation the sample was injected to the flow system for selective retention of Hg(II) on the polyaniline (100–150 mesh particle size) packed in a mini column (30 × 2–4 mm). The column was conditioned with 2% HCl carrier and the sample was loaded (100 µL) and injected to the carrier; in these conditions (pH <3) Hg(II) was retained, whereas eluting MeHg was mixed with 0.8% $NaBH_4$ and MeHgH generated was introduced to ICP-MS. Subsequently, Hg(II) was eluted from the column with 2% HCl containing 0.02% thiourea and transported to CV generator. Alternatively, Hg(II) + MeHg were retained together on mini column when water was used as a carrier (pH >5 required); the successive elution was achieved with 2% HCl and a mixture of 2% HCl and 0.02% thiourea, respectively	Polyaniline was synthesized in laboratory. For different column dimensions (2, 3, 4 mm i.d.), pre-concentration factors were 120, 180 and 240; for the highest enrichment, DLs for Hg(II) and MeHg were 25.2 and 32.4 ng/L, respectively; tuna fish reference materials were analysed for accuracy checking (ERM-CE463, ERM-CE464 and IAEA-350)	[65]

Tomato, mushroom, garlic	Sb(V), Sb(III)*	*FIA/SPE/FAAS* Freeze-dried and pulverized samples were extracted with water (ultrasonic bath, 30 min), centrifuged and the supernatants adjusted to pH 8.0 for Sb(III) determination. In FIA system, sample was mixed with DDTC (diethyl dithiocarbamate) and Sb(III) complex was retained on micro column (8 × 3 mm) packed with nano γ-alumina; at this stage 1 M HNO_3 was introduced to FAAS equipped with a slotted tube atom trapping (STAT). In the next step, the complex was eluted from the column with methanolic 1 M HNO_3 (flow rate 6.0 mL/min, 15 s) and transported to FAAS. For total Sb, pre-reduction was carried out with thiourea solution acidified with HCl	Nano γ-alumina was synthesized and characterized; STAT-FAAS enabled 40 fold higher sensitivity compared with conventional FAAS; DLs evaluated for Sb(III) and Sb(V) standards were 6.0 and 8.2 ng/L, respectively. Food samples were spiked with Sb(III) and Sb(V) standards and the recoveries obtained were in the range 96–107%	[75]
Rice products, seafood	iAs	*SPE with HG-AAS* Homogenized and powdered rice samples were extracted with a mixture 0.1 M HNO_3 + 3% H_2O_2 (water bath, 90°C, 1 h); after centrifugation, pH was adjusted in the range 5.0–7.5 and the sample was loaded onto strong anion-exchange SPE cartridge (Strata SAX) preconditioned with methanol. The cartridge was washed with 0.5 M CH_3COOH and the retained iAs eluted with 0.4 M HNO_3. SPE eluate was mixed with the reduction solution (30 mM KI, 28 mM L-ascorbic acid, 0.1% silicone, 3 M HCl) and, after 1 h at room temperature, arsine was generated in 0.5% $NaBH_4$ and a gaseous phase was transported to AAS. For seafood, MW-assisted extraction was carried out with a mixture of diluted HCl and 3% H_2O_2	Selectivity of iAs separation and pre-concentration was examined by analysing the collected SPE fractions by HPLC/ICP-MS; DLs of 0.02 mg/kg for rice and 0.08 mg/kg for dry seafood mass were reported. For accuracy checking, certified reference materials were analysed; the procedure was also tested collaboratively with participation of various laboratories. In the analysis of 36 rice products, iAs concentration ranged from 0.03 to 0.60 mg/kg, whereas in 20 marine foods concentrations up to 0.14 mg/kg were found	[66, 67]
Tea leaves	As(III), As(V)*	*SPE with HG-AAS* *Alternaria solani* fungal species was used to prepare a biosorbent; dried biomass was mixed with hydrophilic methacrylate ester-based synthetic adsorbent Diaion HP-2MG. After several wetting/drying cycles, the obtained material was ground to 18–50 mesh and packed on a mini column (100 × 5 mm) between two plugs of glass wool. The column was conditioned with phosphate buffer at pH 6–7; As(III) was retained by passing the sample under gravity and then eluted with 1 M HCl. Arsine was generated off-line and transported to AAS. For total As, pre-reduction was accomplished with KI and ascorbic acid, whereas As(V) was evaluated as the difference	Selectivity of As(III) sorption was demonstrated in spiking experiments; the recoveries for As(III) and As(V) were in the range 96–99%. Standard references NIST SRM 1515 and GBW 07605 with certified total As were analysed and black tea leaves were included as for the real world samples. Pre-concentration factor of 35 was reported; DL was 11 ng/L. Only total As was determined in tea	[72]

(Continued)

Table 18.2 (Continued)

Sample type	Elements/species	Analytical procedure	Relevant results	Reference
Se-enriched rice	iSe, tSe	*CPE with ETAAAS* Rice grains were milled to a fine powder and ultrasound water–cyclohexane sequential extraction of iSe was obtained; after heating 2–3 min with 6 M HCl and dilution, an aliquot was mixed with 2 mM dithizone, 1% Triton X-114 was added and the sample was kept at 50°C for 20 min (pH 1). The obtained mixture was centrifuged, cooled in an ice-water bath and the surfactant-rich phase containing Se complex was collected. Finally the extract was diluted with 0.1 M HNO_3 in 10% ethanol and injected to ETAAAS (20 µL). Total Se was determined in acid digested samples and organic Se was evaluated as the difference	Interferences due to the foreign metal ions were not observed up to at least 50 : 1 molar excess. The enrichment factor achieved was 82, the detection limit was 0.08 µg/L as referred to the final solution, and recoveries of inorganic selenium in the selenium-enriched rice samples were between 90.3 and 106.0%. The concentration ranges of iSe and total Se in biofortied rice were 18.2–26.8 and 124.2–143.0 µg/g, respectively	[83]
Canned food: tomato paste, peas, corn, fruit juices and soft drinks	Sn(IV), Sn(II)*	*CPE with FAAAS* Selective cloud point extraction of Sn(IV) complex with CCC (1-(2-hydroxy-4-sulfo-1-naphtylazo)-3-naphtholic acid) was achieved in the presence of a mixture of catonic and non-ionic surfactants; the determination of Sn in surfactant-rich phase was performed by FAAS after dilution with CH_3OH/HNO_3. For total Sn, oxidation with $KMnO_4$ in acidic medium was carried out and Sn(II) was evaluated as a difference between total and Sn(IV)	No significant differences by coexisting ionic species were detected, and analysis of CRM and recovery experiments confirmed acceptable accuracy in the real world samples. ICP-OES was used for comparative purposes. In canned food only total Sn was reported: 1.05–5.65 µg/L in liquid samples and 1.24–4.08 µg/g in solid samples	[84]
Canned tuna and tomato	Sn(IV), Sn(II)*	*Imprinted polymer SPE with ETAAAS* Selective SPE of Sn(IV) was achieved on ion imprinted polymer beads, particle size 50–80 µm; cavities retaining Sn(IV) were obtained by incorporating Sn(IV) complex with 4-(2-pyridylazo)-resorcinol (PAR) in the solid matrix and then removing tin ions with 5 M HCl and 10% CH_3COOH. The extraction/elution conditions were optimized. Sn(IV) eluted from the sorbent and total Sn were determined by ETAAAS and ICP-OES, Sn(II) was evaluated as the difference	Sn(IV) sorption was not affected by foreign cations up to their molar excess (100–200) : 1; DL of the proposed method was 1.3 µg/L. Elution of Sn(IV) was achieved with 4 M HCl in 2 h of contact; sample pretreatment prior to SPE was not described	[79]
Cow milk	Cr(VI), tCr	*SPE and ICP-MS* Selective retention of Cr(VI) was proposed. After buffer addition (pH 3.5), milk sample was sonicated, centrifuged at 4°C and supernatant was collected. Cr(VI) was complexed with alizarin (pH 4.9) and the solution was passed by Chromabond NH2 column for quantitative retention of Cr(VI) complex. Elution was achieved with 2 M HNO_3 and, after dilution, the sample was introduced to ICP-MS; polyatomic interferences were controlled by DRC with O_2	Accuracy was demonstrated by CRM analysis (NIST SRM 8435) and by recovery experiments; DLs for total and Cr(VI) were 0.091 and 0.085 µg/L, respectively; Cr(VI) concentrations found in milk samples were in the range 0.6–1.4 µg/L that corresponded to about 1.35% of total Cr	[71]

Bread	Cr(VI), tCr	*Extraction and ETAAAS* Bread samples were dried and powdered; alkaline extraction of Cr(VI) was accomplished (10 mM NaOH, 17 h with agitation), NH_4NO_3 was added and the mixture was centrifuged for ETAAAS determination of Cr in the supernatant. Total Cr was determined in acid-digested samples	For method validation, recovery experiments were carried out (<94% Cr(VI)). The QLs were 4.95 and 5.60 µg/kg for total chromium and Cr(VI), respectively. 152 bread samples were analysed: total Cr was in the range 5.0–126 µg/kg and Cr(VI) in the range <5.6 to 19.70 µg/kg	[82]
Wheat and rice flour	iAs, As(III)	*Slurry FIA HG-AAS* HG generation from sample slurry in a flow system was designed and coupled with electrothermal quartz tube AAS detection. Samples were ground and sieved yielding powder <54 µm. For total As determination, an aliquot was suspended in a solution containing HCl and KI; after 5–10 min heating in a boiling water bath, the mixture was cooled; 8-hydroxyquinoline (8-HQ), *n*-octanol, agar and water for dilution were added. The obtained slurry, containing 1% KI, 1% 8-HQ, 0.3% agar and 5% HCl, was sonicated. For As(III) determination, a similar procedure was used, but without KI addition. Calibration slurries were prepared from As(V) solution and agar	In the presence of 8-HQ, the sensitivity for As(III) was enhanced, most probably due to the improved physical properties of the solutions. The DLs 4.0 µg/kg for As(III) and 4.5 µg/kg for total inorganic arsenic were obtained. In the spiking experiments, recoveries in the range 94.3–107% were reported. In rice flour, As(III) was the main iAs species; in wheat flour lower iAs was found and As(III) was not detected	[68]
Fish-based baby food	As(III) + As(V) + MMA(V); DMA(V); tAs	*Slurry ETAAAS with different chemical modifiers* An aliquot of powdered sample was sonicated with 10 mM TMAH (80 °C, 10 min) and the slurry was directly injected to a graphite furnace (20 µL) together with chemical modifier (20 µL); 30 µg Pd for tAs; 0.3 µg Ce(IV) for As(III) + As(V) + MMA(V); 5 µg Zr for DMA(V) determination. Ashing temperature applied for tAs and As(III) + As(V) + MMA(V) was 800 °C, whereas for DMA(V) it was set at 600 °C	Complete discrimination among all species could not be achieved; however the procedure enabled fast screening of fish-based baby foods for the most toxic As fraction: As(III) + As(V) + MMA(V). DLs obtained for As(III) + As(V) + MMA(V) and for DMA(V) were 50 and 25 n /g, respectively; AsB was evaluated from the difference. Accuracy was demonstrated analysing four certified reference materials: SRM 1568a (rice flour), 1566a (oyster tissue), DORM-2 (dogfish muscle) and DOLT-2 (dogfish liver)	[92]
Edible oils	iSe: Se(IV), Se(VI) Organic Se: SeCys2 + SeCM + SeMet	*DLLME or SPE with ETAAAS* Dispersive liquid–liquid microextraction was proposed for Se fractionation; total Se was extracted into acidified water phase, organic selenium was present in ionic liquid-containing phase and iSe was evaluated as the difference. The results were compared with SPE procedure, in which total retained Se was eluted in three fractions: non-polar species (hexane used for elution), polar (ethanol) and strong polar, iSe (2% HNO_3). Se(IV)/Se(VI) speciation in strong polar fraction was achieved after Se(IV) complexation with APDC and separation by DLLME. Total Se was determined by ETAAAS and AFS	Pre-concentration by DLLME and solvent evaporation allowed DLs of 0.03 and 0.04 ng/g for total Se and iSe in oil, respectively. Determination of tSe, iSe and speciation Se(IV)/Se(VI) was carried out in commercial oils; the concentration range for tSe was <4 to 52 ng/g, and for iSe 0.38–2.62 ng/g	[85]

(Continued)

Table 18.2 (Continued)

Sample type	Elements/species	Analytical procedure	Relevant results	Reference
Garlic	Se(IV), iSe	*On-line IL-DLLME and ETAAAS* Ultrasound extraction from freeze-dried powder was carried out with 1 M H_2SO_4; hydrochloric acid was added to the extract, 0.5 M for Se(IV) and 1 : 5 concentrated (100°C, 20 min) for total iSe. The pre-concentration was achieved by retention of Se(IV)-APDC complex in ionic liquid (CYPHOS® IL) in a glass column (12 × 4 mm) filled with Florisil®; the subsequent elution was obtained with CH_3OH (10% HNO_3). Pre-concentration and ETAAAS determination was integrated in the two-step on-line system	Pre-concentration factor of ~20 was achieved allowing for DL of 15 ng/L in the analysed solution. Metal cations (Cu, Zn, Ni, Co, Fe) potentially interfering by complexation with APDC were tolerated up to about 100 : 1 molar excess with respect to Se(IV). Accuracy was evaluated by recovery experiments (96–100% for Se(IV) and 98–104% for Se(IV)). The results obtained for garlic samples were reported	[86]
Food additives (table salt, sodium nitrate and sodium acetate)	Cr(VI), tCr	*IL salt-assisted LME and FAAS* After sample dissolution, Cr(VI) reacted with 1,5-diphenylcarbazide and hydrophilic ionic liquid (1-butyl-3-methylimidazolium tetrafluoroborate) was added; pre-concentration of the complex in IL was achieved by salt-assisted phase separation (saturation with K_2HPO_4); 50 µL of the IL phase was aspirated into the flame atomic absorption spectrometer using a home-made microsample introduction system. For total Cr, oxidation with $K_2Cr_2O_7$ was carried out	The DL evaluated was 1.25 µg/L and enrichment factor 100 was achieved. Effect of foreign ions was studied revealing the lowest tolerance for 100 : 1 excess. Accuracy examined by recovery experiments; however Cr was not detected in any of the food additives analysed	[88]
Instant coffee, cappuccino and food integrators	SiO_2 NPs	*SdFFF, PCs, SEM/TEM and ETAAAS* A combination of SdFFF, photon correlation spectroscopy, electron microscopy and ETAAAS was used for characterization of particle size distribution, morphology and quantity of SiO_2 in simple food-related matrices. Extraction of NPs from cappuccino was carried out using typical processes of beverage preparation, followed by multiple centrifugation, washing and reconstitution in low volume under sonication. The sample was injected by Rheodyne valve into a carrier stream containing non-ionic detergent (Triton X-100 0.01% or Fl70 0.1%) and fractograms were obtained with UV/Vis detection	A procedure for SiO_2 NPs from simple powdered food matrices was proposed; the role of the carrier as a dispersion medium in SdFFF was highlighted in terms of its surfactant properties, pH, ionic content, etc. Four types of SiO_2, commercially available as food additives and personal care products, were included in this study. The presence of original nanoparticles (10 nm) was confirmed and their organization in clusters or aggregates of different dimensions was demonstrated. The concentration of Si as SiO_2 in a different powdered products was in the range 0.006 and 0.35% (w/w) according to ETAAAS results	[91]

*Element form evaluated as the difference between total and element form selectively determined.

Table 18.3 Representative examples of analytical speciation procedures based on liquid chromatography separations (2010–2013).

Sample	Element species	Extraction	Chromatographic/detection conditions	Additional comments	Reference
Brazlian seafood	Hg(II), MeHg, EtHg	Homogenization and freeze-drying was followed by extraction with a mixture of mercaptoethanol, L-cysteine and HCl, with sonication for 15 min	C8 column and isocratic elution with a mobile phase containing 0.05% mercaptoethanol, 0.4% L-cysteine and 0.06 M CH_3COONH_4 was followed by on-line ICP-MS detection	Quantitative extraction of three species in 15 min was obtained and LC separation in less than 3 min. The evaluated DLs were 0.25, 0.20 and 0.1 ng/g for Hg(II), EtHg and MeHg, respectively	[120]
DORM-2 dogfish	Hg(II), MeHg, EtHg	Extracting solution contained 0.1% HCl, 0.1% 2-mercaptoethanol and 0.15% KCl; the mixture was either shaken overnight (12 h) or subjected to ultrasonic extraction for 30 min. Supernatant was collected by centrifugation and was filtered prior to analysis	Low-pressure multisyringe chromatography: reversed-phase Onyx Monolithic C18 column (50 × 4.6 mm) with multi-isocratic elution program was used; the two mobile phases contained 0.005% 2-mercaptoethanol in acetate buffer, pH 6 and acetonitrile (1 or 10%). Injection volume was 600 μL. Column effluent was passed through UV radiation for digestion of organic species, and cold vapour was generated before As detection/quantification by AFS	Baseline separation of three species was achieved in 10 min; DLs were in the range 0.1–0.36 μg/L. Capability of multisyringe system for successful delivery of mobile phases and sample for speciation of Hg species in fish extract was demonstrated	[119]
DORM-2 dogfish	Hg(II), MeHg, EtHg, PhHg	An aliquot of reference material was extracted twice with 5 M HCl under sonication (30 min) and, after centrifugation, the supernatant was collected, neutralized with NaOH and injected to the flow system	Reversed-phase MP C18 column (150 × 4.6 mm, 5 μm) was used with acetonitrile–aqueous 0.12% L-cysteine (10 : 90) mobile phase, pH 6.8. Column effluent was mixed with acidified H_2O_2 (pH 2) and transferred to a minicolumn (25 × 5 mm) filled with 0.5 g Fe_3O_4 magnetic nanoparticles for organic species oxidation; afterwards the sample was directly transported to CV-AFS system	Species separation, oxidation and mercury quantification was integrated in on-line flow system. Magnetic nanoparticles were obtained and characterized by SEM. Possible mechanism of organic mercury oxidation by H_2O_2 in acidic medium in the presence of magnetic nanoparticles was presented. Four Hg species were separated in 10 min; DL values were in the range 0.7–1.1 mg/L for Hg(II), MeHg, EtHg and PhHg. For DORM-2, MeHg was the only species detected and its concentration was in agreement with the certified value	[117]

(Continued)

Table 18.3 (Continued)

Sample	Element species	Extraction	Chromatographic/detection conditions	Additional comments	Reference
Fish	Hg(II), MeHg, EtHg, PhHg	Aliquots of fish muscle were homogenized and ultrasound extracted with 0.10% HCl + 0.05% L-cysteine (40 °C, 15 min). After centrifugation the supernatant was collected, filtered (0.45 μm), diluted and introduced to HPLC/ICP-MS system. For total Hg determination, samples were acid digested in MW oven	Two strong cation-exchange guard columns (Zorbax SCX, 2.5 × 4.6 mm, 5 μm) connected in series were used as the analytical column; isocratic elution with 20 mM L-cysteine or thiourea (pH 2.0) at flow rate 1.5 mL/min was applied. For ICP-MS detection, *m/z* 202 for Hg and 209 for Bi (continuously introduced for internal standardization) were monitored	Separation of four Hg species was achieved in 2.5 min (void volume eluted at 17.4 s). DLs were in the range 1.9–3.1 μg/kg. Accuracy was demonstrated by analysing certified reference material GBW 10029 (fish tissue)	[118]
Fish	Hg(II), MeHg	Six frozen commercial fish were analysed. Different drying procedures were tested by combining thawing, freeze-drying and drying in an air circulation oven at temperatures from 50 to 175 °C and time periods 24 and 48 h. After drying, samples were ground in a cryogenic mill. Extraction was carried out with 0.6% L-cysteine at 20 °C for 6–24 h. The supernatant was obtained by centrifugation and, after filtering (0.45 μm), the solution was injected to the column. For total Hg, fish samples were acid digested in MW system or submitted to microwave-induced combustion	Reversed-phase separation of Hg species was obtained on Si-C18 (250 × 4 mm, 5 μm) column with 0.1% L-cysteine, pH 4.0, as mobile phase at a flow rate of 1 mL/min. For post-column cold vapour generation, 0.2% $NaBH_4$ and 1.0 M HCl were added using an X-type connector; after gas–liquid phase separation, detection was carried out by ICP-MS at *m/z* 202. Total Hg was determined by ICP-MS after cold vapour generation	Based on the results obtained, freeze-drying and oven drying below100°C were proposed as reliable procedures for Hg speciation analysis, since species integrity was not altered. However, chemical matrix specific for a given fish type may strongly affect the results of such pretreatment. For two dogfish CRM, the results were in agreement with certified values (97% recovery); DLs of 1.7 and 2.3 ng/g of Hg were obtained for Hg(II) and MeHg, respectively; relative standard deviation was below 5.5%	[121]
Fish	Hg(II), MeHg, EtHg	MW-assisted extraction of As species was carried out from the dried fish muscle	Separation was achieved in 25 min using CE coupled to ICP-MS	The reported DLs were in the range 0.021–0.032 ng/mL as referred to Hg in the injected solution. Speciation recoveries in the range 94–103% were reported	[123]
Soybean oil	As(III), As(V), MMA(V), DMA(V), AsC, AsB	MW-assisted extraction was carried out with 0.5% HNO_3 in 80% CH_3OH (80 °C, 2 h). After centrifugation at 0 °C, supernatant was evaporated to eliminate methanol and diluted with water prior to HPLC/ICP-MS. Total As was determined by ICP-MS after oil emulsification with Triton-X100 and antifoam addition	Separation was achieved on anion-exchange PRP-X100 column (250 × 4.6 mm, 10 μm) using salt gradient elution with 0.5 mM and 50 mM $(NH_4)_2CO_3$ + 1% CH_3OH mobile phases, pH 8.5. To control polyatomic interferences in ICP-MS detection, *m/z* 77 was monitored	Extraction yield was ~92% for total As in oil; six As species were resolved in 8 min (AsC in a void volume). The DLs were 0.015, 0.01, 0.024, 0.008, 0.01 and 0.013 ng/mL for AsC, AsB, As(III), DMA, MMA and As(V), respectively. Based on analysis of unused and used oil it was concluded that As species were leached to oil from food during frying; the major species in used oil were AsC and AsB	[151]

Marine oil and fats	Arsenolipids and their hydrolysis products	Saponification or alkaline hydrolysis (2 N KOH, C_2H_5OH, diethylether) and recovery of aqueous phase. Methanol–hexane extraction, recovery of polar fraction and clean-up by preparative chromatography	Ion-exchange HPLC/ICP-MS was reported for AsC, DMA(V), arsenosugars, TMAO, dimethylarsinoyl acetate. Reversed-phase HPLC/ICP-MS was used for long-chain arseno fatty acids	Critical review, in which a need for further investigations of the abundance and toxicity of As-containing lipids was highlighted in terms of risk assessment and legislation concerning food and feed safety	[147]
Edible anemone: raw and fried	As(III), As(V), MMA(V), DMA(V), AsC, AsB, TMAO, TETRA, DMAS, GPAsC	Crude anemones were battered in wheat flour and fried in olive oil; crude and processed material was freeze-dried for As extraction with CH_3OH/H_2O using ultrasonic bath, ultrasonic probe or focused MW. Each of these extraction modes allowed almost complete recoveries; however, focused MW was finally selected because of its safety, easy operation and feasible automation	Anion-exchange PRP-X100 column with 50 mM $(NH_4)_2CO_3$ at pH 8.5 at a flow rate of 0.8 mL/min was used for separation of As(III), As(V), MMA(V), DMA(V), whereas AB, TETRA and TMAO were separated on cation-exchange LC-SCX column (250 × 4.6 mm, 5 μm) with 20 mM pyridine, pH 2.5, as mobile phase and a flow rate of 1 mL/ min. ICP-MS detection was used for quantification of known and chromatographically resolved species. Anion exchange separation was coupled with triple quadrupole MS; using multiple reaction monitoring, dimethyl-arsinothioic acid (DMAS) and glyceryl phosphorylarsenocholine (GPAsC) co-eluting with AsB, AsC were identified	Combination of two ion-exchange columns, ICP-MS detection and tandem MS enabled speciation analysis of 10 arsenicals. In the raw samples of anemone, the decreasing order of species was DMA(V) > AsB > DMAS > AsC > As(V) > TETRA > GPAsC > MMA(V) > TMAO. After cooking as battered in wheat flour and fried with olive oil, 64% of total arsenic was lost and about 90% was released to the cooking oil, but the order of species was the same (excluding GPAsC and MMA(V) which were not detected after cooking)	[135]
Fish	iAs	Homogenization, drying and three alternative MW-assisted extractions were tested: (i) 70 mM HCl and 3% H_2O_2; (ii) 2% HNO_3; (iii) 1.5 mg/mL NaOH in 50% ethanol. All extracts were treated with H_2O_2, adjusted to pH 7 ± 2 and centrifuged	Anion-exchange PRP-X100 column (250 × 4.6 mm, 10 μm) was used, elution was isocratic with 6.2 mM NH_4NO_3 and 6.5 mM H_3PO_4, pH 6.0, at a flow rate of 1 mL/min. Column effluent was on-line mixed with 3 M HCl and $NaBH_4$ (1.5%) via two T-pieces and passed through a reaction coil to complete hydride generation. Afterwards gas–liquid separator was incorporated and the gas phase transported to ICP-MS. Internal standar (Rh) was nebulized continuously to a cyclonic spray chamber	Polyatomic interferences due to $^{40}Ar^{35}Cl^+$ were checked by monitoring *m/z* 77 ($^{40}Ar^{37}Cl^+$ or $^{77}Se^+$) and *m/z* 82 ($^{82}Se^+$). Accuracy and robustness of HPLC/HG/ICP-MS was evaluated by analysing TORT-2 CRM and by As(V) standard addition. Post-column HG enabled enhanced selectivity toward iAs, eliminating the effect of organic species possibly co-eluting with As(V) in the presence of complex chemical matrix. DL for iAs was <0.002 mg/kg and total chromatographic run 15 min	[132]

(Continued)

Table 18.3 (Continued)

Sample	Element species	Extraction	Chromatographic/detection conditions	Additional comments	Reference
Edible marine algae	As(III), As(V), MMA(V), DMA(V), AsB, TETRA, TMAO, arsenosugars	MW-assisted extraction with three portions of hot water (90 °C, 5 min) was followed by centrifugation and filtration (0.22 μm) prior to HPLC. For total As, MW acid digestion was done	As(III), As(V), MMA(V), DMA(V) and four arsenosugars (Gly-sug, PO_4-sug, SO_3-sug and SO_4-sug) were separated on anion-exchange PRP-X100 column in a gradient elution, using phosphate buffer mobile phases at pH 9.0 and flow rate 1 mL/min. For As(III), As(V), AsB, TETRA and TMAO, cation-exchange PRP-X200 column was used with pyridine mobile phase at pH 2.5 and flow rate 1 mL/min. Column effluent was transferred to an on-line photo-oxidation system, equipped with a UV lamp and a 2% $K_2S_2O_8$ in 2% NaOH, and then transported to continuous HG-AFS. Total As was assessed by ICP-OES	Twelve commercial algae were analysed; for total As, extraction efficiencies ranged from 49 to 98%. As(V) was found as the main specie at concentrations up to 77 μg/g. For detection/confirmation of arsenosugars, previously characterized extract of *Fucus serratus* was run in parallel; phosphate arsenosugars were found in all algae samples	[139]
Spoiled seafood	As(III), As(V), MMA(V), DMA(V)	Freezed-dried shark muscle and DORM-2 were extracted with water in ultrasonic bath (1 h); liquid from the canned product was analysed after filtration (0.2 μm)	Separation was achieved on PRP-X100 column (250 × 4.1 mm, 10 μm) with phosphate mobile phase at pH 6.2; the effluent was introduced to HG-AFS commercial system. For quantification, standard addition of individual species was incorporated in the in-line operating sequential injection system (SIA), in which the mixed sample plus standard solutions were delivered from SIA to fill the HPLC injection loop	Method of standard addition was used to overcome matrix effects typically existing while analysing complex food samples. High throughput was achieved by performing SIA standard addition during HPLC run of the preceding sample. The DLs based on 3σ for As(III), As(V), MMA and DMA were 0.023, 0.39, 0.45 and 1.0 ng/mL, respectively. As(III) was found as a primary As species in liquid from the spoiled can	[133]
Chicken meat	iAs, MMA(V), DMA(V), 4-OH, Rox, PA, p-ASA, 4-NPAA	Homogenization, freeze-drying and CH_3OH/H_2O extraction (50 °C, sonication) was followed by SBSE. A stainless steel magnetic bar coated with high polar extraction phase of TiO_2 immobilized polypropylene hollow fibre was used for retention of As species at pH 6 (25-min stirring); desorption was achieved with 1 M KH_2PO_4, pH 2.5, under sonication	CAPCELL PAK C18 MG column was used; elution was isocratic with 2.5 mM sodium butanesulfonate, 4.0 mM malonic acid (pH 3.0) and 2% CH_3OH at flow rate 1 mL/min, 40 °C; on-line ICP-MS detection	Hollow fibre material and stir bar coating were elaborated and characterized by X-ray diffraction spectrometry and scanning electron microscopy. The enrichment factors were in the range 8.5–22.3 for eight As species; the DLs based on 3σ were in the range 11.4–64.6 ng/L; recoveries evaluated in spiked samples were 78.5–120.4%. In the meat samples, iAs, DMA(V) and 4-NPAA were found in the range 35–108 ng/g	[76]

Liver from chicken treated with roxarsone	As(III), As(V), MMA(V), DMA(V), Rox, 3-amino, N-acetyl	Livers were homogenized in aqueous TMAH (0.625%), additionally vortexed 15–30 min, diluted, centrifuged and the supernatant was taken for speciation analysis. For total As, MW acid digestion was done	Anion-exchange IonPac AS18 column (250 × 4 mm) with 45 mM TMAH + 1% CH_3OH allowed separation of seven As species: DMA(V), As(III), MMA(V), As(V), 3-amino, N-acetyl and Rox	The extraction oxidized most As(III) to As(V) and the procedure was proposed as well suited for iAs determination; however limitations in quantification of other species were highlighted. Roxarsone, its 3-amino and 3-acetamido metabolites and unknown As species were found in livers from Rox-treated chickens	[22]
Porcine and chicken liver	p-ASA, NIT, Rox	Meat samples were freeze-dried and homogenized by grinding; CH_3OH/H_2O (3 : 7) was used for extraction that was accomplished in accelerated solvent extractor (three cycles, 4 min each, 80 °C, 1500 psi). Alternatively, ultrasound extraction was carried out with CH_3OH/H_2O (1 : 1) at 40 °C, 20 min. For fat separation, extracts were kept at –20 °C, 30 min and the withdrawn aliquot was filtered (0.45 μm)	Reversed-phase Luna 5u C18 (250 × 4.60 mm) column was used with the mobile phase CH_3OH + 50 mM KH_2PO_4 (included 0.1% TFA), pH 1.67, 25 °C and flow rate 1.2 mL/min. Column effluent was transferred to the UV/irradiation unit for species oxidation and then to HG-AFS	p-ASA, NIT and Rox were separated in 20 min, with DLs 0.24, 0.74 and 0.41 ng/mL, respectively as referred to As injected on column. Using accelerated solvent extraction, recoveries were higher than 94% for the three species. Species stability during extraction was demonstrated; the three compounds were detected in meat purchased in the local market	[143]
Herbal tea, chicken meat, TORT-2, DORM-3	As(III), As(V), MMA(V), DMA(V), AsC, AsB, 4NPAA, *o*-ASA, Rox	Freeze-dried and homogenized samples were four times extracted with deionized water under sonication and centrifuged	CE separation was carried out on fused silica capillary (100 cm × 50 μm) with phosphate/borate electrolyte at pH 9.2; effluent was on-line introduced to ICP-MS thorough CE-ESI-MS sprayer kit. Mixture CH_3OH/H_2O (95 : 1) with Rh internal standard was introduced as the sheath flow liquid through a built-in active 1 : 100 flow-splitter, providing a constant flow rate of 5 μL/min	In the CE/ICP-MS interface, capillary outlet was directly inserted into the spray chamber enabling reduced dead volume and high transport efficiency. Ten As species were separated in 30 min with RSD of migration times not worse that 1%; DLs were in the range 0.9–3.0 ng/g. The extraction efficiency was examined by analysing fish meat reference materials. In tea samples, As(III), As(V) and DMA(V) were found, whereas in chicken meat As(III) was the sole species	[23]
Marine organisms	Thio-methylated As species, arsenosugars	Seaweeds, gastropods and fish were collected in Australia. Tissues were freeze-dried and homogenized with three portions of CH_3OH/H_2O (1 : 1) either using microwave heating at 70 °C or rotational mixing at 25 °C. Afterwards the samples were centrifuged, roto-evaporated to dryness and redissolved in water	Separation of 14 thio-methylated arsenic species was obtained on reversed-phase Atlantis C18 column (150 × 4.6 mm, 5 μm) with 20 mM $NH_4H_2PO_4$ mobile phase at pH 3 and flow rate 1 mL/min. In ICP-MS detection, potential interference from $^{40}Ar^{35}Cl^+$ was eliminated based on $^{35}Cl^+$, $^{35}Cl^{16}O^+$, $^{35}Cl^{17}O^+$ and $^{40}Ar^{37}Cl^+$ signals; selenium was monitored at *m/z* 82 as a cross check on $^{40}Ar^{35}Cl^+$	In addition to typical standards of As species used in this work, arsenosugars were isolated from certified reference materials and characterized/confirmed by ESI-MS. Thio-methylated As species found in marine organisms included thio-SO_3-riboside, thio-PO_4-riboside, thio-OSO_3-riboside, thio-Gly-riboside sugars. Importance of thio-methylated arsenicals was highlighted in terms of their potential participation in AsB production	[142]

(Continued)

Table 18.3 (Continued)

Sample	Element species	Extraction	Chromatographic/detection conditions	Additional comments	Reference
Seaweeds	As(III), As(V), MMA(V), DMA(V), AsC, AsB, TETRA, arsenosugars, arsenolipids	MW-assisted extraction was carried out with CH_3OH/mobile phase/H_2O mixture (60 °C, 20 min); the obtained extracts were centrifuged, diluted and filtered (0.2 μm). For total As determination, closed-vessel digestion with HNO_3 was done	Separation was obtained on anion-exchange PRP-X100 column (250 × 4.1 mm, 10 μm), using gradient elution with two mobile phases containing 0.5 or 50 mM $(NH_4)_2CO_3$ and 1% CH_3OH at pH 8.5. For controlling $^{40}Ar^{35}Cl^+$ interference in ICP-MS detection, *m/z* 35 and 77 were monitored	Separation of As(III), As(V), MMA(V), DMA(V), AsC and chloride in the standard mix was achieved in less than 9 min. In the analysis of seaweed extracts, more As species were observed; TETRA, AsB, OH-arseno-ribose, and PO_4-arseno-riboside were identified by on-line ESI-MS in multiple reaction monitoring mode. A residue after incomplete extraction of reference material BCR-279 (~14% of total As) was also submitted to ESI-MS suggesting arsenolipid structure	[138]
Wheat, bread and pasta	As(III), As(V), MMA(V), DMA(V)	Four extraction procedures were tested: (i) CH_3OH/H_2O with sonication at 50 °C, (ii) water with ultrasonic probe; (iii) MW-asssisted enzymatic extraction with α-amylase and (iv) MW-assisted extraction with 0.16 M HNO_3; the latter was the most effective	Anion-exchange PRP-X100 column with 10 mM $NH_4H_2PO_4$, 10 mM NH_4NO_3 and 2% CH_3OH at pH 5.5 as the mobile phase was used. On-line ICP-MS detection was carried out and polyatomic interferences were controlled by monitoring *m/z* 77 and 82	Separation was achieved in 12 min and DLs were in the range 0.35–0.46 ng/g; no species interconversion was observed. Accuracy was demonstrated by analysing standard reference materials, by recovery experiments and by the evaluation of mass balance. In food samples, 95% of As was found to be present in inorganic form, As(III) being the most abundant	[129]
Rice	As(III), As(V), MMA(V), DMA(V)	Rice grains were milled avoiding temperatures higher than 40 °C. Extraction was performed with 0.28 M HNO_3 in a heating block (95 °C, 90 min) and the obtained extracts were filtered (0.45 μm). For total As and other elements, 65% HNO_3 digestion in a closed system was performed	Anion-exchange PRP-X100 column (250 × 4.1 mm, 10 μm) at 40 °C and 20 mM $NH_4H_2PO_4$ (pH 5.6) mobile phase with flow rate 1.5 mL/min was coupled to ICP-MS operated at low resolution	121 samples of 12 grain types and three certified reference materials were analysed aiming at validation of the extraction procedure for different rice matrix compositions. Preservation of As(III) and As(V) was demonstrated and satisfactory recoveries for all species were reported. On average, arsenite accounted for 90% of inorganic grain arsenic	[130]

Rice	As(III), As(V), MMA(V), DMA(V), $As(GSH)_3$	Grains were freeze-dried and homogenized by milling. MW-assisted extraction with 2% HNO_3 was carried out (95 °C, 10 min) and the supernatant was collected after centrifugation. For XANES, standards prepared in 30% glycerol and fine powdered rice samples were frozen (~12 K)	Anion-exchange PRP-X100 column (250 × 4.6 mm, 10 μm) with phosphate buffer at pH 4.5, flow rate 1.5 mL/min and 40 °C was used for separation of As(III), As(V), DMA, MA. Cation exchange LC-SCX column (250 × 4.6 mm, 5 μm) with pyridine buffer mobile phase at pH 2.6, flow rate 1.5 mL/min and 40 °C was used to discard cationic As species. Polyatomic interferences in ICP-MS were corrected by monitoring *m/z* values 77 and 82. XANES spectra were collected in fluorescence mode using up to 15 scans. The average spectra were background corrected and analysed by linear combination fitting. Total As was determined by ETAAAS (Pd/Mg modifier) after MW digestion with HNO_3	XANES results gave similar proportions of total As(III), As(V), and DMA compared to HPLC/ICP-MS results; additionally, XANES was able to distinguish between arsenite and As-thiolate. The weak points of XANES were high spectra noise, low sensitivity and possible overestimation of reduced As due to 'in beam' As photoreduction. For HPLC/ICP-MS procedure, the extraction and column recoveries were practically quantitative and, using 2% HNO_3, oxidation of As(III) to As(V) was negligible. Eight certified rice flour reference materials were analysed for accuracy testing	[95]
Rice, wheat, canned tuna fish	iAs, DMA(V), MMA(V)	Rice grains were milled (<0.25 mm), fish was freeze-dried and wheat flour was analysed without pretreatment. Sample aliquot was extracted with 0.02 M TFA + 0.3% H_2O_2 first under sonication (15 min) and then in MW system (95 °C, 1 h); after extraction, inorganic As was in form of As(V). This same extraction was also performed in water bath (95 °C) for comparative purposes	Two PRP-X100 columns were used (250 × 4.1 mm, 5 μm and 150 × 4.1 mm, 5 μm) with malonic acid (2, 5 or 10 mM) mobile phase at pH 5.6. On-line ICP-MS detection was performed monitoring signals at *m/z* 75 and 77 (to check for possible chloride interferences); 1% CO_2 was introduced as optional gas for enhanced sensitivity	TFA was used because it facilitates hydrolysis of complex carbohydrates and starch (better extraction from rice) and does not cause polyatomic interference at *m/z* 75. Recovery was evaluated relating the sum of species to the total As in acid-digested sample; the values obtained for rice were in the range 86–100% and for wheat 93%. Cation-exchange LC was additionally carried out for fish samples to obtain AsB and other organic arsenicals; recoveries of 80–106% were reported (except for one sample where 11% recovery was ascribed to the presence of arsenolipids). Intra-day precision for iAs was 1.8% and DLs for iAs and two methylated species in rice were 1 and 0.5 μg/kg, respectively. The reference material was NIST SRM 1568a	[131]

(Continued)

Table 18.3 (Continued)

Sample	Element species	Extraction	Chromatographic/detection conditions	Additional comments	Reference
Rice	As(III), As(V), MMA(V),DMA(V), AsB	Polished and unpolished white rice flour was pulverized and fractionated to obtain particle sizes <150, 150–250, 250–500 and >500 μm. Hot water extraction was carried out in a heating block (90°C, 3 h); the sample was centrifuged and filtered (0.45 μm). For total As, MW digestion with HNO_3/$HClO_4$/HF was performed	Ion-pair reversed-phase separation was achieved on C18 ODS L (150 × 4.6 mm) and CAPCELL PAK C18 MG (150 × 4.6 mm) columns with the mobile phase containing 10 mM sodium 1-butanesulfonate, 4 mM malonic acid, 4 mM tetramethylammonium hydroxide, 0.05% methanol (pH 3.0) at a flow rate of 0.5 mL/min. On-line ICP-MS detection was used with 3 mL/min He in a CRC	Extraction of rice powder particles with diameters of less 150 μm enabled complete species recovery as compared to total As content in acid-digested samples. Separation of five species on ODS column was achieved in 5 min; DLs evaluated for As(III) and As(V) were 0.2 and 0.1 ng/g for MMA(V) and DMA(V), respectively. Once verified that rice samples did not contain AsB, this compound was added as internal standard enabling enhanced repeatability. SEM observations revealed that large starch particles in the raw material were broken during heating. Analytical performance was compared for unpolished and polished rice	[136]
Edible mushrooms	As(III), As(V), DMA(V)	Whole mushrooms were collected in specific forest area in Poland. After drying (90 °C, 2 h), the samples were ground, homogenized and sieved (0.02 mm); extraction was carried out with 1 M H_3PO_4 with addition of Triton-X100 in ultrasonic bath (room temperature, 30 min). Samples were then filtered and pH adjusted to 6–6.5	Anion-exchange LC-SAX1 (250 × 4.6 mm) column was used with phosphate buffer isocratic elution at 3 mL/min; column effluent was transferred to the hydride generator unit and arsine vapour was passed through gas–liquid separator directly to AAS with quartz tube atomizer. Alternatively, Zorbax SAX column (250 × 4.6 mm) with slightly different mobile phase composition at flow rate 2.5 mL/min was used followed by direct transfer of column effluent to AAS detector	Chromatographic separation was accomplished in less than 5 min. Consistent results were obtained by application of two speciation procedures. In mushrooms collected from sites subjected to anthropogenic emissions, species concentrations were up to 27.1, 40.5 and 88.3 mg/kg for As(III), As(V) and DMA(V), respectively, whereas mushrooms from non-contaminated sites contained below 0.5 mg/kg of As species	[140]
Fruit juices	As(III), As(V), MMA(V), DMA(V), AsB	Juices were filtered (0.45 μm) and diluted with chromatographic mobile phase prior to their injection to HPLC/ICP-MS system. MW acid digestion was carried out for total As determination	Anion-exchange PRP-X100 column (100 × 4.1 mm, 10 μm) was used with 10 mM $(NH_4)_2HPO_4$ mobile phase, pH 8.25, at flow rate 1 mL/min. For ICP-MS detection, CRC was pressurized with 5.0 mL/min He to control polyatomic interferences at *m/z* 75	Separation was achieved in less than 20 min. Chromatographic recovery was in the range 90–107% as compared to total As in acid-digested samples; DLs of 0.35, 0.41, 0.45 and 0.70 ng/g were obtained for As(III), DMA(V), MMA(V) and As(V), respectively. AsB was included to ensure adequate separation from As(III). All juices analysed contained at least one As species at concentration higher than 0.5 ng/g	[128]

NIST SRM 1567a wheat flour and Se supplements	Se(IV), Se(VI), SeMet, MeSeCys, SeCys2	Powdered samples were treated with protease XIV in MW digestion system at 37 °C for 30 min and centrifuged. For total Se determination, wheat flour was MW digested with HNO_3/H_2O_2 and tablets were dissolved in HNO_3	Reversed-phase Econosphere C18 column (250 × 4.1 mm, 5 μm) was used with two mobile phases in gradient elution: 2 mM sodium 1-pentane-sulfonate, 5 mM citric acid in 3% CH_3OH (pH 2.70) and 5 mM NaH_2PO_4, 5 mM citric acid in 3% CH_3OH (pH 2.77). In on-line ICP-MS detection, potentially interfering $^{38}Ar^{40}Ar^+$ and $^{40}Ar^{40}Ar^+$ at Se *m/z* 78, 80, were reduced by using dynamic reaction cell with 1.0 mL/min CH_4	Using ion-pair chromatography, five Se species were resolved in 6 min. DLs of the procedure were in the range 0.04–0.07 μg/L Se. Recoveries of the speciation procedure evaluated by relating the sum of species to total Se in acid-digested sample were in the range 92–104%. SeMe was the main species in wheat flour; γ-glutamyl-Se-methylcysteine was identified in one of the Se tablets using ESI-MS/MS	[168]
Se-enriched food supplements	Se(IV), Se(VI), SeMet, MeSeCys, SeCys2	Ten commercial products were analysed; after homogenization and grinding, protease XIV digestion was carried out under probe ultrasonication (120 s); the samples were centrifuged and filtered (0.22 μm). For comparative purposes, MW-assisted digestion with HNO_3/H_2O_2 was obtained in pressurized closed system	Separation was performed on anion-exchange PRP-X100 column (250 × 4.1 mm, 10 μm) with 3 mM citric acid at pH 5 at a flow rate of 1 mL/min and UV detection. Off-line Se detection/ quantification in the column fractions was done by ETAAAS with Zeeman background correction	DLs evaluated for five Se species were 97 ng/g SeCys2, 90 ng/g MeSeCys, 200 ng/g Se(IV), 299 ng/g SeMet and 265 ng/g Se(VI). Two certified reference materials were used for accuracy checking. The results obtained by ultrasound- and MW-assisted extractions were in good agreement. Based on the labelled supplement composition, SeMet was expected as the main species; however in seven commercial products Se(IV) was found as the most abundant	[166]
Se-enriched edible sprouts	Se(IV), Se(VI), SeMet, SeCys2	Soy, alfalfa and lentil sprouts were enriched with Se in hydroponic cultures (Se(IV)); shoots and roots were dried and ground separately. Aqua regia mineralization and MW digestion with HNO_3/ H_2O_2 were performed. For enzymatic hydrolysis, protease XIV in phosphate buffer at pH 7.5 was used (37 °C, 16 h). The extracts were filtered before analysis (0.45 and 0.22 μm)	Separation was achieved on anion-exchange PRP-X100 column (250 × 4.1 mm, 10 μm) using phosphate buffer mobile phase (pH 7.0) at flow rate 1.5 mL/min. On-line ICP-MS detection was used; CRC was pressurized with H_2 for elimination of polyatomic interferences and *m/z* 78 was monitored (Rh as internal standard). Total Se after acid digestion was determined by HG-AFS after pre-reduction to Se(IV) with 6 M HCl	For accuracy testing, reference materials certified for total Se were analysed. Enzymatic extraction efficiencies obtained for different samples were in the range 67–120%; DLs for Se species were between 0.01 and 0.07 mg/kg. Biofortification of sprouts in hydroponic cultures was demonstrated for all sprout types; the primary species found in alfalfa and soy was Se(VI), whereas for lentil it was SeMet. Oxidized SeMet was also detected; however MeSeCys was not present in any sample	[165]

(Continued)

Table 18.3 (Continued)

Sample	Element species	Extraction	Chromatographic/detection conditions	Additional comments	Reference
Se-enriched edible mushrooms	SeMet, MeSeCys, SeCys2	*Agaricus bisporus* cultures were biofortified with Se by irrigation with Se(IV); caps and stalks were freeze-dried and homogenized separately. Proteins were extracted with 30 mm Tris-HCl, pH 7.5; the supernatant was collected and protein fraction was obtained by precipitation with 90% acetone (4 °C, 48 h) followed by redissolution in Tris-HCl. For enzymatic digestion, trypsin (50 °C, 24 h) and Proteinase K (50 °C, 24 h) were used. In the obtained hydrolysate, amino acids were derivatized with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate prior to LC-ESI-MS analysis. For total Se in the raw biomass and Se in protein fraction, the samples were wet digested with HNO_3/H_2O_2 in a heating block; Se pre-reduction to Se(IV) was carried out with HCl and the sample was transferred to HG-AFS detection system	Separation of derivatized amino acids was carried out on reversed-phase Zorbax Eclipse XDB-C18 Rapid Resolution HT column (50 × 2.1 mm, 1.8 μm) using gradient elution with water and acetonitrile mobile phases, both containing 0.1% HCOOH, at flow rate 0.3 mL/min. HPLC system was coupled to an ESI-triple quadrupole MS (ESI-QqQMS) operated at positive mode with multiple reaction monitoring	Total Se in mushrooms and in protein fractions increased in a dose-dependent mode. In protein digests, selenocysteine detected as SeCys2, SeMet and MeSeCys were separated, identified and quantified by LC/ESI-MS. For the three species, their concentrations significantly increased in Se-biofortified mushrooms compared with controls. SeCys2 was found as the most abundant species in protein fraction, whereas Se-amino acids were not detected in non-protein fraction	[169]
Se-enriched rice	Se(VI), Se(IV), SeMet, SeCys2	Crushed and milled grains were digested in a solution containing lipase and protease (37 °C, 16 h). The mixture was centrifuged and the obtained supernatant was filtered (0.22 μm) prior to its introduction to CE/ICP-MS system	Home made CE (80 cm × 75 μm capillary) coupled to ICP-MS system was used with electromigration injection (10 s) and separation voltage 16 V. Running buffer comprised 20 mM NaH_2PO_4 + 10 mM $Na_2B_4O_7$ + 0.2 mM CTAB (pH 8.60). Between each run, the CE capillary was flushed with Milli-Q water and running buffer for 2 min, respectively. For ICP-MS Se detection, *m/z* 78 and 80 were monitored and reaction cell was pressurized with 3 mL/min H_2 to eliminate polyatomic interferences from argon dimmer ions, 5% CH_3OH was added for increased sensitivity	Separation of four Se species was achieved in 18-min; DLs of 0.1–0.9 ng/mL were reported. Recoveries obtained in spiking experiments were in the range 90–103% for individual species with relative standard deviation for six replicates 3–7%. In Se-enriched samples, only SeMet was detected in the concentration range 0.136–0.143 μg/g (as Se)	[170]

Se-enriched carrot	Se(VI), Se(IV), SeMet, MeSeCys, γ-Glu-MeSeCys, AllSeCys, PrSeCys, SeOMet, TMSe	Foliar application of Se(IV) or Se(VI) was used. After harvesting, plant parts were separated, minced, freeze-dried and homogenized. Non-protein-bound Se species were extracted and proteolytic digests were obtained. All extracts were centrifuged and filtered prior to chromatographic separation. For total Se determination, MW digestion with HNO_3 was done	Strong cation-exchange column Ionospher 5C (100 × 3 mm, 5 μm) was used with salt gradient elution from 0.75 to 10 mM pyridinium formate at pH 3.0 and 3% CH_3OH, flow rate 1 mL/min, 30 °C. For strong anion-exchange column ION-120 (120 × 4.6 mm, 5 μm), a salicylate gradient (0.1–20 mM), pH 8.5, in the presence of 3% CH_3OH was used, flow rate 1 mL/min at room temperature. On-line ICP-MS detection at *m/z* 78 and 80 was carried out with CRC operated at H_2 flow 3.5 mL/min. Cation-exchange mobile phase was changed to volatile $HCOONH_4$ and column effluent was introduced to ESI-MS/MS operated in positive mode. Multiple reaction monitoring transitions were set for the detection of γ-Glu-MeSeCys and SeMet	SeMet, MeSeCys, Se-allylseleno-cysteine (AllSeCys), selenohomocystine (SeHoCys2), γ-glutamyl-Se-methylselenocysteine (γ-glu-MeSeCys), Se(IV) and Se(VI) were separated on cation-exchange column within 12 min. Anion-exchange column in a 16-min run enabled separation of γ-glu-MeSeCys, MeSeCys, AllSeCys, Se-propylselenocysteine (PrSeCys), SeMet, selenomethionine Se-oxide (SeOMet) and trimethylselonium ion (TMSe). Sample extracts were diluted prior to LC in order to counterbalance deteriorating effect of sample matrix on chromatographic performance. Se(VI)-enriched carrot roots accumulated up to 2 μg/g Se: SeMet and γ-Glu-MeSeCys were the main organic Se species; SeMe was confirmed by HPLC/ESI-MS/MS, although the concentration of second species was too low for positive confirmation	[167]
Cactus pear grown on Se-rich sediment	Se(VI), Se(IV), SeMet, MeSeCys, SeCys2	Three different cultivares were harvested from agricultural drainage sediment rich in salts, boron and selenium; control plants were also included. Fruit and clacode were freeze-dried and homogenized, and water extracts and protease XIV digests were obtained for chromatographic analyses. For bulk XANES analysis, samples were macerated in liquid nitrogen, packed into 2-mm path length sample cells	Strong anion-exchange PRP-X100 (250 × 4.1 mm, 10 μm) was used with 5 mM ammonium citrate, pH 5.2, mobile phase, containing 2% CH_3OH and flow rate 1 mL/min. For on-line ICP-MS detection, *m/z* 78 and 80 were monitored, octopole reaction cell was pressurized with 5.5 mL/min H_2 + 0.5 mL/min He for interference elimination. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was run to check for Se-containing proteins. Bulk XANES spectra were collected	There was no change in total Se between aqueous and enzymatic fruit extracts whereas for clacode, protease extracts contained 1.5–1.9 times more Se than water extracts. The results of gel electrophoresis showed Se incorporation to proteins; HPLC/ICP-MS enabled quantification of Se(VI), SeMet, MeSeCys, Se-cystathionine (SeCyst) and Se(IV). XANES analysis showed one higher energy peak ascribed to Se(VI) and lower energy feature of organic C–Se–C forms. Linear combination fitting enabled assessment of profiles of Se distribution in Se(VI), orgSe, Se^0 and Se(IV). Differences between cultivars were quantitatively evaluated. HPLC/ICP-MS and XANES provided complementary data. Elaboration of Se-biofortified nutraceutical food products from Cactus pear was suggested	[164]

(Continued)

Table 18.3 (Continued)

Sample	Element species	Extraction	Chromatographic/detection conditions	Additional comments	Reference
Brazil nuts	Se-containing peptides and proteins	Ground nuts were defatted by extraction with two *n*-hexane portions, and protein extract was obtained with 30 mM Tris-HCl, pH 7.5, containing polyvinylpyrrolidone (PVP), EDTA, dithiothreitol (DTT) and phenylmethylsulfonyl fluoride (PMSF) at room temperature, 12 h. In separate aliquots of the supernatant, bulk, fractional or Se-containing proteins were precipitated by controlling concentration of $(NH_4)_2SO_4$; after 30 min in ice-water bath, the supernatants were separated and the pellets were dissolved in 30 mM Tris-HCl (pH 7.5). Se-containing protein fraction was additionally desalted, concentrated by ultrafiltration and fractionated by SEC. Tryptic digests were also obtained	Size exclusion fractionation on Superdex TM peptide column with 30 mM Tris-HCl, pH 7.5, and 30 mM CH_3COONH_4, pH6.8 mobile phases was coupled to ICP-MS detection; column fractions were collected for trypsin digestion. The obtained digests were desalted by C-18 Zip-Tip, mixed with α-cyano-4-hydroxycinnamic acid in acetonitrile and 1% TFA, and analysed by MALDI-MS. Alternatively, trypic digests were acidified with 1% HCOOH and loaded on a C-18 capillary column (10 cm × 75 μm) for peptide separation in gradient elution of mobile phases composed of water, acetonitrile and 0.1% HCOOH at a flow rate of 7 μL/min (split 50 : 50). Column effluent was introduced to QTOF mass spectrometer; mass spectra and fragmented mass spectra were obtained	It was demonstrated that fractional precipitation by ammonium sulfate in selenoproteomics studies enables matrix reduction and sample concentration simultaneously, under non-denaturing conditions. SEC/ICP-MS showed Se association with both HMM and LMM proteins. Results of LC/ESI-MS and MALDI-MS analyses of tryptic digests allowed identification of several Brazil nut proteins in LMM Se-containing protein fractions. In particular, methionine-rich 2S albumins were identified as a most probable target for non-specific SeMet incorporation. Based on slightly modified isotope pattern observed for Se-containing peptides, the occurrence of their partial deamidation was proposed	[109]
Brazil nut, coco pulp, Cupuassu seeds	Molecular mass fractions of Cu, Fe, Mn and Zn	Nuts were milled, defatted with $CHCl_3$/CH_3OH (2 : 1); water extracts were obtained and after centrifugation the supernatants were introduced to HPLC system. For total element determination, MW acid digestion was carried out	Size-exclusion fractionation was obtained using Superdex 75 10/300 GL (3.0–70 kDa) column with 0.2 M Tris-HCl mobile phase (pH 7.5) and UV detection; 0.5-mL column fractions were collected for element determination by ETAAAS (Pd/Mg modifier). Water extracts and column fractions were dialysed, mixed with a saturated solution of synapic acid in 50% acetronitrile and 0.5% TFA and 0.5 mL was spotted onto the MALDI plate for MALDI-TOF-MS analysis	A combination of UV and ETAAAS detection enabled detection of element association with HMM compounds present in water extracts. Based on the fine structure of the MALDI-TOF mass spectra in 12 kDa region, it was proposed that Cu was associated with 11 kDa species, Fe with 6 kDa, Mn with 6 and 11 kDa and Zn with 6 kDa. For Se, the major peak eluted in molecular mass region corresponding to 11 kDa	[199]
Seafood	Se(IV), Se(VI), SeMet, SeCys, TMSe, Se-proteins	Brief description of extraction procedures reported in literature	Different LC separations with ICP-MS detection, molecular mass spectrometry techniques, X-ray absorption spectrometry	Comprehensive review describing knowledge of Se-proteins and non-protein Se compounds in edible marine organisms as compared with terrestrial food	[163]

Human milk and commercial infant formulas	Fe-binding proteins	Human milk was collected from mothers of preterm and full-term infants; respective infant formulas were dissolved in water, following standard hospital procedure. Samples were ultracentrifuged at 31,000*g* for 90 min at 4 °C. Total Fe was also assessed	Superdex 200 HR 130/10 column with 0.1 M Tris-HCl buffer, pH 7.0, mobile phase and flow rate 0.5 mL/min was used. The purity of the SEC fractions was checked by anion- or cation-exchange chromatography; Mono Q HR 5/50 column was used with salt gradient elution, 0–50 mM $CH_3COOHNH_4$, in Tris-CH_3COOH at pH 7.4. For cation exchange chromatography, the column was Mono S HR 5/50 and gradient elution was carried out with three mobile phases containing $CH_3COOHNH_4$ or 2-(*N*-morpholino) ethanesulfonic acid at pH 4.1, 6.2 and 6.3, respectively. Column fractions were freeze-dried and digested with trypsin under antioxidant protection. Column fractions and tryptic digests were desalted, mixed with matrix (synapic acid or α-cyano in acetonitrile + TFA aqueous solution) and deposited on a plate for MALDI-TOF analysis	Preterm and full-term formulas showed total Fe concentrations of 9.5 and 6.0 μg/mL, respectively; both values were significantly higher compared with the human samples (0.4 and 0.5 μg/mL). Differences observed between binding profiles of Fe to proteins in breast milk and in formulas were discussed within the context of Fe bioavailability. In particular, Fe was mostly associated with HMM region of biomolecules in human milk samples, whereas in formulas it was eluting in LMM region. Iron binding to α-lactalbumin, albumin, transferrin and lactoferrin was confirmed by peptide mass fingerprinting after tryptic digestion	[185]
Human milk	Zn-citrate	Frozen samples were thawed and left at 4 °C for 16 h, yielding separation of fat in the upper part; defatted milk was collected in a plastic syringe and centrifuged for 10 min. LMM fraction was obtained for speciation analysis by protein elimination in micro-ultrafiltration device. Total Zn was determined in defatted milk and in LMM fraction by FAAS or ICP-MS, after appropriate dilution	Weak CIM DEAE-1 anion-exchange tube monolithic column (6.7 × 4.2 mm, volume 1 mL) was used with aqueous/1.2 M NH_4NO_3 linear gradient elution at flow rate 1.0 mL/min; off-line FAAS or on-line ICP-MS detection was used. For species quantification, column fractions were off-line introduced to ICP-MS. Polyatomic interferences at *m/z* 66 ($^{32}S^{34}S^+$, $^{34}S^{16}O^{16}O^+$, $^{32}S^{16}O^{18}O^+$, $^{31}P^{35}Cl^+$, $^{54}Fe^{12}C^+$) were eliminated in CRC pressurized with He. For identification of Zn-binding ligand, mass spectra were obtained by orthogonal Z-spray ESI coupled to hybrid QTOFMS spectrometer operated in negative mode	Zn-citrate complex was obtained and its solution was stable for at least 5 days if stored in HEPES, MOPS or MES buffer, pH 5–7, at 4 °C. Separation of $[Zn(Cit)]^-$ and $[Zn(Cit)_2]^{4-}$ was achieved in 10 min and both species were confirmed by ESI-MS/MS analysis of respective column fractions; DLs for CIM DEAE FAAS and CIM DEAE ICP-MS were 10 and 0.5 ng/mL Zn, respectively. The reported column recoveries were 94–102% and relative standard deviation did not exceed 3%. In two samples provided by volunteers at different stage of lactation, about 23% of total Zn was present in LMM milk fraction and the citrate complex was the main species. It was suggested that citrate addition to infant formulas might enhance element bioavailability	[186]

(Continued)

Table 18.3 (Continued)

Sample	Element species	Extraction	Chromatographic/detection conditions	Additional comments	Reference
Edible seaweed	I^-, IO_3^-, MIT, DIT	Dehydrated commercial seaweeds were dried, ground in liquid N_2 and stored pulverized; ultrasound-assisted enzymatic extraction with pancreatin in phosphate buffer at pH 8 (50 °C, 12 h) was applied. Total iodine was determined after MW alkaline extraction with 25% TMAH	Dionex IonPac AS7 anion-exchange column was used with 175 mM NH_4NO_3 containing 15% CH_3OH mobile phase in the flow rate gradient and at room temperature. Gradient elution was also applied for reversed-phase Zorbax XDB-C8 column, using $H_2O/CH_3COOH/CH_3OH$ mobile phases. For ICP-MS detection, ^{125}Te was added as internal standard and CRC was pressurized with a mixture of H_2 and He, 8 : 92, at flow rate 4.5 mL/min	Inorganic iodine, MIT and DIT were separated by reversed-phase chromatography with DLs of 1.1 and 4.3 ng/g; elution of unknown I species was also observed. Extractable inorganic iodide and iodate were separated on anion-exchange column	[180]
I-fortified microalgae as food supplement	MM fractions, I^-, IO_3^-	Cells were collected by centrifugation and freeze-dried. The fractionation scheme was applied yielding water soluble iodine compounds and iodine macromolecular fraction. For total I, biomass was MW digested with TMAH	Two size-exclusion columns were used: Superdex-75 and Superdex-200 (300 × 10 mm, 13 μm) with effective separation range of 0.5–80 kDa and 10–600 kDa, respectively. Elution was carried out with Tris-HCl buffer (pH 7.0) at a flow rate of 0.75 mL/min. For anion-exchange separation, G3154A/101 (150 × 4.6 mm) column was applied wih 20 mM NH_4NO_3 mobile phase (pH 5.6), at flow rate 1.0 mL/min, 20 °C. On line ICP-MS detection was used	SEC/ICP-MS enabled detection of three molecular mass fractions of I in aqueous extracts, whereas anion-exchange chromatography confirmed that iodide was a primary water-soluble species (75% vs. 25% iodate). The iodine macromolecular fraction corresponded to about 13% of total I in the microalgae biomass. Possible application of biofortified algae as dietary supplement was suggested	[181]
Fish	As(III), As(V), MMA(V), DMA(V), Se(IV), Se(VI), SeMet	Aliquots of different fish types were freeze-dried; MW-assisted enzymatic hydrolysis with pronase E and lipase was carried out at pH 7.25, 37 °C for 30 min. The obtained extracts were centrifuged and filtered (0.22 μm). For total element determination, MW digestion with HNO_3 and H_2O_2 was applied	For size-exclusion chromatography, TSK–gel QC-PAK GFC (150 × 7.8 mm, 5 μm) column was used with 50 mM phosphate buffer containing 2% CH_3OH, pH 7.5, at flow rate 0.7 mL/min. For Metrosep Anion Dual 3 column (100 × 4.0 mm, 6 μm), a salt gradient was used from 5 to 50 mM NH_4NO_3 with 2% CH_3OH as organic modifier, pH 8.7 and flow rate 1 mL/min. On line ICP-MS detection was carried out at *m/z* 75, 77, 80	In the mixed standard solution, the following species were resolved by anion-exchange HPLC/ICP-MS: AsB, As(III), SeMet, DMA(V), MMA(V), As(V), Se(IV), Se(VI) in less than 7 min. After standard addition, the recovery values in dogfish liver DOLT-3 reference material were 99–106% for As, and 97–104% for Se species. Extraction revoveries in the real world samples were in the range 63–106% for As and 46–98% for Se; AsB and SeMet were found as the primary species. SEC/ICP-MS results indicated that Se-peptides or Se-containing proteins remained intact after MW-assisted enzymolysis	[178]

Shellfish	TMT, DBT, TBT, DPhT, TPhT	Different shellfish were purchased; for each type, soft tissues were pooled, freeze-dried and powdered. Fresh material was also analysed. Extraction with the chromatographic mobile phase was carried out under ultrasonication (30 min), the samples were centrifuged and filtered (0.45 μm). Alternatively, CH_3OH/acetate buffer, pH 4.5 (1 : 1) was used as extractant. For total Sn determination, samples were MW digested with HNO_3/H_2O_2	Reversed-phase TC-C18 (250 × 4.6 mm, 5 μm) column was used with acetonitrile/H_2O/CH_3COOH/TEA (65 : 23 : 12 : 0.05), pH 3 and flow rate 0.4 mL/min. To reduce plasma solvent loading in ICP-MS detection, the double-pass spray-chamber was Peltier cooled to −5 °C; make up gas (O_2/Ar 30%) was added to convert organic carbon to CO_2; ^{118}Sn was monitored in on-line ICP-MS detection	Baseline separation of TMT, DPhT, DBT, TPhT and TBT was achieved in 35 min; in shellfish samples additionally two unidentified species were eluted. DLs evaluated based on 3S/N were in the range 0.24–0.37 μg/L for five species. The recoveries evaluated after standard addition for TMT, TBT and TPhT were ~80%; however, the recoveries for DPhT and DBT were lower (about 40% and 50%). Better recoveries were obtained using mobile phase for extraction. Primary species found in marine organisms were TBT and TPhT	[197]
Tea, bread	Cr(III), Cr(VI)	Tea infusions were prepared with boiling water doubly spiked with 10 μg/L ^{50}Cr(VI) and 10 μg/L ^{53}Cr(III), left to cool, filtered and immediately analysed; in parallel, alkaline extraction with Na_2CO_3 was also performed. Spiked bread samples were extracted with 10 mM NaOH with agitation (17 h). The supernatants were filtered	Strong anion-exchange Mono Q HR 5/5 column (50 × 5 mm, 10 μm) was used with linear gradient elution from 100% water to 100% 0.7 M NaCl, flow rate 1.5 mL/min, separation in 10 min. For ICP-MS detection, *m/z* 50, 52 , 53 were monitored. Species interconversion was followed by isotope ratios measurements. Polyatomic interferences were controlled by high energy collision mode CRC	Cr(VI) was not detected in any of the samples analysed; oxidation of Cr(III) in the tea and bread samples was shown. Almost all added ^{50}Cr(VI) was reduced in tea infusions to ^{50}Cr(III), due to the presence of antioxidants. Partial reduction of ^{50}Cr(VI) was observed even in highly alkaline bread extracts (pH 12). It was concluded that the antioxidants and organic matter present in the food matrices analysed prevent Cr(VI) formation	[196]
Spinach, onions, carrots	Sb(III), Sb(V), TMSb(V)	Agitation or sonication-assisted extraction with four different extractants (water, citric/oxalic/formic acids, EDTA, methanol/water) was tested; 0.01 M EDTA at pH 2 was finally selected. The mixture was centrifuged and supernatant taken for LC-HG-AFS analysis	Anion-exchange PRP-X100 column (100 × 4.1 mm, 5 μm) and gradient elution with the mobile phases containing EDTA, potassium hydrophthalate and phosphate at pH 8.3 was used; before hydride generation in a commercial HG-AFS system, a post-column pre-reduction to Sb(III) was performed by mixing the column effluent on-line with 2% L-cysteine in HCl	Detection limits for Sb(V), Sb(III), TMSb(V) were 0.08, 0.07 and 0.9 μg/L, respectively. Extraction yield of total Sb from vegetables was in the range 50–70% and the only specied detected in vegetables was Sb(V)	[198]
Different brands of tea	Ni(II), Ni-quinate, Ni-citrate	Aliquots of ground homogenized raw material was acid digested in MW oven; hot water infusions were left 3, 5 and 10 min, then cooled, filtered and both sample types were analysed for total Ni, whereas speciation analysis was carried out in infusions	Weak anion-exchange CIM DEAE-1 monolithic column with aqueous/0.6 M NH_4NO_3 linear gradient elution was applied for 10 min at flow rate 1.0 mL/min and the effluent was on-line introduced to ICP-MS. Identification of Ni species after LC was performed by QTOFMS equipped with two types of ESI sources (negative mode)	LC/ICP-MS detection limit based on 3σ criterion was 0.15 μg/L for the three Ni species (0.006 μg/L for total Ni by ICP-MS). Total concentration in teas was about 5.4 mg/kg and up to 85% was found in infusions. The main species in different teas was Ni associated with quinic acid (except hibiscus)	[195]

Table 18.4 Representative examples of analytical speciation procedures based on gas chromatography separations (2010–2013).

Sample	Element species	Pretreatment	Chromatographic/detection conditions	Additional comments	Reference
Standard solution	MeHg	Standard MeHgCl aqueous solution was used; this species was collected in a sorption tube packed with a mixture of Tenax TA at –10 °C for 5 min. Afterwards the tube was heated at 320 °C for 20 min for thermal desorption of the analyte to GC system	*SPE-GC-TOFMS* CP-Wax column (30 m × 0.25 mm, 0.25 μm) was used with the temperature rise from 100 to 210 °C; He carrier gas at flow rate 1.2 mL/min and a splitless injection were applied. Interface and ion source were kept at 230 °C. In total ion chromatogram mode (TIC), the *m/z* range was 35–260; extracted ion chromatograms were then obtained in the four mass ranges overlapping MeHgCl	This work has been presented as proof of concept for the quantitative analysis of MeHg by cryotrapping from liquid phase followed by thermal desorption and GC-TOFMS analysis. The method DL was 1.86 pg and relative standard deviation 1.82%	[222]
Fish	Hg(II), MeHg	Fish meat was homogenized, dried and ground. MSPD was as follows: the sample was blended with SiO_2 to obtain a homogeneous mixture and the extraction solution (0.5 M NaCl and 4.2 M HCl) was added, the mixture was vortexed (1 min) and centrifuged. Ethylation reaction was carried out in the supernatant (1% $NaBEt_4$, pH 5), the derivatized species were extracted to hexane and introduced to GC-MS. Total Hg was determined after MW nitric acid digestion by flow injection cold vapour generation coupled to ICP-MS	*MSPD-GC-MS* Separation was carried out on Rtx-5MS column (30 m × 0.25 mm, 0.25 μm) with He carrier gas and temperature gradient from 130 to 270 °C; ion source and interface were maintained at 230 and 250 °C, respectively. MS was operated in the selective ion monitoring mode, *m/z* values for MeHgEt were 51, 77, 91, 217, 279, 294, and for $Hg(Et)_2$ 51, 77, 154, 279, 356	Fractional factorial design was applied for the optimization of extraction conditions. The DLs of the method evaluated based on S/N = 3, were 0.06 μg/g MeHg and 0.12 μg/g Hg(II). Satisfactory resultas were obtained in the analysis of two certified materials DOLT-3 and DORM-2. Recoveries of the speciation procedure, evaluated by comparing total Hg in acid-digested samples with the sum of species determined by GC-MS, were close to 100%. Due to suppressive matrix effects, it was recommended to run the calibration solutions and the samples using this same matrix solid-phase extraction procedure	[74]
Fish	MeHg	Edible tissues of marine and freshwater fish collected in Ghana were homogenized; extraction with 1 M KOH was carried out in a water bath (100 °C, 1 h). MeHg complex with dithizone was formed in toluene and, after clean-up by liquid–liquid extraction (LLE), toluene phase was injected to GC system	*LLE-GC-ECD* Glass column packed with Hg-20 A on Uniport HP AW-DMCS (3 m × 3 mm, 60–80 mesh) was used with 2–3 cm length of the injection section packed with NaCl for on-column conversion of MeHg–dithizone complex to chloride; N_2 carrier gas at 40 mL/min was used; column oven was kept at 150 °C, injector port and electron capture detector oven at 200 °C	The DL was evaluated as 0.5 μg/kg. For accuracy testing, DORM-2 dogfish reference material was analysed and recovery experiments in the real world samples were performed.The concentration range found in analysed fish tissues ranged from 0.009 to 0.107 mg/kg based on the wet mass	[219]

Fish	Hg(II), MeHg	Extraction was carried out with KOH/C_2H_5OH at 100°C, 1 h and after further purification and pH adjustment (pH4.9), dithizone complex was formed in toluene. Derivatization by ethylation was accomplished after mixing of back-extracted water solution, $NaBEt_4$ and acetate buffer in a syringe. Finally, an aliquot was injected to the purge-and-trap system (Tenax A trap), the sample was purged with He gas at 40 mL/min (40 °C, 15 min) and desorbed 200 °C for 3 min	*CVG-GC-MS* DB-5MS capillary column (30 m × 0.5 mm, 0.25 μm) was used with He carrier gas at flow rate 1 mL/min. The column temperature was programmed from 40 °C up to 280 °C; the injection port and detector were operated at 220 and 230 °C, respectively. MS was operated in selected ion monitoring mode, using *m/z* 202, 217 and 246 for MeHgEt; and *m/z* 202, 231 and 260 for HgEt2	In addition to development of a purge-and-trap procedure, this study was undertaken in order to describe the current status of total mercury and methylmercury levels in the freshwater fish and sediment from Korean peninsula	[223]
DORM-2 DOLT-3	Hg(II), MeHg	Alkaline extraction with TMAH was carried out in a closed vessel MW system. For derivatization, pH was adjusted to 3.9 and $NaBEt_4$ (0.3%) was added together with hexane. The sample was shaken (5 min), centrifuged and organic layer was collected for GC analysis	*CVG-GC-pyro-AFS* Non-polar capillary column TRB-5 (30 m × 0.25 mm, 0.25 μm) with He as a carrier gas was used; column effluent was transferred to pyrolysis oven for generation of Hg^0, Ar was added as a make-up and a sheath gas prior to ASF detection *CVG-GC-MS* The column was DB-5MS (30 m × 0.25 mm, 0.25 μm) with He as a carrier gas; in MS detection the *m/z* 246 and 260 were monitored for MeHgEt and HgEt2, respectively *GC/ICP-MS* The outlet of Crossbond column (30 m × 0.53 mm, 1 μm) was connected through a transfer line (170 °C) to ICP-MS	Robustness of each procedure was tested varying chromatographic conditions in a Plackett–Burman fractional factorial model. The three procedures showed sufficient sensitivity and selectivity for the speciation of mercury in the real world matrices. The recoveries of Hg species in CRM dogfish were in the range 95–101%	[220]

(Continued)

Table 18.4 (Continued)

Sample	Element species	Pretreatment	Chromatographic/detection conditions	Additional comments	Reference
Terrestrial animals	Hg(II), MeHg	Red deer liver and kidney and wild boar kidney were freeze-dried and MW extraction with TMAH was carried out, followed by derivatization with $NaBEt_4$ at pH3.9. The derivatives were recovered by headspace SPME using fused silica fibre coated with a100-μm film of PDMS. For sorption, solution was kept at 35 °C for 10 min; the desorption conditions were 200 °C, 0.5 min	*CVG-HS-SPME-GC-pyro-AFS* DB-5 (30 m × 0.25 mm, 0.25 μm) was used with splitless injection and He carrier gas at 3 mL/min. For ASF detection, Hg^0 was generated in pyrolyser at 800 °C	The procedure DLs evaluated for liver were 31.8 ng/g and 52.5 ng/g for MeHg and Hg(II); for kidney the DLs were 35.3 and 58.1 ng/g, respectively. The headspace SPME extraction enabled lowering of DLs 5.5–6 times as referred to the procedure without SPME. The two Hg species were quantified in animals from the mining district area	[221]
Marine organism reference materials: SRM 1947, 1566b, TORT-2	MeHg, EtHg, PrHg	Samples were defatted with acetone, internal standard was added and pancreatin digestion was carried out at 37 °C, 12 h, and followed by acid extraction (HCl) for 3 h. Derivatization with $NaBPh_4$ was accomplished at pH 4–5 on the ice bath. The derivatized species were extracted to iso-octane or collected by headspace SPME on PDMS fiber for 50 min at 65 °C and then desorbed at 220 °C, 1 min	*CVG-HS-SPME-GC-ICP-MS* DB-5MS column (30 m × 25 mm, 0.25 μm) was used with He carrier gas containing 1% Xe at flow rate 1 mL/min, and splitless injection; 50–280 °C temperature program, injector temperature was 220 °C. For ICP-MS detection, a transfer line was maintained at 280 °C; *m/z* monitored were 200, 201 and 202	Phenylation of Hg species yielded better chromatographic performance as compared with propylated compounds. ICP-MS detection provided better analytical performance compared with HS-SPME-GC-MS. PrHg was used as surrogate standard and enriched $Me^{101}Hg$ was used as internal standard. The DL evaluated corresponded to 0.3 μg/kg Hg in dry food matrix. Using PrHg, slightly worse recoveries and precision were obtained as compared to isotope dilution method	[224]
Fish, mollusc, crustacean; fresh and cooked	Hg(II), MeHg	Aliquots of $^{199}Hg(II)$ and $^{201}MeHg$ were added to the freeze-dried samples, TMAH 25% was used for extraction and three closed-vessel procedures were tested: (1) heating at 85 °C, 2 h; (2) MW-assisted extraction, 3 min; (3) room temperature extraction, 24 h. Three types of agitation were also evaluated. After pH adjustment (pH 4), alkylation with $NaBPr_4$ and extraction to iso-octane was accomplished; the samples were centrifuged and placed in GC vials	*CVG-GC-ICP-MS* MXT-1 column (30 m × 0.53 mm, 1 μm) was used with He flow rate 25 mL/min and temperature gradient between 60 and 280 °C; transfer line was maintained at 280 °C. Isotope ratios $^{199}Hg/^{202}Hg$ and $^{201}Hg/^{202}Hg$ were measured for both Hg species, mass bias was monitored by $^{203}Tl/^{205}Tl$ measurement	Solid–liquid extraction (procedure 1), derivation by propylation and rotary agitation were selected based on the results obtained for the certified reference materials (DOLT-4, TORT-2, BCR-464). Among the three isotope dilution methods tested, double species-specific IDA and isotope pattern deconvolution provided Hg(II) and MeHg concentrations simultaneously and enabled control of methylation and demethylation transformations. Method quantification limits were 1.2 μg/kg for MeHg and 1.4 μg/kg for total Hg (as referred to Hg in dry mass). Results obtained in the analysis of real world samples were presented and discussed	[18]

Fish oil	Arsenic-containing hydrocarbons	Several oil samples were decontaminated by activated carbon and steam deodorization; afterwards As species were partitioned into hexane and H_2O/CH_3OH phases. The latter fraction was cleaned up on a weak anion-exchange SPE cartridge; after elution, the solvent mixture was evaporated, the residue dissolved in hexane and filtered prior to GC/ICP-MS, GC/MS-MS and HPLC-qTOF-MS. Total As was determined in the whole oils and all their fractions by ICP-MS after MW digestion with HNO_3/H_2O_2	*GC/ICP-MS* HP-5 (30 m × 0.32 mm, 0.25 μm) column was used with 2 mL/min He as a carrier gas; 1-μL injection was in a splitless mode. Temperature program 50–280 °C was applied and the effluent was transported via heated transfer line to ICP-MS; triphenylarsine was used for external calibration and for internal control of the retention behaviour of the chromatographic system *GC/MS-MS* The column was Rtx®-5SIL MS (30 m × 0.25 mm, 0.25 μm), He flow 0.8 mL/min; MS spectra were registered in the *m/z* range 80–450 in a positive mode. Collision induced dissociation in a selected reaction monitoring mode was used for MS/MS measurements *HPLC-qTOF-MS* EC-C8 Poroshell 120 (100 × 2.1 mm, 2.7 μm) column was used with a gradient of mobile phases containing formic buffer and acetonitrile, at flow rate 0.3 mL/min. The scan range was from *m/z* 100 to 1000 in positive mode	GC/ICP-MS enabled detection and quantification of As-containing compounds in SPE extracts. GC/MS-MS results provided identification of the molecular structures. HPLC-qTOF-MS was used for determination of accurate masses of the As hydrocarbons. Three dominant As-containing hydrocarbons found in all fish oils analysed were dimethylarsinoyl hydrocarbons ($C_{17}H_{38}AsO$, $C_{19}H_{42}AsO$ and $C_{23}H_{38}AsO$); these species accounted for 13–35% of the total arsenic in the fish oils. Not all species were identified	[227]

(Continued)

Table 18.4 (Continued)

Sample	Element species	Pretreatment	Chromatographic/detection conditions	Additional comments	Reference
Canned cod liver	Arsenic-containing hydrocarbons	Fish tissue was separated from can oil, cut in small pieces and arsenolipids were ultrasound extracted with CH_3OH (room temperature, 24 h). Once solvent was evaporated, the obtained oil was fractionated by SPE; the column was packed with silica gel and the retained As species were eluted in 12 fractions, using ethyl acetate/methanol mixtures of increasing CH_3OH percentage. Each fraction was evaporated and redissolved in CH_3OH for GC analysis	*GC* Separation was carried out on HP-5MS (30 m × 0.25 mm, 0.25 μm) column with 2 mL/min He as carrier gas, in a splitless mode and 40–280 °C temperature gradient; transfer line was maintained at 280 °C *GC/ICP-MS* Column outlet was located at the torch tip and Ar/O_2 make-up gas was used *GC-MIP-OES* Column effluent was transferred to MW-induced plasma optical emission system *GC-MS* The feasibility of MS detection after electron impact ionization and single quadrupole mass filter was examined. The extracts were injected hydrodynamically to ESI source of TOF-MS for exact mass acquisition in positive mode	Numerous As compounds were detected in SPE fractions and the results obtained by ICP-MS and MIP-OES were consistent. The presence of As compounds was also confirmed by GC-MS, yet their concentration levels were too low for molecular mass assessment. ESI-TOF-MS allowed accurate mass determination of three As-containing hydrocarbons with the following molecular formulas: $C_{17}H_{37}AsO$, $C_{19}H_{41}AsO$ and $C_{23}H_{37}AsO$. Suggestions for the corresponding structures were discussed	[226]
Se-yeast	Se-amino acids	MW-assisted hydrolysis with 4 M methanesulfonic acid was carried out in a closed system; extract was adjusted to pH3 (NaOH) and DTT was added. For derivatization, pyridine/methanol (1 : 3) and methyl chloroformate were added. After extraction to chloroform, and centrifugation, organic layer was transferred to GC vials	*CVG-GC-MS* DB-5MS (30 m × 0.25 mm) column was used with 1.5 mL/min He carrier gas, 1 μL splitless injection and 120–290 °C temperature gradient; transfer line was kept at 260 °C and MS ion source at 250 °C. MS spectra were acquired in the *m/z* range 50–500 and selected ion monitoring was used for final quantification	Standards of amino acids were efficiently derivatized and then separated in 12 min; 17 compounds could be determined without any interference from concomitant substances with DLs in nanomolar range. Spike recovery of all 17 compounds showed values in the range 95.4–106.8%. SeCys was not detected in yeast samples	[235]

Potatoes	SeMet, MeSeCys, SeEt	Commercial potatoes enriched with Se and I, and non- enriched, were homogenized and freeze-dried; water extract (1 min) was obtained and centrifuged. For derivatization, propanol and pyridine mixture and then propyl chloroformate were added to the supernatant; after acidification to pH 2 and NaCl addition (10%), the derivatized species were sampled by immersion SPME. For desorption, the fibre was placed in the injector set at 270°C for 10 min	*CVG-SPME-GC-ITMS* Ion trap device was used for method development. For quantification, triple quadrupole instrument (GC-QqQMS) was used and operated in electron ionization and selected reaction monitoring mode. The column was TR-5MS (30 m × 0.25 mm, 0.25 μm), 1 mL/min He carrier gas and Ar as collision gas were used; temperatures set for transfer line and ionization source were 270 and 250°C. The *m/z* transitions for SeMet, MeSeCys anf SeEt were 325 → 188, 311 → 208 and 339 → 188, respectively	Variables influencing the efficiency of SPME analysis were optimized by the multivariate approach using central composite design; divinylbenzene/carboxen/PDMS fibre was selected. The DLs evaluated on dry matter basis were 0.143 μg/kg for SeMeCys and 0.210 μg/kg for SeMet; SeEt was used as internal standard. The application of the method to commercial samples of selenized and non-selenium-enriched potatoes proved that Se fertilization increased significantly the concentration of seleno amino acids	[236]
Se-yeast, selenium supplements	MeSeCys, SeMet, SeMetO, DMDSe, DMSe	Powdered tablets were dried and protease XIV hydrolysis was carried out at room temperature for 24 h with shaking. The obtained extracts were centrifuged and filtered (0.45 μm and10 kDa). Ethyl chloroformate derivatization was carried out in H_2O/C_2H_5OH/pyridine; the solutes were recovered in $CHCl_3$ and after evaporation redissolved in this same solvent. SPME fibre with PDMS coating was introduced into the headspace for extraction of volatile compounds (20 min) and then desorbed into GC injection port	CVG-HS-SPME-GC-AED Separation was obtained on SE-30 capillary column (30 m × 0.32 mm, 0.25 μm) with He 1 mL/min as a carrier gas, with split injection 30 : 1, 60–300°C temperature program; wavelengths monitored were 196 nm (Se), 181 nm (S) and 193 nm (C). For GC-MS, the column was DB-5MS (30 m × 0.25 mm, 0.25 μm) with the same temperature gradient and 1 mL/min He flow rate. Non-derivatized extracts were analysed by HPLC/ICP-MS, using reversed-phase column Symmetry ShieldTM RP-C8 (150 × 3.9 mm, 5 μm) and 0.1% heptafluorobutyric acid as ion-pair reagent. Dynamic reaction cell was pressurized with CH_4, 0.6 mL/min	Analytical results obtained in this work were discussed in relation to the previous hypotheses that MeSeCys found in yeast-based Se supplements might be formed from a reaction between dimethyldiselenide (DMDSe) and cysteine or cystine on heating. The need for species stability evaluation in commercial supplements was highlighted	[232]

(Continued)

Table 18.4 (Continued)

Sample	Element species	Pretreatment	Chromatographic/detection conditions	Additional comments	Reference
Milk, milk powder, yoghurt, fruit juices	DMSe, DMDSe	A headspace hollow fibre protected liquid-phase microextraction was optimized by the Box–Behnken design. The hollow fibre was located in the vial containing the sample; 3 μL of 1-decanol as extracting solvent were disposed to the fibre and withdrawn in a continuous mode at a speed of 20 μL/min by means of the mounted syringe. The obtained extract was introduced to GC system	*LLME-GC-MS* The column DB-624 (60 m × 0.25 mm, 1.40 μm) was used with 1.2 mL/min He as a carrier gas; 1-μL injection in a splitless mode was applied, temperature program was designed starting from 50 to 250 °C; temperature of GC-MS interface, MS source and quadrupole was 150, 250 and 150 °C, respectively. The scan range of *m/z* 30–300 was used. Ethylbenzene was used as internal standard	Under optimum conditions, pre-concentration factors up to 1250 and 1170 were achieved for DMSe and DMDSe respectively. The DLs for DMSe and DMDSe were 65 and 57 ng/L, respectively. Volatile Se compounds were not detected in the real world samples, but satisfactory recoveries were obtained after spiking the samples with 15 and 30 μg/L of the two species	[233]
Canned tuna fish	iSn, TeMT	Ultrasound extraction with aqueous 3% KOH/CH_3OH (5 : 1) mixture was carried out for 1 h; the obtained extracts were transferred to vials and pH was adjusted to 5.3; the vials were closed and $NaBEt_4$ (10%) was added via syringe. The PDMS fibre was exposed to the headspace for 15 min (25 °C, stirring) and then transferred to GC injector port	*CVG-HS-SPME-GC-MS* Desorption was carried out at 250 °C, 4 min; DB-5MS column (30 m × 0.25 mm, 0.1 μm) was used with 5 mL/min He as a carrier gas, the injection was splitless, column temperature gradient from 50 to 250 °C; the MS transfer line and ion source temperatures were 250 and 150 °C, respectively. Quantification was carried out in selective ion monitoring mode; *m/z* values monitored for TeMT were 161 and 165, while for TeET the *m/z* 205 and 207 were used	The derivatization of iSn and headspace SPME of both TeMT and iSn as TeET were performed in one step. The DLs evaluated for the mixed standards were 24 ng/L for TeET and 45 ng/L for TeMT. After standard addition to the certified reference material IAEA-433, the recovery for TeMT was in the range 87–97% and the recovery for iSn as TeET 88–94%. In the analysis or fish samples, only iSn was found	[239]

Shellfish	MBT, DBT, TBT, TPrT	Muscle from commercial shellfish was crushed; TPrT was added as internal standard and HCl 0.12 M/CH_3OH were added for ultrasound extraction (30 min); after centrifugation supernatant was adjusted to pH 5 for $NaBEt_4$ derivatization and to pH 3 for $NaBH_4$. For headspace SDME, 2 μL decane was introduced through a needle and volatile compounds were collected for 20 min at room temperature while stirring	*CVG-HS-SDME-GC-ICP-MS* HP-5 (30 m × 0.32 mm, 0.25 μm) column with 2 mL/min He as a carrier gas, temperature program from 55 to 200 °C and splitless injection were used. Injector and transfer line temperature were different for the two derivatization reagents; ^{118}Sn and ^{120}Sn were monitored in ICP-MS detection	TPrT was used as internal standard; derivatization with $NaBEt_4$ enabled better chromatographic separation and more sensitive detection compared with HG. The four species were resolved in 7 min; the DLs were 1.4 ng/L for MBT, 1.8 ng/L for DBT and 0.8 ng/L for TBT. The concentration ranges found in different shellfish samples were 11.6–30.4 ng/g MBT, 11.8–8.9 ng/g DBT and 12.8–52.6 ng/g TBT	[240]
Honey	MBT, DBT, TBT, MPhT, DPhT, TPhT, TCT, FBTO	An aliquot of honey was diluted with acetate buffer (pH 5); NaCl , diphenyltin (DPhT as internal standard) and $NaBEt_4$ (2%) were added; the vials were sealed and the mixture was kept at 90 °C with stirring for 1 min. Afterwards PDMS fibre was exposed to a headspace for 20 min (90 °C, stirring). The fibre was transferred to the injector port, thermal desorption was done at 270 °C for 2 min	*CVG-HS-SPME-GC-AED* Column (25 m × 0.32 mm, 0.17 μm) with 4 mL/min He carrier gas, temperature gradient 35–308 °C was used, transfer line temperature was 325 °C. For MS detection, HP-5MS (30 m × 0.25 mm, 0.25 μm) column with 1 mL/min He as carrier gas and pulsed splitess injection was used. Temperature gradient was 60–280 °C; transfer line and ion source temperature were maintained at 300 and 230 °C	Eight Sn species were resolved within 15 min; using GC the DLs were in the range 0.6–4.3 pg/g and for AED the range was 2.0–22 ng/g as referred to Sn in honey (internal standard used for quantification). For HS-SPME different sizes and fibre materials were tested. None of the organotin compounds was detected in honey samples analysed; the recovery experiments yielded average 89 ± 12% values. The procedure was also applied for the analysis of wines	[241]

by chemical vapour generation (CVG) owing to the low background and high tolerance to excessive hydrogen produced in the reaction with tetrahydroborate [19].

In addition to the well-established species and food matrices, the area of speciation analysis also involves innovative applications. To this end, the production of selenium biofortified food requires thorough characterization of accumulated element species [4]; arsenic species and their transformations must be fully characterized to evaluate possible health hazards due to consumption of seaweed containing arsenosugars [20, 21]; and studies aimed at the fate of As-containing feed additives in the food chain also require explorative chemical speciation [22, 23]. Another task for speciation analysis has been imposed by the increasing applications of phytochemicals for controlled delivery of mineral elements [24–26]. Since the presence of unknown or unexpected species/forms is often observed, a combination of atomic spectrometry with other techniques providing structural information becomes necessary.

Finally, a variety of micro- or nano-structured materials are incorporated in dietary products; food additives such as TiO_2, SiO_2 or micronutrient nano-additives should be mentioned. Furthermore, food is often exposed to nano-structured antimicrobial salts, oxides or elemental colloids (Ag) used for disinfection and also through contact with packing polymers containing nano-forms of Ti, Si, Cu, Zn, Sn and Ag integrated in order to extend food shelf-life [27]. Environmental contamination with nano-dimensional materials is another source of element forms/species in food [28]. Detection, quantitative characterization of size distribution, studies on potential bioavailability and chemical transformations of elements contained in these materials fall within the domain of food speciation analysis. In particular, the analytical information generated is crucial for a better understanding of potential risks associated with intentional use of nanomaterials in food elaboration or with food contamination.

18.3 Analytical procedures in food speciation analysis

18.3.1 Fractionation procedures used to assess element/species bioaccessibility or bioavailability

Food ingredients of plant or animal origin contain mineral elements associated with high molecular mass constituents (proteins, polysaccharides, lipids) and also present as low molecular mass species such as free ions, organic complexes or amino acids; this variety of forms and species is the main reason for inefficient delivery of elements from food to the organism. In this sense, the results of speciation analysis allow for assessment of bioaccessible and bioavailable fractions. It has already been commented that natural species distribution may be altered in the course of food preparation, since different ingredients are mixed, pH conditions and moisture content are altered, and elevated temperature can be applied. For the evaluation of changes occurring during food elaboration, analysis of the raw material and of the processed food has been undertaken [10, 24, 29–32]. Those food components acting as enhancers or inhibitors of element uptake from food have been included in a few the speciation studies (Table 18.1) [26, 32–36]. In particular, accessibility of elements from transgenic plants exhibiting different metabolite profile compared with their natural varieties has received attention [37].

On the other hand, the solubilization of elements/species from food during gastrointestinal digestion and subsequent absorption through gastrointestinal membranes has been extensively studied. The most complete *in vitro* models involve simulation of the processes occurring during passage of food from mouth to the bloodstream and consist of the successive treatment with saliva, gastric and intestinal juices, exposure to intestinal microbiota, and absorption through a suitable gastrointestinal membrane; for better imitation of *in vivo* conditions, normal human body temperature, corresponding time periods and agitation have been applied at each stage [12, 37, 38]. Aliquots have been withdrawn for analysis of elements/species at different stages of digestion and, depending on food type and element/species of interest, some authors have reported saliva or gastric enzymolysis as the most efficient process [30, 39, 40]. Salivary enzymes, amylase and lipase, initiate degradation of polysaccharides and fats, so that solubilization of element forms associated with these food ingredients can be expected. In the stomach, acid gastric juice contains different proteases that yield release of metal/metalloid species bound to proteins or contained in metalloproteins, thus increasing their bioaccessibility [31]; accordingly, some authors simulated food digestion by only applying gastric juice [41]. Direct enzymolysis with amylase or proteolytic enzymes has also been explored [40, 42, 43]; the results of gastrointestinal digestion have been compared with those obtained after extraction with aqueous buffer solutions at physiological pH

[31, 42–45]. It is worth noting that the increase in pH in the duodenum may favour rearrangement of species distribution established previously, thus altering actual species bioaccessibility in the small intestine; such is the case with the inhibitory effect of phytic acid on iron and zinc absorption [46]. To evaluate element/species absorption through the gastrointestinal tract, dialysis [10, 38, 47], liposome [26, 37] or Caco-2 cell cultures [35, 48, 49] have been used as a model membrane. It should be noted that the design of continuous leaching is an interesting alternative to batch fractionation, since it enables shortening and simplification of the procedure and also facilitates transfer of the obtained fractions to chemical speciation system [30, 39].

In terms of speciation analysis after sample fractionation, the main effort is still concentrated om Se, As, Hg and also I [50]; typical speciation schemes involve chromatographic separation followed by atomic mass spectrometry detection, as shown in Figure 18.1 and Table 18.1 and further discussed in sections 18.3.3 and 18.3.4 [24, 29–31, 39, 40, 44, 45, 47, 51, 52]. On the other hand, total element determination after fractionation is also relevant since such approaches allow multi-element determination to be easily accomplished, thus providing information on possible similarities or differences in the behaviour of individual elements [10, 26, 32, 36, 41]. Examination of total element concentrations may also contribute to a better understanding of possible interactions among elements and their species during food processing and digestion, as established for Fe, Zn, Cu and Mn [17, 25, 32, 37], for As in the presence of Fe [33], and in relation to Se antagonism of heavy metals extensively studied in human nutrition [53, 54].

Finally, the application of enzyme-aided fractionation and/or membrane models in studies on nanoparticles in food is a relatively new area of research. Analytical data of potential interest for bioaccessibility/bioavailability assessment include determination of total element content derived from nanoparticles, their size distribution, morphology, solubilization and possible chemical transformations. Enzymolysis experiments have been combined with field-flow fractionation and atomic spectrometry detection; the application of electron microscopy tools and X-ray spectrometric techniques should also be highlighted [27, 42, 43, 48].

Another approach for evaluation of species bioavailability relies on *in vivo* studies; basic concepts, procedures and analytical methodologies, as well as a comprehensive literature survey, can be found elsewhere [12]. However, it is worth noting that the ability of ICP-MS for sensitive isotopic analysis makes this analytical technique particularly attractive for such studies, since it allows assessment of the fate of element species enriched with stable isotope after ingestion with food [55].

18.3.2 Procedures based on non-chromatographic separations

Evaluation of food quality and safety is an important goal of speciation analysis; in this sense, relatively simple and fast procedures for the determination of known element forms/species are needed for their inclusion in routine practice. Non-chromatographic fractionation enabling separation of different oxidation states, for discrimination between organic and inorganic forms or yielding species-selective extraction or derivatization are of special interest. Integrating such procedures in high-throughput or sequential injection schemes offers further benefits in terms of miniaturization, possible automation, high precision and environmental friendliness [56]. Expensive and sophisticated equipment is not needed and, most importantly, a many-fold pre-concentration of target form/species can be relatively easily achieved. Two recent review articles have discussed non-chromatographic speciation and provide a systematic description of separation methods [57, 58]; these include different modes of liquid–liquid extraction (LLE) and solid-phase extraction (SPE), co-precipitation, distillation, field-flow fractionation (FFF), selective reduction, derivatization and CVG. However, one should bear in mind that such separation schemes are susceptible to matrix effects and are well suited for the analysis of simple environmental samples, whereas their application to chemically complex food products has been less common. It is worth noting that non-chromatographic separations are often coupled with atomic fluorescence, atomic absorption or optical emission spectrometries. These techniques are less sensitive but also less demanding as compared with ICP-MS, which otherwise is considered the best specific detector in the area of trace element analysis.

Without doubt, the most extensively explored non-chromatographic separation is selective generation of chemical vapour followed by introduction of gaseous phase to an atomic spectrometry detector; it offers separation of analytes from the matrix, high transport efficiency, easy pre-concentration and speciation with minimum sample handling. Conveniently, hydride generation (HG) or cold vapour (CV) generation can be applied for elements of particular importance for food

safety/quality: arsenic, selenium, antimony and mercury, respectively [19, 59]. During HG, selectivity to element forms/species can be controlled by adjusting the acidity of the solution and tetrahydroborate concentration; typically in the first step, lower oxidation states of the element are determined; afterwards a reducing agent is added for total element determination and the fraction corresponding to the higher oxidation states is evaluated as the difference [57]. Similarly, using $NaBH_4$ or $SnCl_2$, cold vapour generation can be achieved in two runs: first for Hg(II) and after appropriate oxidation also for MeHg [60, 61]. For arsenic, the determination of As(III) + As(V), referred to as inorganic fraction, iAs, is of special interest. Since the two species are readily interconverted in the body and also in the course of analytical procedures, their sum has been adopted as an indicator of health risk associated with food consumption [3, 21, 62] and an oxidative mixture of dilute nitric acid and hydrogen peroxide has been used for extraction [3, 62]. Rice, wheat and seafood are the most important dietary sources of As; non-toxic arsenobetaine is the main species in the majority of fish, whereas the primary form in rice and wheat products is iAs. It is worth noting that the two unique standard methods approved so far by European legislation for this element form are based on HG-AAS; one of these methods refers to iAs in seaweed and the second to iAs in animal feed composed of marine organisms [63, 64]. A few recent applications of HG and CV generation, to As and Hg speciation in food respectively, are described in Table 18.2 [65–68]. In addition to direct element fractionation, CVG has been incorporated as a post-separation derivatization step yielding enhanced analytical performance of element quantification by atomic spectrometry techniques [69]. Despite important advantages of this approach, there are also some limitations, such as instability of plasma-based detection systems due to introduction of hydrogen excess or possible interferences caused by redox active species present in the solution (mainly transition metals). In an effort to circumvent the difficulties observed when tetrahydroborate is applied, alternative CVG systems have been developed. In particular, photochemical- or ultrasound-mediated CVG, electrochemical CVG as well as the use of $SnCl_2$ or amineboranes have been critically reviewed by Sturgeon and colleagues [70], including some applications in food analysis. It should be noted that different schemes for alkylation reactions were also included in this review, although their utility in non-chromatographic speciation is limited [70].

SPE has been a popular technique in the field of redox speciation, mainly due to its versatility and easy inclusion in both batch and flow systems. It consists of two steps: (i) during loading, the retention of metal species or its derivatives occurs on the appropriate solid sorbent packed in a small column and (ii) the analyte is eluted with a suitable solvent. Depending on the element of interest, a wide variety of sorbents has been used, including typical reversed-phase chromatographic phases, ion exchangers, chelating resins, phases modified with complexing agents or biomaterials [57, 58, 65–67, 71, 72]. In the last few years, the application of sorbents with nano-dimensions has markedly increased because of their intrinsic properties, such as homogeneity of particle size, large surface area, physical and chemical stability and also relatively easy functionalization. For carbon nanotubes, a systematic description of their synthesis, characterization and modification by oxidation/funtionalization can be found in a recent review by Herrero Latorre *et al*. [73]. Several non-chromatographic applications in speciation analysis of vegetables and beverages were included in this review, yet practically all of them focused on the sorption of metal ions from the sample digest. Other nanoparticles, such as nano γ-alumina, SiO_2, TiO_2 and magnetic materials, have also been explored as SPE sorbents in food speciation analysis [74–77]. An interesting trend in SPE involves molecularly imprinted polymers (MIPs); these sorbents are synthesized by polymerizing functional and cross-linked monomers around a target species and then eliminating this species. The material obtained is capable of specific recognition based on shape, size and functional groups previously 'imprinted' [78]. Elaboration of MIPs is challenging, because fine particles must be homogeneous in shape and size and should possess a high density of evenly distributed binding sites. To cope with these requirements, MIPs can be deposited on spherical silica or polymer particles. Table 18.2 includes recent applications of MIPs for selective determination of Sn(IV) in canned food as a representative example [79].

Classic liquid extraction is less attractive compared with SPE because it is operationally more complex, offers lower pre-concentration factors and generates more chemical residues; however, some miniaturization approaches have been successfully applied to redox speciation in food products [58, 80–82]. In particular,

cloud point extraction (CPE) is a very simple and environmentally friendly technique, in which target species concentrates in micelles of non-ionic surfactant formed in aqueous sample solution; afterwards a small volume of surfactant-rich phase is efficiently separated by heating to reach the 'cloud point' temperature. This phase is collected, diluted and introduced to the element-specific detection system, typically atomic absorption spectrometry, as described in two recent reports included in Table 18.2 [83, 84]. Dispersive liquid–liquid microextraction (DLLME) is another system of potential interest in food speciation analysis, and is based on a three-component solvent system. Specifically, the extracting solvent of high density (tetrachloromethane, chloroform, etc.) is mixed with the disperser solvent, which is miscible with the extractant and water (methanol, ethanol, acetonitrile or acetone), and a small aliquot is rapidly poured into the aqueous sample solution. Turbulent mixing promotes emulsification and gives rise to a high surface area of contact thus facilitating fast and efficient mass transfer in the extraction process; after centrifugation, organic phase containing the target species is collected for element-specific determination [80, 85, 86].

With regard to 'green' analytical chemistry, ionic liquids constitute a promising class of solvents for species separation. An interesting review of their applications in food analysis is available [87]. The desired physicochemical properties of ionic liquids in terms of viscosity, miscibility with other solvents and affinity to target pecies can be adjusted by selecting organic cations and anions for their combination. For metals, imidazolium and phosphonium ionic liquids have been considered; however the main applications so far cover total element determination in liquid and solid foods after their suitable pretreatment [87]. Ionic liquids can be used in dispersive liquid separation systems or as retained on solid-phase particles [81]; two examples related to element speciation in food are briefly described in Table 18.2 [86–88].

In summary, a thorough literature survey demonstrates that microextraction systems have often been used as a unique separation/pre-concentration step for total element determination and also in speciation analysis; however, their application to element speciation in food products has been less common compared with other areas [57, 81, 87]. Otherwise, SPE, solid-phase microextraction (SPME), DLLME and single-drop microextraction (SDME) are important tools for sample pretreatment prior to species separation by liquid or gas chromatography.

It has already been commented that FFF in combination with atomic spectrometry is a useful instrument in characterization of mineral element containing nanoparticles in food [89]. In such an approach, the separation of macromolecules, colloids, and nano- or micro-particles (load mass range, nanogram to microgram) relies on their retention in an FFF channel with respect to the average flow velocity of the carrier. Depending on the type of external field applied, different modes of fractionation can be obtained: in the asymmetric flow technique (AF^4), a secondary perpendicular flow is the driving force of separation; centrifugal force is used in sedimentation FFF; and an electrical or magnetic field in electrical FFF and magnetic FFF, respectively. A detailed description of basic concepts and applications of FFF can be found elsewhere [90]; however very few applications to metal/non-metal-based nanoparticles in food are mentioned there. To highlight the potential of such fractionation in food speciation analysis, recent examples are given in Tables 18.1 and 18.2 [43, 86, 91].

Finally, it is worth noting analytical procedures that enable element speciation in food with minimum or even without any pretreatment. In this regard, fish-based baby foods and also wheat or rice flour were prepared for analysis as slurry [68, 92]. In the former study, selective determination of three As fractions (As(III) + As(V) + MMA(V); DMA(V); tAs) was achieved by electrothermal atomization atomic absorption spectrometry (ETAAAS) using three different analyte modifiers and varying temperature conditions in the ashing step [92]. With reference to more sophisticated instrumental tools, X-ray absorption spectroscopy has proved its potential utility for *in situ* element speciation in food-related materials, including plant tissues, rice, Se-yeast and seafood [9, 93–96].

18.3.3 Analytical procedures based on liquid chromatography separations

The most efficient speciation procedures are those based on hyphenated systems, where a powerful separation of chemical species is coupled on-line to atomic or molecular spectrometric detection for element-specific quantification or for assessment of species identity and quantity, respectively [97]. Since the great majority of small elemental species of interest in food analysis are known and can be classified as polar or ionic compounds, the first

hyphenation technique of choice for speciation analysis is liquid chromatography (LC) coupled to an element-specific detector. A variety of separation mechanisms, a wide range of column dimensions and stationary phases in combination with suitable selection of mobile phases offer exceptional versatility. Additionally, interfacing LC separations with atomic spectrometry detectors with continuous sample introduction is relatively straightforward; typical flow rates for standard-sized analytical columns (0.5–1.2 mL/min) are compatible with the aspiration rate of conventional pneumatic nebulizers (concentric Meinhard, Babington, or cross-flow), so the column outlet can be connected directly to the liquid inlet of the nebulizer, using a short polymeric or stainless steel tubing [98]. However, it should be mentioned that optimum separation conditions cannot always be used, since the chemical lability of element species and their interactions with the stationary phase and/or the components of the mobile phase (buffers, organic modifiers) may cause unwanted alterations of the original species distribution in the sample. Even though the capability of atomic spectrometry for element-specific detection usually compensates for incomplete chromatographic resolution, another concern arises due to low transport efficiency and to the potentially detrimental effect that chemical components of the column effluent may exert on analytical performance in these detection systems. It is well established that ICP-MS does not tolerate large amounts of organic solvents since they cause lower plasma stability and formation of carbon deposits on the torch and the sampling cone. Plasma stability is also compromised by hydrogen excess introduced when HG is included; the high salt concentration is undesired because of nebulizer and cone clogging, plasma destabilization and a potential increase in polyatomic interferences [2, 99, 100]. As already mentioned, other atomic spectrometry detectors are less demanding and, in particular, coupling AFS with CVG has gained general acceptance [19]. Applications of optical emission spectrometry (ICP-OES) have been less common because of lower detection power, susceptibility to spectral interferences and the same limitation regarding sample introduction and plasma stability as ICP-MS [98]. It should be stressed, however, that ICP-MS is the technique of choice when microbore (0.5–2.0 mm i.d.), nanobore or capillary (<0.5 mm i.d.) columns are used. Even though not very popular yet in food analysis, miniaturization in LC has been an important trend enabling increased mass sensitivity, better resolving power, smaller sample volumes, no sample dilution and less solvent consumption as compared with conventional (4.6 mm i.d.) columns [99, 101]. The lower peak volume and higher peak concentration of the eluted species (by a factor of 10–100 times) generally improve the absolute detection limits with respect to normal bore columns. However, due to the small injection volume, the high sensitivity of ICP-MS is needed to cope with low mass flow eluting from the column. In such an instrumental configuration, the volumetric flow drops from millilitres per minute to microlitres per minute and the composition of column effluent has negligible effect on plasma stability, so that even harsh optimum separation conditions can be effectively used with capillary-based LC/ICP-MS providing enhanced separation performance. On the other hand, micro LC/ICP-MS coupling requires specialized interfacing devices and, in particular, low-flow nebulizers such as microconcentric nebulizers, direct injection nebulizers, high-efficiency nebulizers, direct-injection high efficiency nebulizers and total consumption nebulizers [2, 102–105].

Confirmation and structural analysis of target compounds in speciation studies relies on LC coupling to molecular mass spectrometry and for such analytical tasks there is a wide range of ionization sources and mass filters of analysers available [106]. In particular, electrospray ionization (ESI) has been the most commonly used, because this soft ionization source is suitable for characterization of ionic species, labile organometallic compounds and biomolecules [107]. ESI in combination with high-resolution MS and tandem MS/MS devices (quadrupole with time of flight or QTOF, QqTOF or Orbitrap) furnishes accurate mass, isotopic pattern and fragmentation spectra required for ultimate species identification [108]. Matrix-assisted laser desorption/ionization (MALDI) is another soft ion source that is usually operated off-line with LC, so it is less susceptible to ion suppression effects compared with ESI and enables more harsh chromatographic conditions [11]. On-line LC-MALDI-MS has also been explored using capillary columns and separations on microfluidic chips [109, 110]. The interesting review by Sola-Vázquez *et al.* [111] describes recent advances in the development of integrated instruments enabling simultaneous acquisition of elemental and molecular information.

Considerable effort has been dedicated to improving the analytical capabilities of hyphenated techniques based on LC separation. The most important strategies described in recent reviews involve (i) advances in

sample pretreatment [1, 73, 80, 81, 112]; (ii) application of on-line multidimensional separation systems [113]; (iii) application of fast chromatography [114]; (iv) high transport efficiency between column outlet and detector [69, 102]; and (v) interference-free highly sensitive detection of fast transient peaks [19, 100, 115].

Capillary electrophoresis (CE) is another separation technique with many applications in speciation analysis [116]. Several features are usually listed when comparing CE with LC separations; these include high efficiency, ability to separate high numbers of solutes of different physicochemical characteristics, no stationary phase, simple composition of running buffer, mild separation conditions favouring species integrity, and also the economic and 'green' aspects of CE. There are also some limiting factors, such as sensitivity, problematic coupling between the capillary outlet and atomic/molecular detector, and potential detrimental effect of chemical matrix on separation performance. The exhaustive review of literature published before 2010 has revealed many original articles dealing mainly with Hg, As, Sn and I speciation analysis in marine food; however, in more recent studies, the interest in CE has slightly decreased, as also commented by Timerbaev [116].

In line with the previous sections of this chapter, an overview of recent LC/CE applications confirms that arsenic, mercury and selenium have been the most studied elements in food speciation analysis (see Table 18.3).

Mercury

Methylmercury, inorganic mercury and, to lesser extent, ethyl- or phenyl-mercury are primary species of this element determined in seafood [117–121]. Using liquid chromatography, there is no need for pre-column derivatization and separation can be achieved at room temperature; reversed-phase columns are typically used with mobile phases containing L-cysteine or 2-mercaptoethanol as a complexing agent, pH range 2–6, and acetonitrile or methanol as organic modifier [122]. Since a high throughput is usually required, fast chromatographic separations have been proposed. In particular, shorter than typical analytical columns can be used as well as reduced particle diameter (less than 2 or 3 μm), porous shell or monolithic columns [114]. In the procedure reported by Guzmán-Mar *et al*. [119], RP C18 monolithic column was used with a multi-isocratic elution performed in a sequential injection system. The advantage of monolithic columns relies on much higher separation efficiency achieved at a similar pressure drop as compared with particle-packed columns; monolithic columns can work at high flow rates providing fast mass transfer, yet without generating high back-pressures [114]. The above features make these columns also attractive for low-pressure flow-based methodologies as shown in the cited study [119]. It has already been highlighted that post-column cold vapour generation can be used to enhance transport efficiency in both ICP-MS and AFS applications; however, an additional step is then required in order to convert organic species to Hg(II) thus assuring uniform and high detection sensitivity for all eluting analytes [19, 69, 117, 119, 121]. The recent application of CE in mercury food speciation is also included in Table 18.3 [123].

The continuing challenge in mercury speciation is the quantitative extraction of all species in a single procedural step, while preserving their integrity. A wide range of conditions has been examined, including alkaline or acid extractions, addition of different thiol ligands (L-cysteine, thiourea, 2-mercaptoethanol or others), organic solvents (methanol, toluene), enzymatic treatments (protease), as well as extractions assisted by conventional heating, microwave energy or ultrasonication [7]. In recent studies, a tendency toward ultrasound, thiol-aided extraction in acidic medium (HCl) or in alkaline medium (tetramethylammonium hydroxide, TMAH) can be noted [117–120, 122], in agreement with earlier reports on species instability at elevated temperature or in potentially oxidizing media [124].

Arsenic

Several procedures recently proposed for arsenic speciation in edible marine organisms, rice, wheat, mushrooms, products derived from vegetables and animal meat are briefly presented in Table 18.3. Depending on the raw material and its preparation, more than 50 As compounds have been characterized in food-related products; however, for assessment of the health risk associated with the consumption of these products, the list of species is much shorter and typically includes arsenate As(III), arsenite As(V), methylarsonous acid [MMA(V)], dimethylarsinic acid [DMA(V)], arsenobetaine (AsB) and arsenocholine (AsC) [9, 62, 125]. At neutral pH, As(III) (pK_{a1} 2.3), MMA(V) (pK_{a1} 3.6) and DMA(V) (pK_{a1} 6.2) are present as anions, AsC as cation and AsB (pK_{a1} 2.18) as zwitterion, so their LC separation can be achieved by anion exchange or ion-pair reversed-phase mechanism [126]. When anion-exchange separation is

performed at near neutral conditions with phosphate, Tris buffer or malate mobile phase, the elution order AsC, AsB, As(III), DMA(V), MMA(V) and As(V) is usually reported; however, elution in a void volume or co-elution of the first three species has been reported. For mobile phases containing nitric acid at pH <3, the typical order is As(III), MMA(V), DMA(V), As(V), AsB and AsC, whereas with strongly basic eluents like carbonate or hydroxide (pH >8.5) this order is generally reversed, depending on column choice and the specific elution conditions [127]. It can be seen in Table 18.3 that, in the recent years, typical size polymeric PRP-X100 column (250×4.1 or 4.6 mm, 5 or 10 μm) and isocratic elution with phosphate mobile phase has been most frequently used in analyses of rice, wheat, fruit juices and vegetable oil [95, 127–131]. It has already been mentioned that iAs is often determined instead of individual As(III) and As(V) species; since arsenate is much easier to determine by anion-exchange HPLC than weakly or non-retained As(III), some authors have proposed slightly oxidative conditions to convert any arsenite to arsenate [22, 76, 131, 132]. Similar chromatographic conditions have been applied in the analysis of seafood extracts [127, 131, 133]; however, these products usually contain more organic species with respect to food derived from terrestrial plants, and co-elution of As(III), AsB, trimethylarsine oxide (TMAO) or tetramethylarsonium ion (TETRA) in early peaks has been observed [134]. Some authors used a combination of anion- and cation-exchange columns for resolution and quantification of all the above species [127, 135]. It is worth noting that cation-exchange separations require relatively low pH (pH <4) and typical mobile phases contain pyridine [131]. Ion-pair reversed-phase separation has also been employed in As food speciation, the most important feature being that resolution of positive, negative and uncharged solutes of different polarity are possible in a single run. On the other hand, separation becomes more susceptible to sample matrix, mobile phase contains more solids compared with other mechanisms, and resolution deteriorates if the concentration of ion-pairing agent is outside optimum range. Both cation and anion ion-pairing agents have been used, depending on pH applied [127, 136]. Even without direct application to real-world samples, hydrophilic interaction chromatography (HILIC) with zwitterionic column (150×2.1 mm, 3.5 μm) has proved its feasibility for separation of nine As species of different polarity/charge [137].

When column effluent is directly introduced to conventional quadrupole ICP-MS, monoisotopic arsenic suffers from $^{40}Ar^{35}Cl^{+}$ interference that can be eliminated by application of interference equations, using collision/reaction cell technology, either with helium as neutral gas or with reactive gases (O_2, CH_4) in dynamic reaction cells, or by chromatographic separation of chloride [62, 95, 128, 129, 131, 135, 138]. An additional concern with the use of ICP-MS is due to species-dependent changes in sensitivity of As detection and also because of changes in sensitivity related to the composition of mobile phases, especially in gradient elution programs. Introduction of post-column HG counterbalances practically all the above-mentioned limitations. The oxyanions arsenite and arsenate and the monomethyl and dimethyl arsenic species generate volatile arsines when reduced with $NaBH_4$ in acidic media; however, for other organic species such as AsB, AsC, TMAO or TETRA, their oxidation is required before HG. Taking advantage of this selectivity, the most toxic As species can be safely determined even if they co-elute with AsB or AsC in chromatographic separation [59, 69, 132]. Atomic fluorescence detection is an alternative detection system in As speciation based on LC [133, 139]; atomic absorption spectrometry with a quartz-type atomizer has also been used [140].

Ribofuranosides form another group of As species analysed in marine food [141]. These compounds, particularly abundant in algae, were considered non-toxic; however, studies revealing their metabolism to DMA(V) has warned of possible health threats due to consumption of seaweeds [20, 21]. Fractionation and speciation procedures focusing on possible transformation of arsenosugars during gastrointestinal digestion and their bioavailability have already been mentioned (see Table 18.1) [44, 49]; further applications of LC are included in Table 18.3 [139, 142]. In the cited studies, a combination of element-specific detection with MS techniques has been used for structural characterization and quantification of both arsenosugars and their metabolites.

Within the domain of food safety, LC and CE have been used in the analysis of As-based feed additives and their metabolites in chicken or porcine meat [22, 23, 76, 143] (see Table 18.3). The compounds of interest include the arsenic drug roxarsone (4-hydroxy-3-nitrophenyl arsonic acid, Rox), its transformation products 3-amino-4-hydroxyphenylarsonic acid (3-amino) and

4-hydroxyphenylarsonic acid (4-OX) as well as 4-aminophenylarsonic acid (p-ASA), 4-nitrophenylarsonic acid (4-NPAA), phenylarsonic acid (PA) and 4-nitrophenylarsonic acid (NIT). It has been demonstrated that transformation of the above compounds to inorganic As occurs in treated animals and also in poultry wastes used for soil fertilization, so the related food products may become contaminated with the most toxic As species [144]. The analytical challenge is to obtain efficient separation of arsenic drugs and their metabolites, including As(III), As(V), MMA(V) and DMA(V), in a single run. The procedure based on CE with phosphate/borate buffer at pH 9.20 enabled separation of 10 As species within 30 min; its utility in food speciation was demonstrated by analysing fish reference materials and herbal teas [23]. Animal meat is a difficult chemical matrix and low concentrations of As species can be expected; in this regard an interesting procedure has been reported, based on stir bar sorptive extraction using titania immobilized polypropylene hollow fibre as a stir coating. Quantification of nine As compounds with enrichment factor 8.5–22.3 was reported [76].

The most critical step in food As speciation is sample pretreatment; this topic has been extensively discussed in several reviews [62, 125, 141, 145, 146] and there is a consensus that complete extraction of all and intact species present in the sample is not realistic in a single step. Furthermore, pre-analytical operations such as grinding, drying, freeze-drying, homogenization and storage may introduce changes in native species distribution; the stability of As compounds in the obtained extracts has also been discussed with specific focus on rice and wheat [3, 62, 129, 131]. Depending on the sample matrix, water, methanol–water, enzymatic, acid or alkaline extractions have been used [62, 125, 145, 146].

In Table 18.3, some examples from the recent literature related to marine food are presented; there have been several applications of water–methanol mixture [135, 142], but diluted nitric acid and hot water have also been used as extractants [23, 133, 139]. In a comparative evaluation of HCl/H_2O_2, $NaOH/C_2H_5OH$ and 2% HNO_3, the latter provided the best extraction efficiency in fish analysis [132]. Arsenic speciation in fish oil, or more generally in lipids, requires an additional procedural step prior to LC separation [147]. As reviewed in the cited article, after chloroform–methanol extraction of lipids, alkaline hydrolysis or saponification has to be performed to release water-soluble As species such as derivatives of AsC, arsenosugars, dimethylarsinoyl acetate (DMAA), TMAO or DMMA(V). It should be emphasized that identification of arsenolipids relies on the complementary applications of ICP-MS and organic MS[147].

Rice and wheat are two extremely difficult chemical matrices, and incomplete extraction and species interconversion have been observed [3, 62]. A tendency toward extraction with dilute nitric acid can be noted in recent studies [95, 129, 130]; it was demonstrated that this extractant was more effective in wheat analysis compared with water and methanol–water or α-amylase treatment [129]. Even though preservation of As(III)/As(V) species is difficult, it can be achieved if the concentration of nitric acid is maintained in the range 0.28–0.70 M [125]. For monitoring of iAs, hot water extraction has been used [148]. The recent study by Raber *et al.* [131] should be mentioned, since the authors propose ultrasound extraction with a mixture of trifluoroacetic acid and hydrogen peroxide and demonstrate its feasibility in the analysis of iAs, MMA(V) and DMA(V) in different matrices (fish, wheat and rice). For the analysis of animal meat, methanol–water extraction is still preferred [22, 76]; for mushrooms a mixture of 1 M phosphoric acid with Triton-X100 was applied [140].

Arsenic extraction from food products has usually been assisted by mechanical agitation, conventional heating, ultrasonication, accelerated solvent extraction or microwave energy [62]. In a few studies, preference was given to traditional heating blocks [130, 149, 150], whereas other authors have used ultrasonic probe or bath [23, 76, 129, 131, 133, 135, 140], microwave oven or microwave-focused radiation [95, 129, 132, 139, 142, 151]; accelerated solvent extraction system has also been included [143].

Even though the majority of LC applications to As speciation in food refer to known species with application of ICP-MS or AFS element-specific detection, studies focusing on species identification/confirmation should also be mentioned. In this regard, characterization of thio-methylated species, arsenosugars [44, 138, 142] and arsenolipids [152–154] has been undertaken using molecular MS.

Selenium

Unlike mercury and arsenic, selenium is an essential element whose main sources in human nutrition are food products naturally rich in Se, biofortified products and dietary supplements. In this regard, food analysis has

been aimed at species profiling in nuts, milk, seafood and other natural element sources. Furthermore, the efficiency of the enrichment process, usually carried out on yeast, vegetables or mushrooms, has been evaluated. As presented in section 18.3.1, studies focused on leaching processes and the bioavailability of Se species in the gastrointestinal tract are also of interest (see Table 18.1). However, it should be emphasized that the beneficial and adverse health effects of selenium are very narrowly separated, depending on the type of compounds present in food and their ingested dose; hence, food speciation studies may also warn of possible toxicological risk [155, 156]. In this regard, the quantitative aspect of speciation analysis is very important and indeed several studies have focused on the development and application of isotope dilution techniques [11, 55, 157–159]. A wide variety of selenium compounds can be found in food products; among the species analysed by LC with atomic or molecular spectrometric detection are the inorganic oxoanions selenite Se(IV) and selenate Se(VI), selenium-containing amino acids and low molecular mass species such as selenomethionine (SeMet), selenocysteine (SeCys), selenocystine (SeCys2), Se-methylselenocysteine (MeSeCys), Se-methylselenomethionine (MeSeMet), γ-glutamyl-Se-methylselenocysteine (γ-Glu-MeSeCys), and high molecular mass compounds such as selenium-containing proteins, selenoenzymes and selenosugars. In general, the sample has to be initially fractionated, based either on molecular mass of selenium-containing compounds or on species solubility in water or Tris-HCl buffer (with or without addition of sodium dodecyl sulfate). In further procedural stages, speciation of small water-soluble molecules is carried out by a suitable hyphenated technique; conversely, for high molecular mass compounds, the fractions of interest are purified by precipitation/redissolution or by orthogonal chromatographic separations, then hydrolysed and speciation analysis is performed in the obtained digests. When protein hydrolysis is employed, typical proteolytic enzymes include proteinase K, protease XIV, pepsin, trypsin or their mixtures; otherwise acid hydrolysis with methanesulfonic acid has proved to be an efficient procedure for protein cleavage. For the release of Se species bound to polysaccharide or lipid structures, driselase, chitinase or lipase have been used, respectively. At different stages of sample treatment, fractions can be collected, purified and analysed by MS, as a standard approach providing structural information [4, 6, 11, 145, 160]. As with other elements, liquid extraction and hydrolysis of high molecular mass compounds have often been carried out under sonication, microwave energy or in accelerated solvent extraction systems in order to enhance extraction efficiency and speed up the analysis. Several comprehensive review articles and book chapters have been dedicated to selenium speciation, including applications of LC in food analysis [4, 6, 11, 55, 160–163]. Tables 18.1 and 18.3 list selected examples of LC separations from the recent literature.

In addition to molecular mass profiling by size-exclusion chromatography (SEC) [51, 55, 109], separation of individual compounds has been obtained using different separation mechanisms. Specifically, Se(IV), Se(VI), SeMet, SeCys2, MeSeCys were separated on anion-exchange columns with mobile phases at pH 5–7 [164–166]. On the other hand, at lower pH, selenium-containing amino acids and peptides and trimethylselonium ion are present as cations, which enables their separation on cation-exchange columns [167]. Different perfluorinated and alkylsulfonic acids have been extensively used as ion-pair reagents for separation of Se compounds on reversed-phase columns with mobile phases containing pyridine or ammonium formate at pH 2–5.7 [168, 169]. With regard to liquid separation techniques used in hyphenated techniques, food selenium speciation can also be performed by CE, although in recent years this technique has been less popular than chromatography [116, 170].

A typical problem in Se speciation is the high risk of selenomethionine oxidation to selenomethionine Se-oxide (SeMetO); reducing agents such as dithiothreitol (DTT) or 2-mercaptoethanol are often added during sample treatment to prevent this conversion. SeMetO can also be added to the standard mixtures, which helps to identify this species in chromatograms of real samples and to correct for negative systematic error in SeMet quantification. It is also difficult to avoid oxidation of selenocysteine to selenocystine, so in many cases SeCys2 is referred to instead of SeCys [161]. ICP-MS is well established as the most popular element-specific detection technique in Se speciation by hyphenated techniques; however, several issues have to be considered, such as relatively low ionization efficiency of Se (about 30% in Ar-sustained plasma), polyatomic interference on the most abundant ^{80}Se isotope caused by argon dimer ($^{40}Ar^{40}Ar^{+}$), and detrimental influence of the mobile phase on detection sensitivity. Typical approaches for compensation of these limitations include

application of collision/reaction cell technology, addition of methane gas for increased sensitivity and adoption of the compromised chromatographic conditions giving preference to isocratic elution with diluted mobile phases and low concentration of organic modifier [6, 11, 161, 171]. As to post-column HG coupled on-line with AFS detection, all organic Se compounds eluting from the column have to be converted to Se(VI) and then reduced to Se(IV) to ensure efficient H_2Se generation; even using harsh chemical conditions (5 M HCl, heating) the reduction of Se(VI) to Se(IV) is relatively slow [19, 162].

With regard to food matrices, Se-enriched products derived from plants have received major attention both in earlier applications [4, 6] and in recent studies [29, 51, 83, 165, 167, 169, 170, 172]. The goal of speciation analysis is to assess conversion of inorganic Se, typically used for fertilization, into organic forms thus providing data on species identity and quantity; accordingly, LC separation of Se(IV), Se(VI), SeMet, MeSeCys, SeCys2 and γ-Glu-MeSeCys has been carried out. An interesting study has been reported in which edible cactus pear grown on sediment rich in selenium was evaluated in terms of its feasibility as a nutraceutical food [164]. Analysis of Se-containing food supplements, more frequently selenized yeast (*Saccharomyces cerevisiae*), involves similar analytical tasks [40, 166, 168, 173].

Selenium speciation in Brazil nuts has been an active research area in the past decade [6]. Initial interest was focused on the evaluation of major Se species within the context of possible utility of nuts as a dietary source of Se and with special emphasis on efficient extraction and reliable species quantification. In further development, Se-proteomics studies have also been undertaken in order to understand the related biochemical aspects [109].

Selenium content in fish and shellfish is generally higher compared with terrestrial organisms. The same core Se proteins have been found in organisms from both habitats, although Se speciation appears to be more complex in the marine world. In the recent review presented by Yoshida *et al*. [163], several earlier speciation studies are briefly described. Specifically, typical water/buffer extraction and hydrolysis procedures were followed by size-exclusion or reversed-phase chromatography with ICP-MS detection and MS [174–177]. Seafood can be considered a valuable dietary source of Se, yet there is a concern related to As content in these products. In this regard, an interesting procedure based on a mild microwave-assisted enzymatic digestion and anion-exchange HPLC coupled to ICP-MS has been proposed for simultaneous determination of As(III), As(V), MMA(V), DMA(V), Se(IV), Se(VI) and SeMet [178]. Similarly, there has also been interest in simultaneous assessment of Se and Hg species in fish [96].

Milk is an important source of selenium for humans at every age, but special attention has been paid to speciation of selenium and other essential elements in breast milk as compared to infant formulas [55]. It was demonstrated that about 70% of total essential elements are present in milk whey; hence fat and caseins have usually been eliminated prior to speciation studies. In the cited article, Sanz-Medel *et al*. presented a comprehensible summary of their studies, organized into the following three phases: (i) multi-element (Ca, Mg, Fe, Cu, Zn, Se, I) screening in milk whey by SEC/ICP-MS; (ii) qualitative proteomic analysis by MALDI-MS and/or ESI-$(MS)^n$, performed in SEC column fraction additionally purified by orthogonal cation-exchange LC; and (iii) quantitfication of target peptide/protein by ICP-MS. The results obtained revealed drastic differences in chemical speciation between breast milk and formulae; α-lactalbumin was found as an important heteroelement-binding protein but it was stressed that application of ICP-MS for protein quantification would be feasible only for known metal–protein stoichiometry and if all the binding sites in protein are saturated with the monitored element [55].

Miscellaneous

The state of the art in the field of iodine analysis, with an emphasis on environmental, biological and nutritional applications, has been presented in a recent review, from which it is clear that the determination of total element content has been the most common approach [50]. In the frame of analytical speciation by LC, studies have been performed in seafood and in fortified products such as table salt, milk and eggs. An interesting contribution was presented by the Caruso research group [179]; they analysed different commercially available seaweeds using 0.1 M sodium hydroxide, 30 mM Tris-HCl buffer at pH 8.5 and 75% ethanol for the extraction of water-soluble organic species, proteins and polyphenols, respectively. Enzymolysis with Proteinase K was also carried out to release iodinated amino acids. The obtained extracts were analysed by size-exclusion, reversed-phase and anion-exchange chromatography with ICP-MS detection; iodide was found as the predominant

inorganic species, whereas organic compounds reported were monoiodotyrosine (MIT) and diiodotyrosine (DIT) [179]. A few recent reports regarding marine organisms, enriched eggs and salt are included in Tables 18.1 and 18.3 [45, 47, 180, 181]. Iodine has also been considered in speciation analysis of human milk [182, 183]; as for other matrices, molecular mass fractionation by size exclusion was combined with anion-exchange separation of iodide and iodate; iodide has always been reported as the main species in milk. Because of the extreme importance of breast milk for infant nutrition, not only iodine and selenium but also other micronutrients (Cu, Zn, Fe, Ca, Mg, Mn) and toxic Cd have been studied [182, 183]. As described before, defatted samples are usually prepared and the analytical approach relies on multidimensional chromatography based on size exclusion, anion or cation exchange and/or reversed-phase separations. SEC with ICP-MS detection was also used for multi-element profiling in bovine whey [184]. In more recent studies, molecular mass spectrometry has been integrated in the analytical scheme for characterization of metal binding to proteins [55, 185] and to confirm formation of zinc citrate complexes [186].

Tea is another food-related matrix of increasing interest in speciation analysis. Infusions made from *Camellia sinensis* leaves and herbal teas are recognized as a rich source of micronutrients, whose concentrations depend on geographical region of plant origin, degree of fermentation process, element content in water and particular recipes for beverage preparation; elevated concentrations of potentially toxic element have also been reported [187–189]. Organic sample constituents such as polyphenols, alkaloids, proteins, amino acids, enzymes, aroma-forming substances, carbohydrates and vitamins may interact with metals/metalloids, thus affecting their bioaccessibility/bioavailability [190–192]. Pretreatment procedures as applied for total element determination in solid material have been discussed in a recent review [192]. LC speciation analysis has been performed on hot water infusions in both earlier [193, 194] and very recent [195,196] studies. As can be seen in Table 18.3, stable isotopes were used to follow possible species interconversion in the determination of two oxidation states of chromium, ^{50}Cr(VI) and ^{53}Cr(III), [196], whereas two Ni(II) complexes were identified by ESI-Q-TOF-MS [195].

Finally, recent applications of LC for the determination of organotin compounds in shellfish [197] and for Sb speciation in vegetables [198] confirm the well-established importance of such analyses in food safety assessment. The increasing interest in multi-element speciation should also be mentioned [9, 47, 55, 178, 184, 199].

18.3.4 Analytical procedures based on gas chromatography separations

Gas chromatography (GC) has been extensively used for analytical speciation purposes, not only because of its high resolving power but also because the gaseous column effluent can be efficiently transported to the element-specific or molecular mass detection system [200]. Even though the conversion of target element species to non-polar and thermally stable compounds is usually required, this procedural step facilitates extraction of the analytes from the sample and their many-fold pre-concentration. In consequence, the procedures based on GC are characterized by high sensitivity and high tolerance to sample matrix. It is worth noting that ICP-MS as a detection system is of particular interest, since isotope dilution methods can be used for reliable quantification at low concentration levels and for correction of analytical errors [105, 159, 200, 201]. Other element-specific techniques in use include ICP-OES, microwave-induced plasma optical emission (AED or MIP-OES, depending on the configuration), AFS and, to lesser extent, atomic absorption spectrometry (AAS); on the other hand, different molecular mass spectrometry techniques may serve as the sole detection system or combination with other detectors. It is worth remembering that electron impact ionization and total ion count mode in traditional GC-MS instruments equipped with a single quadrupole cannot compete with the ICP-MS detector in terms of sensitivity; however, enhanced performance for target solutes can be obtained while switching to selective ion monitoring (SIM) [200]. In this regard, GC-MS instruments with ion trap (in particular Orbitrap) or triple quadrupole can also be operated in a full-scan or SIM mode and, additionally, collision-induced dissociation (CID) can be integrated for fragmentation of selected ions and multiple reaction monitoring (MRM) in MS/MS mode becomes possible. These features make modern GC-MS an attractive tool for structural characterization and determination of low analyte concentrations immersed in chemically complex matrices [202, 203]. Time of flight mass analyser combines full spectral sensitivity with speed and

high mass resolution, hence GC-HRTOF-MS coupling has been shown to be specifically well suited for identification of non-target unknown compounds; however, the detrimental influence of matrix components on TOF performance should be noted [204, 205].

The most common derivatization procedures prior to GC separation include (i) conversion of inorganic and small organometallic ions into volatile covalent compounds in aqueous media (hydrides, fully ethylated, propylated or phenylated species); (ii) conversion of larger alkylated cations to saturated non-polar species using Grignard reagents; and (iii) conversion of ionic species to volatile chelates (dithiocarbamate, trifluoroacetone, etc.) [70, 112, 206]. The remarkable progress in sample pretreatment prior to GC analysis is reflected in several recent reviews, specifically focused on derivatization reactions [70,201], different modes of immersed or headspace SPME [207, 208] and liquid–liquid microextractions [209, 210].

In relation to the element species present in food matrices, only very few of them are volatile (e.g. alkyl selenides or As-containing hydrocarbons) and not all non-volatile species can be converted into the compounds amenable to GC analysis. Traditionally, organometallic species of Hg, Sn, Pb and Sb as well as organic species of As and Se have been analysed by GC and earlier methodological development has been described in comprehensive reviews [7, 122, 211–213]. In Table 18.4, several recent studies are included in order to present the actual state of the art and the research trends.

Mercury

GC with electron capture detection (GC-ECD) started to be used for mercury speciation in marine organisms approximately 50 years ago, when Westö [214] proposed conversion of alkyl or aryl mercury species to respective chlorides, followed by their extraction to benzene, purification by re-extraction to aqueous L-cysteine solution, and finally back to benzene. The main limitation of that system was its low sensitivity and instability of chloride derivatives; however, progress in analytical instrumentation and methodology soon enabled enhanced analytical performance. In this sense, GC coupling to atomic and molecular mass spectrometric techniques enabled improved detection and the use of capillary columns helped to eliminate the undesirable interaction of mercury species with packing material. Prior to GC separation, extraction with strong acid (HCl) or alkaline extraction with TMAH has been the most common procedure [122]. When tetraethylborate ($NaBEt_4$) is used as derivatizing agent, the reaction is carried out in aqueous sample extract and Hg(II) and MeHg are converted to $HgEt_2$ and MeHgEt, respectively; if EtHg needs to be included in the analysis, tetrapropylborate ($NaBPr_4$) enables differentiation of the derivatives formed for Hg(II) and EtHg. Commercially available tetraphenylborate ($NaBPh_4$) has also been proposed for alkylation in aqueous media, with the advantage of higher hydrophobicity of phenylated Hg species [70]. Alternatively, Grignard reagents can be applied, but non-aqueous reaction medium is then necessary [215]. Once volatile and thermally stable compounds are formed, purge-and-trap or SPME techniques are used for species collection and/or pre-concentration [215, 216] followed by their thermal desorption for on-column introduction [122]. On the other hand, in low-temperature chromatography, hydrides or alkylated species can be cryogenically trapped and then separated during the controlled column heating, according to their boiling points [217, 218]. The advantage of HG with $NaBH_4$ relies on the fast and efficient reaction and the facilitating role of abundant *in situ*-formed hydrogen, in purging of Hg^0, MeHgH and EtHgH to the cryotrap, although a risk of organomercury species decomposition has been commented [70, 201].

As can be observed in Table 18.4, mercury speciation in marine organisms is still an active research area. A new addition to the classic derivatization followed by GC-ECD has been reported [219]; comparative study based on ethylation reaction prior to GC separation coupled to element-specific or MS detection systems has been carried out [220]. In further development, these same authors applied headspace SPME extraction for the determination of Hg(II) and MeHg in terrestrial animals from a Spanish mine district [221]. The TOF-MS instrument enabled accurate sub-picogram determination of MeHg [222] and there has been progress in design of novel trapping/pre-concentration devices [74, 222, 223]. An interesting study has been presented in which enzymatic hydrolysis was combined with acid extraction; derivatization by phenylation or propylation were compared and it was shown that ICP-MS detection provided higher sensitivity with respect to headspace SPME followed by traditional GC-MS [224]. Finally, studies dedicated to quantification improvement by means of isotopic dilution have been progressing [18, 224, 225].

Arsenic

Even though typical As species addressed in food analysis can be derivatized to volatile compounds by means of HG, alkylation or substitution with thioglycol [201], LC has been the dominant separation technique in the analysis of these compounds in foods [62]. On the other hand, GC has been recently applied for characterization and quantification of volatile As-containing hydrocarbons in edible oils, as briefly described in Table 18.4 [226, 227].

Selenium

There have been relatively few applications of GC to selenium speciation in food-related products [4, 15, 228, 229]. In the early GC/ICP-MS procedure for volatile compounds released by edible plants to a headspace, it was possible to simultaneously monitor S and Se species and the two selenium compounds found in the analysis of real world samples corresponded to dimethylselenide (DMSe) and dimethydiselenide (DMDSe) [230]. In further development, SPME with multicapillary GC was applied to the analysis of alkyl selenides in selenium-enriched yeast and vegetable samples [231]. Using different detection systems (ICP-MS, MIP-OES, AFS), at least seven different volatile species were detected in this work, DMDSe being the predominant species. The two recent studies focusing on volatile Se compounds in dietary supplements are included in Table 18.4 [232, 233].

Seleno amino acids are the second group of compounds amenable for GC; Vonderheide *et al*. [234] used isobutyl chloroformate as derivatizing agent for acylation of the amino group and esterification of the carboxylic group. In the cited work, the volatile derivatives of SeMet, SeEt and SeCys were extracted by SPME prior to GC/ICP-MS. In the recent review by Pedrero and Madrid [11], earlier applications of methyl chloroformate and cyanogen bromide have been described. As shown in Table 18.4, methyl formate and ethyl chloroformate were used for derivatization in the analysis of Se amino acids in yeast, other dietary supplements and potatoes by GC-MS [232, 235, 236].

Other elements

Determination of organotin compounds has been mainly carried out in environmental samples and in marine or freshwater organisms. Analytical methodologies focusing on inorganic tin and its alkylated species have been described in comprehensive reviews [201, 237, 238]. In Table 18.4, three examples related to food analysis are presented from the recent literature [239–241]. On the other hand, leaching of organotin compounds from packing materials to food has been studied using liquid food simulators [242]. Within the context of food safety, alkylated compounds of lead and antimony have also been addressed and relevant information can be found elsewhere [70, 213, 215, 243]; however, speciation analysis has been mainly focused on environmental and biological samples, whereas recent studies in food matrices are scarce.

18.4 Quantification and quality control

Correct interpretation of analytical data is compromised by their quality and in this regard reliable quantification is still a bottleneck in food speciation analysis. Each procedural step becomes a potential source of error, though the pretreatment operations are usually responsible for the major contribution. For a given species/sample, random errors can be relatively easily evaluated and also reduced, by increasing the number of independent replicates and by fine-tuning of the experimental and instrumental conditions. On the other hand, accuracy testing is much more challenging. The first choice for such a purpose is to run suitable certified reference materials in parallel with the sample; however, the assortment of such materials includes very few food-related matrices and the certified values rarely match those existing in the real world samples; hence traceability is practically impossible to attain. The survey of recent literature (Tables 18.1–18.4) shows that the most frequently used are the materials based on marine organisms, rice and wheat, available from National Institute of Standards and Technology, USA (NIST), Institute for Reference Materials and Measurements, Belgium (BCR, Community Bureau of Reference; ERM, European Reference Materials; IMEP, International Measurement Evaluation Program), National Research Council of Canada (NRCC), International Atomic Energy Agency, Austria (IAEA) and National Institute of Environmental Studies, Japan (NIES). In particular, these materials include: tuna fish (BCR 464, BCR 627, ERM-CE 463, IAEA 350) [39, 65, 178, 223], dogfish muscle or liver (DORM-2, DORM-3, DOLT-2, DOLT-3, DOLT-4) [39, 60, 65, 117, 121, 135, 178, 219, 220], lobster hepatopancreas (TORT-2) [197, 224], oyster

tissue (NIST 1566a, NIST 1566b) [39, 142, 214, 224] seaweed (NIES-09, IAEA-140) [5, 10, 47, 142, 166], rice (ERM-BC211, SRM 1568d, CRM 10-a, CRM 10-b, CRM 10-c, CRM 10-d, IMEP-107) [41, 44, 67, 95, 130, 131], wheat flour (NIST 1567a) [29, 167, 168] and selenium-enriched yeast SELM-1 [5, 166]. Among these products, BCR 463, DOLT-3, DORM-2, IAEA-350, IAEA-140 and ERM-CE have been certified for MeHg and total Hg; BCR 627, IMEP-107, ERM-BC21 and DORM-2 have been certified for As species/forms; and SELM-1 for SeMet and for total Se. It is worth mentioning the rice flour material, elaborated by National Metrology Institute of Japan, NMIJ-CRM-7503a, which is certified for As(III), As(V), DMA(V) and tAs [166]. The full list of available reference materials can be accessed through the website of the European Virtual Institute for Speciation Analysis (EVISA), although only a small part can be used for accuracy checking in species-specific determinations in food products. It is common practice though to use materials certified for total element concentration in speciation studies and assuming the consistency between the sum of species and the certified value as a validation criterion. However, it has been noted that the obtained results cannot be considered a means of ultimate validation [149, 166].

Other internal approaches for accuracy testing include (i) assessment of mass balance during the entire procedure (in both fractionation and chemical speciation); (ii) standard addition and recovery evaluation; (iii) stability studies; (iv) comparison of quantification results obtained by external calibration with those obtained by the method of standard addition; (v) interference studies and blank measurements; (vi) confirmation of the results by means of independent method, preferentially based on different physicochemical principles; and (vii) the use of appropriate internal standards.

Various forms of internal standard (IS) have been exhaustively used in different speciation schemes, both for enhanced performance of the detection system and for compensation of errors committed during the preceding operations. In particular, internal standardization is routinely used in ICP-MS in order to control variations originating from transport, nebulization, ionization and matrix effects and also for correction of instrumental drift. Suitable IS can be continuously introduced post-column, thus allowing real-time correction of fluctuations in the ICP-MS response; alternatively, IS can be added directly to the sample with the additional advantage of compensation for procedural errors. This latter approach is especially attractive in the complex multistage procedures often required in food speciation analysis; however, nearly similar behaviour of IS and target compounds throughout the entire procedure needs to be assured. Some examples of internal standards reported in the recent literature include arsenobetaine for As speciation in rice [136]; propylmercury, methylpropylmercury or bismuth ion for Hg speciation in marine organisms [224, 244]; selenoethionine for Se speciation in dietary supplements [232]; and triphenyltin for Sn speciation in shellfish [240] or diphenyltin for Sn speciation in honey [241]. However, it should be stressed that the most reliable 'internal standards' in speciation analysis with MS detection are those enriched with stable isotopes [245]. Among different techniques of isotope dilution analysis (IDA), speciated isotope dilution mass spectrometry (SIDMS) has been recommended as a definitive quantification technique and also as a diagnostic tool that enables evaluation/correction for species transformations and non-quantitative recovery [246]. Isotope dilution is of no use for monoisotopic elements such as arsenic, but it has been extensively used for quantitative speciation of mercury [225], selenium [11], tin [245] and chromium [196, 247].

For further information, the reader is encouraged to consult comprehensive reviews and opinion papers specifically focused on the reliability of speciation analysis and the use of IDA [248–254].

18.5 Future perspectives in speciation

Over four decades, speciation analysis has evolved into an important and well-established research area, with innumerable real-world applications. The advances in analytical methodology have been boosted by the continuous progress in instrumentation and by the expanding knowledge of the role that element species have in human nutrition and toxicology. For the assessment of food safety, there has been incessant effort in the development of simple and robust procedures enabling quantification of element form/species that impose a health risk. In this regard, progress in non-chromatographic procedures should be emphasized. On the other hand, hyphenated techniques have gained undisputed priority in the analysis of known species. ICP-MS is considered the most powerful detection technique owing to its exceptional sensitivity, relatively few and easy to

eliminate interferences, compatibility with all dimensions of chromatographic/electrophoretic separations and, most importantly, its unique isotopic capabilities. Full characterization of element species is often required, especially in the analysis of dietary supplements, highly processed foods, new products elaborated to achieve enhanced bioavailability of mineral micronutrients, and those elaborated from biofortified raw material or genetically modified plants. For such purposes, the identification/confirmation of new or unexpected species relies on accurate mass measurements and structural information furnished by molecular mass spectrometry. Typically, structural characterization is obtained in a separate analytical run; however, integrated instrumental systems have also been designed to provide elemental and molecular information simultaneously. The main issue in speciation analysis is to assess 'native' species distribution, so it is not surprising that nuclear magnetic resonance and techniques based on synchrotron radiation have been incorporated, since they require minimum or no sample treatment.

As to future trends, effort will continue in reinforcement of reliability in food speciation analysis by means of further methodological development and production of suitable certified reference materials. In particular, standardization of speciation methods is necessary to promote more specific food regulations. On the other hand, progress in the use of small dimension techniques for efficient extraction and pre-concentration of target species, further incorporation of miniaturized fast chromatography and implementation of ICP-MS compound-independent calibration and quantification can be anticipated. Studies evaluating species bioavailability from food will continue, with special focus on the role of other food components acting as potential enhancers or inhibitors of element uptake. Relatively new challenges related to the analysis of transgenic foods and the presence of nano-dimensional metal/metalloid forms will be addressed. Finally, as in other field of chemical analysis, a preference will be given to environmentally friendly procedures.

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Abbreviations

3-amino: 3-Amino-4-hydroxy-phenylarsonic acid
4-NPAA: 4-Nitrophenylarsonic acid
4-OH: 4-Hydroxyphenylarsonic acid
AED: Atomic emission detector
AF^4: Asymmetric flow field fractionation
AFS: Atomic fluorescence spectrometry
AgNPs: Silver nanoparticles
AllSeCys: Se-allylselenocysteine
As(III): Arsenite
As(V): Arsenate
AsB: Arsenobetaine
AsC: Arsenocholine
CE: Capillary electrophoresis
CPE: Cloud point extraction
CID: Collision-induced dissociation
CRC: Collision/reaction cell
CRM: Certified reference material
CTAB: Cetyltrimethylammonium bromide
CV: Cold vapour
CVG: Chemical vapour generation
DBT: Dibutyltin
DIT: 3,5-Diiodo-L-tyrosine, diiodotyrosine
DL: Detection limit
DLLME: Dispersive liquid–liquid microextraction
DMAA: Dimethylarsinoyl acetate
DMA(V): Dimethylarsinic acid
DMAE(V): Dimethylarsinoyl ethanol
DMAS(V): Dimethylarsinothioic acid
DMDSe: Dimethyldiselenide
DMSe: Dimethylselenide
DPhT: Diphenyltin
DRC: Dynamic reaction cell
DTT: Dithiothreitol
ECD: Electron capture detector
EDTA: Ethylenediaminetetracetic acid
ESI: Electrospray ionization
ETAAAS: Electrothermal atomization atomic absorption spectrometry
EtHg: Ethylmecrury
FIA: Flow injection analysis
FFF: Field-flow fractionation
GPAsC: Glyceryl phosphorylarsenocholine
GC: Gas chromatography
γ-Glu-MeSeCys: γ-Glutamyl-Se-methylselenocysteine
HEPES: 4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid

HFBA: Heptafluorobutyric acid
HG: Hydride generation
HMM: High molecular mass
HPLC: High performance liquid chromatography
HR: High resolution
HS: Headspace
IAs: Inorganic arsenic
ICP: Inductively coupled plasma
IDA: Isotopic dilution analysis
IL: Ionic liquid
IS: Internal standard
ISe: Inorganic selenium
ISn: Inorganic tin
LC: Liquid chromatography
LLE: Liquid–liquid extraction
LMM: Low molecular mass
MALDI: Matrix-assisted laser desorption/ionization
MBT: Monobutyltin
MeHg: Methylmercury
MES: 2-(*N*-Morpholino)ethanesulfonic acid
MeSeCys: Se-methylselenocysteine
MeSeMet: Se-methylselenomethionine
MIP: Microwave-induced plasma
MIP-OES: Microwave-induced plasma optical emission spectrometry
MIPs: Molecularly imprinted polymers
MIT: 3-Iodo-L-tyrosine, monoiodotyrosine
MMA(V): Monomethylarsonic acid
MMSe: Monomethylselenide
MOPS: 3-(*N*-Morpholino)propanesulfonic acid
MRM: Multiple reaction monitoring
MS: Mass spectrometry
MSPD: Matrix solid phase dispersion
MW: Microwave
N-acetyl: *N*-Acetyl-4-hydroxy-*m*-arsanilic acid ((3-acetamido-4-hydroxyphenyl)arsonic acid)
NIT: Nitarsone, 4-nitrophenylarsonic acid
OES: Optical emission spectrometry
PA: Phenylarsonic acid
p-ASA: 4-Aminophenylarsonic acid, arsanilic acid
PDMS: Polydimethylsiloxane
PIPES: Piperazine-*N*,*N*′-bis(2-ethane-sulfonic acid) disodium salt
PhHg: Phenylmercury
PMSF: Phenylmethylsulfonyl fluoride
PrSeCys: Se-propylselenocysteine
PVP: Polyvinylpyrrolidone
Q: Quadrupole
QL: Quantification limit
Rox: Roxarsone, 3-nitro-4-hydroxyphenylarsonic acid
SBSE: Stir bar solid extraction
SdFFF: Sedimentation field-flow fractionation
SDME: Single-drop microextraction
Se(IV): Selenite
Se(VI): Selenate
SEC: Size-exclusion chromatography
SeCM: Selenocystamine
SeCys: Selenocysteine
SeCys2: Selenocystine
SeHoCys2: Selenohomocystine
SeMet: Selenomethionine
SeMetO: Selenomethionine Se-oxide
SEM-EDS: Scanning electron microscopy energy dispersive X-ray spectroscopy
SIDMS: Speciated isotope dilution mass spectrometry
SIA: Sequential injection analysis
SIM: Selected ion monitoring
SPE: Solid-phase extraction
SPME: Solid-phase microextraction
TBT: Tributyltin
TEA: Triethylamine
TEM: Transmission electron microscopy
TeET: Tetraethyltin
TeMT: Tetramethyltin
TETRA: Tetramethylarsonium ion
TFA: Trifluoroacetic acid
TIC: Total ion chromatogram
TMAH: Tetramethylammonium hydroxide
TMAO: Trimethylarsine oxide
TMSe: Trimethylselonium ion
TMT: Trimethyltin
TOF: Time of flight
TPhT: Triphenyltin
TPrT: Tripropyltin

References

1 Ruzik L. Speciation of challenging elements in food by atomic spectrometry. *Talanta* 2012:**93**:18–31.
2 Wrobel K, Wrobel K, Caruso JA. Liquid chromatography with inductively coupled plasma mass spectrometric detection for element speciation: clinical and toxicological applications. In: Winecker RE, Bertholf R (eds) *Chromatographic Methods in Clinical Chemistry and Toxicology*. John Wiley & Sons Ltd, Chichester, 2007, pp. 217–275.

3 de la Calle MB, Baer I, Robouch P *et al*. Is it possible to agree on a value for inorganic arsenic in food? The outcome of IMEP-112. *Anal Bioanal Chem* 2012;**404**:2475–2488.

4 Pyrzynska K. Selenium speciation in enriched vegetables. *Food Chem* 2009;**114**:1183–1191.

5 Thiry C, Ruttens A, De Temmerman L, Schneider YJ, Pussemier L. Current knowledge in species-related bioavailability of selenium in food. *Food Chem* 2012;**130**:767–784.

6 Wrobel K, Wrobel K, Caruso JA. Application of ICP-MS for the evaluation of Se species in food related products and dietary supplements. In: Caroli S (ed.) *The Determination of Chemical Elements in Food. Applications for Atomic and Mass Spectrometry*. John Wiley & Sons, Hoboken, NJ, 2007, pp. 671–706.

7 Krystek P, Rob R. Determination of Hg species in seafood. In: Caroli S (ed.) *The Determination of Chemical Elements in Food. Applications for Atomic and Mass Spectrometry*. John Wiley & Sons, Hoboken, NJ, 2007, pp. 707–726.

8 Nakazato T. Analytical methods for speciation of organic arsenicals. *Biomed Res Trace Elem* 2007;**18**:63–72.

9 Carey AM, Lombi E, Donner E *et al*. A review of recent developments in the speciation and location of arsenic and selenium in rice grain. *Anal Bioanal Chem* 2012;**402**:3275–3286.

10 Romarís-Hortas V, García-Sartal C, Barciela-Alonso MC, Domínguez-González R, Moreda-Piñeiro A, Bermejo-Barrera P. Bioavailability study using an *in-vitro* method of iodine and bromine in edible seaweed. *Food Chem* 2011;**124**:1747–1752.

11 Pedrero Z, Madrid Y. Novel approaches for selenium speciation in foodstuffs and biological specimens: a review. *Anal Chim Acta* 2009;**634**:135–152.

12 Moreda-Piñeiro J, Moreda-Piñeiro A, Romarís-Hortas V *et al*. *In-vivo* and *in-vitro* testing to assess the bioaccessibility and the bioavailability of arsenic, selenium and mercury species in food samples. *Trends Anal Chem* 2011;**30**:324–345.

13 Khouzam RB, Szpunar J, Holeman M, Lobinski R. Trace element speciation in food: state of the art of analytical techniques and methods. *Pure Appl Chem* 2012;**84**:169–179.

14 Caparand SG, Szefer P. Determination and speciation of trace elements in foods. In: Otles S (ed.) *Methods of Analysis of Food Components and Additives*.CRC Press, Boca Raton, FL, 2011.

15 Meija J, Caruso JA. Plasma spectrometry for element speciation and characterization in beverages. *J AOAC Int* 2004;**87**:205–224.

16 Welna M, Szymczyha-Madeja A, Stelmah E, Pohl P. Speciation and fractionation of elements in tea infusions. *Crit Rev Anal Chem* 2012;**42**:349–365.

17 World Health Organization. Vitamin and mineral requirements in human nutrition. World Health Organization and Food and Agriculture Organization of the United Nations, Hong Kong, 2004.

18 Clémens S, Monperrus M, Donard OF, Amouroux D, Guerin T. Mercury speciation analysis in seafood by species-specific isotope dilution: method validation and occurrence data. *Anal Bioanal Chem* 2011;**401**:2699–2711.

19 Sanchez-Rodas D, Corns WT, Chen B, Stockwell PB. Atomic fluorescence spectrometry: a suitable detection technique in speciation studies for arsenic, selenium, antimony and mercury. *J Anal Atom Spectrom* 2010;**25**:933–946.

20 Molin M, Ulven SM, Dahl L *et al*. Humans seem to produce arsenobetaine and dimethylarsinate after a bolus dose of seafood. *Environ Res* 2012;**112**:28–39.

21 Feldmann J, Krupp EM. Critical review or scientific opinion paper. Arsenosugars: a class of benign arsenic species or justification for developing partly speciated arsenic fractionation in foodstuffs? *Anal Bioanal Chem* 2011;**399**:1735–1741.

22 Conklin SD, Shockey N, Kubachka K, Howard KD, Carson MC. Development of an ion chromatograpgy-inductively coupled plasma-mass spectrometry method to determine inorganic arsenic in liver from chicken treated with roxarsone. *J Agric Food Chem* 2012;**60**:9394–9404.

23 Liu L, He B, Yun Z, Sun J, Jiang G. Speciation analysis of arsenic compounds by capillary electrophoresis on-line coupled with inductively coupled plasma mass spectrometry using a novel interfase. *J Chromatogr A* 2013;**1304**:227–233.

24 He M, Wang WX. Factors affecting the bioaccessibility of methylmercury in several marine fish species. *J Agric Food Chem* 2011;**59**:7155–7162.

25 Pizarro F, Boccio J, Salgueiro M *et al*. Bioavailability of stabilised ferrous gluconate with glycine in fresh cheese matrix: a novel iron compound for food fortification. *Biol Trace Elem Res* 2013;**151**:441–445.

26 Zheng FY, Chen LH, Li SX, Qiu JQ. Effect of edible plants combination on mineral bioaccessibility and bioavailability, using *in vitro* digestion and liposome-affinity extraction. *Food Res Int* 2013;**53**:174–179.

27 Llorens A, Lloret E, Picouet PA, Trbojevich R, Fernandez A. Metallic-based micro and nanocomposites in food contact materials and active food packaging. *Trends Food Sci Technol* 2012;**24**:19–29.

28 Klaine SI, Alvarez PJJ, Batley GE *et al*. Nanomaterials in the environment: behavior, fate, bioavailability, and effects. *Environ Toxicol Chem* 2008;**27**:1825–1851.

29 Hart DJ, Fairweather-Tait SJ, Broadley MR *et al*. Selenium concentration and speciation in biofortified flour and bread: retention of selenium during grain biofortification, processing and production of Se-enriched food. *Food Chem* 2011;**126**:1771–1778.

30 Horner NS, Beauchemin D. The effect of cooking and washing rice on the bio-accessibility of As, Cu, Fe, V and Zn using an on-line continuous leaching method. *Anal Chim Acta* 2013;**758**:28–35.

31 Koplik R, Linkova M, Mestek O. Changes of phosphorus and trace elements species in rye and oat flakes and oat porridge induced by simulated digestion. *Eur Food Res Technol* 2011;**232**:1007–1016.

32 Proietti I, Mantovani A, Mouquet-Rivier C, Guyot JP. Modulation of chelating factors, trace minerals and their estimated bioavailability in Italian and African sorghum

(*Sorghum bicolor* (L.) Moench) porridges. *Int J Food Sci Technol* 2013;**48**:1526–1532.

33 Alava P, Du Laing G, Odhiambo M, Verliefde A, Tack F, Van de Wiele TR. Arsenic bioaccessibility upon gastrointestinal digestion is highly determined by its speciation and lipid–bile salt interactions. *J Environ Sci Health A* 2013;**48**:656–665.

34 Gautam S, Platel K, Srinivasan K. Higher bioaccessibility of iron and zinc from food grains in the presence of garlic and onion. *J Agric Food Chem* 2011;**58**:8426–8429.

35 Sanz-Penella JM, Laparra JM, Sanz Y, Haros M. Bread supplemented with amaranth (*Amaranthus cruentus*): effect of phytates on *in vitro* iron absorption. *Plant Foods Hum Nutr* 2012;**67**:50–56.

36 Moreda-Piñeiro J, Moreda-Piñeiro A, Romarís-Hortas V *et al*. ICP-MS for the determination of selenium bioavailability from seafood and effect of major food constituents. *Microchem J* 2013;**108**:174–179.

37 Li SX, Chen LH, Zheng FY, Li YC. Effect of the cp4–epsps gene on metal bioavailability in maize and soybean using bionic gastrointestinal tracts and ICP-MS determination. *J Agric Food Chem* 2013;**61**:1579 – 1584.

38 Laird BD, Shade C, Gantner N, Chan HM, Siciliano SD. Bioaccessibility of mercury from traditional northern country foods measured using an *in vitro* gastrointestinal model is independent of mercury concentration. *Sci Total Environ* 2009;**407**:6003–6008.

39 Leufroy A, Noel L, Beauchemin D, Guerin T. Bioaccessibility of total arsenic and arsenic species in seafood as determined by a continuous online leaching method. *Anal Bioanal Chem* 2012;**402**:2849–2859.

40 Thiry C, Schneider YJ, Pussemier L, De Temmerman L, Ruttens A. Selenium bioaccessibility and bioavailability in Se-enriched food supplements. *Biol Trace Elem Res* 2013;**152**:152–160.

41 Signes-Pastor AJ, Al-Rmalli SW, Jenkins RO, Carbonell-Barrachina AA, Haris PI. Arsenic bioaccessibility in cooked rice as affected by arsenic in cooking water. *J Food Sci* 2012;**77**:T201–T206.

42 Beltrami D, Calestani D, Maffini M *et al*. Development of a combined SEM and ICP-MS approach for the qualitative and quantitative analyses of metal microparticles and submicroparticles in food products. *Anal Bioanal Chem* 2011;**401**:1401–1409.

43 Loeschner K, Navratilova J, Kobler K *et al*. Detection and characterization of silver nanoparticles in chicken meat by asymmetric flow field flow fractionation with detection by conventional or single particle ICP-MS. *Anal Bioanal Chem* 2013;**405**:8185–8195.

44 Garcia-Sartal C, Taebunpakul S, Stokes E, Barciela-Alonso MC, Bermejo-Barrera P, Goenaga-Infante H. Two-dimensional HPLC coupled to ICP-MS and electrospray ionisation (ESI)-MS/MS for investigating the bioavailability *in vitro* of arsenic species from edible seaweed. *Anal Bioanal Chem* 2012;**402**: 3359–3369.

45 Lipiec E, Warowicka O, Ruzik L, Zhou Y, Jarosz M, Połec-Pawlak K. Investigation of iodine bioavailability from chicken eggs versus iodized kitchen salt with *in vitro* method. *Eur Food Res Technol* 2012;**234**:913–919.

46 Troesch B, Jing H, Laillou A, Fowler A. Absorption studies show that phytase from *Aspergillus niger* significantly increases iron and zinc bioavailability from phytate-rich foods. *Food Nutr Bull* 2013;**34**:90S–101S.

47 Romarís-Hortas V, Bermejo-Barrera P, Moreda-Piñeiro A. Development of anion-exchange/reversed-phase high performance liquid chromatography–inductively coupled plasma-mass spectrometry methods for the speciation of bio-available iodine and bromine from edible seaweed. *J Chromatogr A* 2012;**1236**:164–176.

48 Brun E, Jugan ML, Herlin-Boime N *et al*. Investigation of TiO_2 nanoparticles translocation through a Caco-2 monolayer. *J Phys Conf Series* 2011;**304**:012048.

49 Leffers L, Wehe CA, Huewel S *et al*. *In vitro* intestinal bioavailability of arsenosugar metabolites and presystemic metabolism of thio-dimethylarsenic acid in Caco-2 cells. *Metallomics* 2013;**5**:1031–1042.

50 Moreda-Pineiro A, Romarís-Hortas V, Bermejo-Barrera P. A review on iodine speciation for environmental, biological and nutrition fields. *J Anal Atom Spectrom* 2011;**26**:2107–2152.

51 Bhatia P, Aureli F, D'Amato M *et al*. Selenium bioaccessibility and speciation in biofortified *Pleurotus* mushrooms grown on selenium-rich agricultural residues. *Food Chem* 2013;**140**:225–230.

52 Ovca A, van Elteren JT, Falnoga I, Selih VS. Speciation of zinc in pumpkin seeds (*Cucurbita pepo*) and degradation of its species in the human digestive tract. *Food Chem* 2011;**128**:839–846.

53 Schrauzer GN. Selenium and selenium-antagonistic elements in nutritional cancer prevention. *Crit Rev Biotechnol* 2009;**29**:10–17.

54 Cabanero AI, Madrid Y, Camara C. Mercury–selenium species ratio in representative fish samples and their bioaccessibility by an *in vitro* digestion method. *Biol Trace Elem Res* 2007;**119**:195–211.

55 Sanz-Medel A, Fernández-Sánchez ML, González Iglesias H, López-Sastre JB. Speciation and isotope pattern deconvolution for inductively coupled plasma-mass spectrometry quantitative studies of mineral metabolism and supplementation. *Pure Appl Chem* 2010;**82**:447–460.

56 Bendicho C, Lavilla I, Pena-Pereira F, Romero V. Green chemistry in analytical atomic spectrometry: a review. *J Anal Atom Spectrom* 2012;**27**:1831–1857.

57 Vieira MA, Grinberg P, Bobeda CRR, Reyes MNM, Campos RC. Non-chromatographic atomic spectrometric methods in speciation analysis: a review. *J Hazard Mater* 2009;**64**:459–476.

58 Gonzalvez A, Armenta S, Cervera ML, de la Guardia M. Non-chromatographic speciation. *Trends Anal Chem* 2010;**29**: 260–268.

59 Anawar HM. Arsenic speciation in environmental samples by hydride generation and electrothermal atomic absorption spectrometry. *Talanta* 2012;**88**:30–42.
60 Shah AQ, Kazi TG, Baig JA, Afridi HI, Arain MB. Simultaneous determination of methyl and inorganic mercury in fish species by cold vapour generation atomic absorption spectrometry. *Food Chem* 2012;**134**:2345–2349.
61 Zhang Y, Adeloju SD. Speciation of mercury in fish samples by flow injection catalytic cold vapour atomic absorption spectrometry. *Anal Chim Acta* 2012;**721**:22– 27.
62 Tyson J. The determination of arsenic compounds: a critical review. *ISRN Anal Chem* 2013;Article ID 835371. doi:10.1155/2013/835371.
63 European Standard EN 15517:2008. Determination of trace elements: determination of inorganic As in seaweed by hydride generation atomic absorption spectrometry (HG-AAS) after acid digestion.
64 European Standard EN 16278:2012. Animal feeding stuffs: determination of inorganic arsenic by hydride generation atomic absorption spectrometry (HG-AAS) after microwave extraction and separation by solid phase extraction (SPE).
65 Balarama Krishna MV, Chandrasekaran K, Karunasagar D. On-line speciation of inorganic and methyl mercury in waters and fish tissues using polyaniline micro-column and flow injection-chemical vapour generation-inductively coupled plasma mass spectrometry (FI-CVG-ICPMS). *Talanta* 2010;**81**:462–472.
66 Rasmussen RR, Hedegaard RV, Larsen EH, Sloth JJ. Development and validation of an SPE HG-AAS method for determination of inorganic arsenic in samples of marine origin. *Anal Bioanal Chem* 2012;**403**:2825–2834.
67 Rasmussen RR, Qian Y, Sloth JJ. SPE HG-AAS method for the determination of inorganic arsenic in rice: results from method validation studies and a survey on rice products. *Anal Bioanal Chem* 2013;**405**:7851–7857.
68 Sun H, Liu X. A slurry sampling hydride generation atomic absorption spectrometric method for speciation analysis of trace inorganic arsenic in flours. *J Environ Chem Ecotoxicol* 2011;**3**:9–16.
69 Arslan Y, Yildirim E, Gholami M, Bakirdere S. Lower limits of detection in speciation analysis by coupling high-performance liquid chromatography and chemical-vapour generation. *Trends Anal Chem* 2011;**30**:569–585.
70 Wu P, He L, Zheng C, Hou X, Sturgeon RE. Applications of chemical vapor generation in non-tetrahydroborate media to analytical atomic spectrometry. *J Anal Atom Spectrom* 2010;**25**:1217–1246.
71 Ambushe AA, McCrindle RI, McCrindle CME. Speciation of chromium in cow's milk by solid-phase extraction/dynamic reaction cell inductively coupled plasma mass spectrometry (DRC-ICP-MS). *J Anal Atom Spectrom* 2009;**24**:502–507.
72 Tuzen M, Saygi KO, Karaman I, Soylak M. Selective speciation and determination of inorganic arsenic in water, food and biological samples. *Food Chem Toxicol* 2010;**48**:41–46.
73 Herrero Latorre C, Álvarez Méndez J, Barciela García J, García Martín S, Peña Crecente RM. Carbon nanotubes as solid-phase extraction sorbents prior to atomic spectrometric determination of metal species: a review. *Anal Chim Acta* 2012;**749**:16– 35.
74 Duarte FA, Soares BM, Vieira AA *et al*. Assessment of modified matrix solid-phase dispersion as sample preparation for the determination of CH_3Hg^+ and Hg^{2+} in fish. *Anal Chem* 2013;**85**:5015 – 5022.
75 Tian J, Huang T, Lu J. Speciation of inorganic antimony in food and water samples by flow injection on-line nano γ-alumina micro-column solid-phase extraction coupled with slotted tube atom trapping flame atomic absorption spectrometry. *Food Anal Method* 2013;**6**:1080–1089.
76 Mao X, Chen B, Huang C, He M, Hu B. Titania immobilized polypropylene hollow fiber as a disposable coating for stir bar sorptive extraction–high performance liquid chromatography–inductively coupled plasma mass spectrometry speciation of arsenic in chicken tissues. *J Chromatogr A* 2011;**1218**:1–9.
77 Giakisikli G, Anthemidis AN. Magnetic materials as sorbents for metal/metalloid preconcentration and/or separation. A review. *Anal Chim Acta* 2013;**789**:1–16.
78 He C, Long Y, Pan J, Li K, Liu F. Application of molecularly imprinted polymers to solid-phase extraction of analytes from real samples. *J Biochem Biophys Method* 2007;**70**:133–150.
79 Abedi H, Ebrahimzadeh H. Imprinted polymer-based extraction for speciation analysis of inorganic tin in food and water samples *React Funct Polym* 2013;**73**:634–640.
80 Asensio-Ramos M, Ravelo-Pérez LM, González-Curbelo MA, Hernández-Borges J. Liquid phase microextraction applications in food analysis. *J Chromatogr A* 2011;**1218**:7415– 7437.
81 Pena-Pereira F, Lavilla I, Bendicho C. Miniaturized preconcentration methods based on liquid–liquid extraction and their application in inorganic ultratrace analysis and speciation: a review. *Spectrochim Acta B* 2009;**64**:1–15.
82 Soares ME, Vieira E, Bastos ML. Chromium speciation analysis in bread samples. *J Agric Food Chem* 2010;**58**:1366–1370.
83 Sun M, Liu G, Wuc Q. Speciation of organic and inorganic selenium in selenium-enriched rice by graphite furnace atomic absorption spectrometry after cloud point extraction. *Food Chem* 2013;**141**:66–71.
84 Ulusoy S, Ulusoy HI, Akçay M, Gürkan R. Inexpensive and versatile method for trace Sn(II) and Sn(IV) ions in food samples by CPE/FAAS. *Food Chem* 2012;**134**:419–426.
85 Lopez-García I, Vicente-Martínez Y, Hernández-Córdoba M. Nonchromatographic speciation of selenium in edible oils using dispersive liquid – liquid microextraction and electrothermal atomic absorption spectrometry. *J Agric Food Chem* 2013;**61**:9356–9361.
86 Martinis EM, Escudero LB, Berton P, Monasterio RP, Filippini MF, Wuilloud RG. Determination of inorganic selenium

species in water and garlic samples with on-line ionic liquid dispersive microextraction and electrothermal atomic absorption spectrometry. *Talanta* 2011;**85**:2182– 2188.

87 Martín-Calero A, Pino V, Afonso AM. Ionic liquids as a tool for determination of metals and organic compounds in food analysis. *Trends Anal Chem* 2011;**30**:1598–1619.

88 Majidi B, Shemirani F. Salt-assisted liquid–liquid microextraction of Cr(VI) ion using an ionic liquid for preconcentration prior to its determination by flame atomic absorption spectrometry. *Microchim Acta* 2012;**176**:143–151.

89 Dubascoux S, Le Hécho I, Hassellov M, Von Der Kammer F, Potin Gautiera M, Lespes G. Field-flow fractionation and inductively coupled plasma mass spectrometer coupling: history, development and applications. *J Anal Atom Spectrom* 2010;**25**:613–623.

90 von der Kammer F, Legros S, Larsen EH, Loeschner K, Hofmann T. Separation and characterization of nanoparticles in complex food and environmental samples by field-flow fractionation. *Trends Anal Chem* 2011;**30**: 425–436.

91 Contado C, Ravani L, Passarella M. Size characterization by sedimentation field flow fractionation of silica particles used as food additives. *Anal Chim Acta* 2013;**788**:183– 192.

92 López-García I, Briceño M, Hernández-Córdoba M. Non-chromatographic screening procedure for arsenic speciation analysis in fish-based baby foods by using electrothermal atomic absorption spectrometry. *Anal Chim Acta* 2011;**699**:11–17.

93 Li YF, Wang X, Wang L, Li B, Gao Y, Chen C. Direct quantitative speciation of selenium in selenium-enriched yeast and yeast-based products by X-ray absorption spectroscopy confirmed by HPLC-ICP-MS. *J Anal Atom Spectrom* 2010;**25**:426–430.

94 Lombi E, Scheckel KG, Kempson IM. In situ analysis of metal(loid)s in plants: state of the art and artefacts. *Environ Exp Bot* 2013;**72**:3–17.

95 Maher W, Foster S, Krikowa F, Donner E, Lombi E. Measurement of inorganic arsenic species in rice after nitric acid extraction by HPLC-ICPMS: verification using XANES. *Environ Sci Technol* 2013;**47**:5821 – 5827.

96 George GN, Singh SP, Prince RC, Pickering IJ. Chemical forms of mercury and selenium in fish following digestion with simulated gastric fluid. *Chem Res Toxicol* 2008;**21**: 2106–2110.

97 Heumann KG. Hyphenated techniques: the most commonly used method for trace elemental speciation analysis. *Anal Bioanal Chem* 2002;**373**:323–324.

98 Wrobel K, Wrobel K, Caruso JA. Metal analysis (ICP-OES and ICP-MS). In: Scott RE, Lukehart CM (eds) *Applications of Physical Methods to Inorganic and Bioinorganic Chemistry*. Encyclopedia of Inorganic Chemistry, Vol. 1. John Wiley & Sons, Chichester, 2007, pp. 205–225.

99 Montes-Bayón M, DeNicola K, Caruso JA. Liquid chromatography-inductively coupled plasma mass spectrometry. *J Chromatogr A* 2002;**1000**:457–476.

100 Agatemor C, Beauchemin D. Matrix effects in inductively coupled plasma mass spectrometry: a review. *Anal Chim Acta* 2011;**706**:66–86.

101 Szpunar J. Advances in analytical methodology for bioinorganic speciation analysis: metallomics, metalloproteomics and heteroatom-tagged proteomics and metabolomics, *Analyst* 2005;**130**:442–465.

102 Meyer G. Nebulizers for inductively coupled plasma sepctroscopy. *Spectroscopy* 2013;**28**:40–46.

103 Popp M, Hann S, Koellensperger G. Environmental application of elemental speciation analysis based on liquid or gas chromatography hyphenated to inductively coupled plasma mass spectrometry. *A review. Anal Chim Acta* 2010;**668**:114–129.

104 Profrock D. Progress and possible applications of miniaturised separation techniques and elemental mass spectrometry for quantitative, heteroatom-tagged proteomics. *Anal Bioanal Chem* 2009;**398**:2383–2401.

105 Profrock D, Prange A. Inductively coupled plasma-mass spectrometry (ICP-MS) for quantitative analysis in environmental and life sciences: a review of challenges, solutions, and trends. *Appl Spectrosc* 2012;**66**:843–868.

106 Holcapek M, Jirásko R, Lísa M. Recent developments in liquid chromatography–mass spectrometry and related techniques. *J Chromatogr A* 2012;**1259**:3– 15.

107 Jirásko R, Holcapek M. Sructural analysis of organometallic compounds with soft ionization mass spectrometry. *Mass Spectrom Rev* 2011;**30**:1013–1036.

108 Rosen AL, Hieftje GM. Inductively coupled plasma mass sectromerty and electrospray mass spectrometry for speciation analysis: applications and instrumentation. *Spectrochim Acta B* 2004;**59**:135–146.

109 Jayasinghe SB, Caruso JA. Investigation of Se-containing proteins in *Bertholletia excelsa* H.B.K. (Brazil nuts) by ICP-MS, MALDI-MS and LC–ESI-MS methods. *Int J Mass Spectrom* 2011;**307**:16–27.

110 Hölttä M, Zetterberg H, Mirgorodskaya E, Mattson N, Blennov K, Gobom J. Peptidome analysis of cerebrospinal fluid by LC-MALDI MS. *PloS ONE* 2012;**7**:e42555.

111 Sola-Vázquez A, Costa-Fernández JM, Pereiro R, Sanz-Medel A. Plasma-based mass spectrometry for simultaneous acquisition of elemental and molecular information. *Analyst* 2011;**136**:246–256.

112 Mesko MF, Hartwig CA, Bizzi CA, Pereira JSF, Mello PA, Flores EM. Sample preparation strategies for inorganic analysis by inductively coupled plasma mass spectrometry. *Int J Mass Spectrom* 2011;**307**:123–136.

113 Groskreutz SR, Swenson MM, Secor LB, Stoll DR. Selective comprehensive multi-dimensional separation for resolution enhancement in high performance liquid chromatography. Part I: Principles and instrumentation. *J Chromatogr A* 2012;**1228**:31–40.

114 Núñez O, Gallart-Ayala H, Martins CPB, Lucci P. New trends in fast liquid chromatography for food and environmental analysis. *J Chromatogr A* 2012;**1228**:298– 323.

115 Engelhard C. Inductively coupled plasma mass spectrometry: recent trends and developments. *Anal Bioanal Chem* 2011;**399**:213–219.

116 Timerbaev AR. Element speciation analysis using capillary electrophoresis: twenty years of development and applications. *Chem Rev* 2013;**113**:778–812.

117 Ai X, Wang Y, Hou X, Yang L, Zheng C, Wu L. Advanced oxidation using Fe_3O_4 magnetic nanoparticles and its application in mercury speciation analysis by high performance liquid chromatography-cold vapor generation atomic fluorescence spectrometry. *Analyst* 2013;**138**:3494–3501.

118 Chen X, Han C, Cheng H *et al*. Rapid speciation analysis of mercury in seawater and marine fish by cation exchange chromatography hyphenated with inductively coupled plasma mass spectrometry. *J Chromatogr A* 2013;**1314**:86– 93.

119 Guzmán-Mar JL, Hinojosa-Reyes L, Serra AM, Hernández-Ramírez A, Cerdá V. Applicability of multisyringe chromatography coupled to cold-vapor atomic fluorescence spectrometry for mercury speciation analysis. *Anal Chim Acta* 2011;**708**:11–18.

120 Lemos Batista B, Rodrigues JL, de Souza SS, Oliveira Souza VC, Barbosa F Jr. Mercury speciation in seafood samples by LC–ICP-MS with a rapid ultrasound-assisted extraction procedure: application to the determination of mercury in Brazilian seafood samples. *Food Chem* 2011;**126**:2000–2004.

121 Schmidt L, Bizzi CA, Duarte FA, Dressler VL, Flores EMM. Evaluation of drying conditions of fish tissues for inorganic mercury and methylmercury speciation analysis. *Microchem J* 2013;**108**:53–59.

122 Gao Y, Shi Z, Long Z, Wu P, Zheng C, Hou X. Determination and speciation of mercury in environmental and biological samples by analytical atomic spectrometry. *Microchem J* 2012;**103**:1–14.

123 Zhao Y, Zheng J, Fang L *et al*. Speciation analysis of mercury in natural water and fish samples by using capillary electrophoresis-inductively coupled plasma mass spectrometry. *Talanta* 2012;**89**:280–285.

124 Reyes LH, Mizanur Rahman GM, Fahrenholz T, Skip Kingston HM. Comparison of methods with respect to efficiencies, recoveries, and quantitation of mercury species interconversions in food demonstrated using tuna fish. *Anal Bioanal Chem* 2008;**390**:2123–2132.

125 Amaral CDB, Nóbrega, JA, Nogueira ARA. Sample preparation for arsenic speciation in terrestrial plants: a review. *Talanta* 2013;**115**:291–299.

126 Leermakers M, Bayens W, De Gieter M *et al*. Toxic arsenic compounds in environmental samples: speciation and validation. *Trends Anal Chem* 2006;**25**:1–10.

127 Amman AA. Arsenic speciation analysis by ion chromatography: a critical review of principles and applications. *Am J Anal Chem* 2011;**2**:27–45.

128 Conklin SD, Chen PE. Quantification of four arsenic species in fruit juices by ion-chromatography-inductively coupled plasma-mass spectrometry. *Food Addit Contam A* 2012;**29**:1272–1279.

129 D'Amato M, Aureli F, Ciardullo S, Raggi A, Cubadda F. Arsenic speciation in wheat and wheat products using ultrasound- and microwave-assisted extraction and anion exchange chromatography-inductively coupled plasma mass spectrometry. *J Anal Atom Spectrom* 2011;**26**:207–213.

130 Huang JH, Fecher P, Ilgen G, Hud KN, Yang J. Speciation of arsenite and arsenate in rice grain: verification of nitric acid based extraction method and mass sample survey. *Food Chem* 2012;**130**:453–459.

131 Raber G, Stock N, Hanel P, Murko M, Navratilova J, Francesconi KA. An improved HPLC–ICPMS method for determining inorganic arsenic in food: application to rice, wheat and tuna fish. *Food Chem* 2012;**134**:524–532.

132 Petursdottir AH, Gunnlaugsdottir H, Jorundsdottir H, Mestrot A, Krupp EM, Feldmann J. HPLC-HG-ICP-MS: a sensitive and selective method for inorganic arsenic in seafood. *Anal Bioanal Chem* 2012;**404**:2185–2191.

133 Jesus JP, Suárez CA, Ferreira JR, Giné MF. Sequential injection analysis implementing multiple standard additions for As speciation by liquid chromatography and atomic fluorescence spectrometry (SIA-HPLC-AFS). *Talanta* 2011;**85**:1364–1368.

134 Huang JH, Ilgen G, Decker B. Sample pre-treatment to eliminate cationic methylated arsenic for determining arsenite on an anion-exchange column by high performance liquid chromatography-inductively coupled plasma mass spectrometry. *Anal Chim Acta* 2008;**611**: 48–55.

135 Contreras-Acuña M, García-Barrera T, García-Sevillano MA, Gómez-Ariza JL. Speciation of arsenic in marine food (*Anemonia sulcata*) by liquid chromatography coupled to inductively coupled plasma mass spectrometry and organic mass spectrometry. *J Chromatogr A* 2013;**1282**: 133– 141.

136 Narukawa T, Chiba K. Heat-assisted aqueous extraction of rice flour for arsenic speciation analysis. *J Agric Food Chem* 2010;**58**:8183–8188.

137 Xie D, Mattusch J, Wennrich R. Retention of arsenic species on zwitterionic stationary phase in hydrophilic interaction chromatography. *J Sep Sci* 2010;**33**:817–825.

138 Hsieh YJ, Jiang SJ. Application of HPLC-ICP-MS and HPLC-ESI-MS procedures for arsenic speciation in seaweeds. *J Agric Food Chem* 2012;**60**:2083 – 2089.

139 Garcia-Salgado S, Quijano MA, Bonilla MM. Arsenic speciation in edible algae samples by microwave-assisted extraction and high performance liquid chromatography coupled to atomic fluorescence spectrometry. *Anal Chim Acta* 2012;**714**:38–46.

140 Niedzielski P, Mleczek M, Magdziak Z, Siwulski M, Kozak L. Selected arsenic species: As(III), As(V) and dimethylarsenic acid (DMAA) in *Xerocomus badius* fruiting bodies. *Food Chem* 2013;**141**:3571–3577.

141 Niegel C, Matysik FM. Analytical methods for the determination of arsenosugars: a review of recent trends and developments. *Anal Chim Acta* 2010;**657**:83–99.

142 Maher WA, Foster S, Krikowa F *et al*. Thio arsenic species measurements in marine organisms and geothermal waters. *Microchem J* 2013;**111**:82–90.

143 Cui J, Xiao Y, Dai L, Zhao X, Wang Y. Speciation of organoarsenic species in food of animal origin using accelerated solvent extraction (ASE) with determination by HPLC-hydride generation-atomic fluorescence spectrometry (HG-AFS). *Food Anal Method* 2013;**6**: 370–379.

144 Nachman KE, Raber G, Francesconi KA, Navas-Acien A, Love DC. Arsenic species in poultry feather meal. *Sci Total Environ* 2012;**417–418**:183–188.

145 Wrobel K, Wrobel K, Caruso JA. Pretreatment procedures for characterization of arsenic and selenium species in complex samples utilizing coupled techniques with mass spectrometric detection. *Anal Bioanal Chem* 2005;**381**:317–331.

146 Francesconi KA, Kuehnelt D. Determination of arsenic species: a critical review of methods and applications, 2000–2003. *Analyst* 2004;**129**:373–395.

147 Sele V, Sloth JJ, Lundebye AK, Larsen EH, Berntssen MHG, Amlund H. Arsenolipids in marine oils and fats: a review of occurrence, chemistry and future research needs. *Food Chem* 2012;**133**:618–630.

148 Narukawa T, Hioki A, Chiba K. Speciation and monitoring test for inorganic arsenic in white rice flour. *J Agric Food Chem* 2012;**60**:1122–1127.

149 Batista BL, Souza JMO, De Souza SS, Barbosa F Jr. Speciation of arsenic in rice and estimation of daily intake of different arsenic species by Brazilians through rice consumption. *J Hazard Mater* 2011;**191**:342–348.

150 Huang JH, Ilgen G, Fecher P. Quantitative chemical extraction for arsenic speciation in rice grains. *J Anal Atom Spectrom* 2010;**25**:800–802.

151 Chu YL, Jiang SJ. Speciation analysis of arsenic compounds in edible oil by ion chromatography–inductively coupled plasma mass spectrometry. *J Chromatogr A* 2011;**1218**:5175– 5179.

152 Fukuda S, Terasawa M, Shiomi K. Phosphatidylarsenocholine, one of the major arsenolipids in marine organisms: synthesis and metabolism in mice. *Food Chem Toxicol* 2013;**49**:1598–1603.

153 Lischka S, Arroyo-Abad U, Mattusch J, Kuhn A, Piechotta C. The high diversity of arsenolipids in herring fillet (*Clupea harengus*). *Talanta* 2013;**110**:144–152.

154 Amayo KO, Petursdottir A, Newcombe C *et al*. Identification and quantification of arsenolipids using reversed-phase HPLC coupled simultaneously to high-resolution ICPMS and high-resolution electrospray MS without species-specific standards. *Anal Chem* 2011;**83**:3589–3595.

155 Kieliszek M, Blazejak S. Selenium: significance, and outlook for supplementation. *Nutrition* 2013;**29**:713–718.

156 Rayman MP, Infante HG, Sargent M. Food-chain selenium and human health: spotlight on speciation. *Br J Nutr* 2008;**100**:238–253.

157 Goenaga Infante H, Ovejero Bendito MC, Camara C, Evans L, Hearn R, Moesgaard S. Isotope dilution quantification of ultratrace gamma-glutamyl-Se-methylselenocysteine species using HPLC with enhanced ICP-MS detection by ultrasonic nebulisation or carbon-loaded plasma. *Anal Bioanal Chem* 2008;**390**:2099–2106.

158 Rodríguez-Castillón JA, Hinojosa-Reyes L, Marchante-Gayón JM, Moldovan M, García Alonso JI. Internal correction of spectral interferences and mass bias in ICP-MS using isotope pattern deconvolution: application to the determination of selenium in biological samples by isotope dilution analysis. *J Anal Atom Spectrom* 2008;**23**:579–582.

159 Yang L, Maxwell P, Mester Z. Microwave-assisted acid digestion protocol for the determination of methionine and selenomethionine in selenium-enriched yeast by species specific isotope dilution GC-MS. *Anal Method* 2013;**5**:525–529.

160 B'Hymer C, Caruso JA. Selenium speciation analysis using inductively coupled plasma-mass spectrometry. *J Chromatogr A* 2006;**1114**:1–20.

161 Wang W, Chen ZL, Davey DE, Naidu R. Speciation of selenium in biological samples by ion chromatography with inductively coupled plasma mass spectrometry. *J Liq Chromatogr Rel Technol* 2010;**33**:1151–1173.

162 Chen YW, Belzile N. High performance liquid chromatography coupled to atomic fluorescence spectrometry for the speciation of the hydride and chemical vapour-forming elements As, Se, Sb and Hg: a critical review. *Anal Chim Acta* 2010;**671**:9–26.

163 Yoshida S, Haratake M, Fuchigami T, Nakayama M. Selenium in seafood materials. *J Health Sci* 2011;**57**: 215–224.

164 Bañuelos GS, Stushnoff C, Walse SS *et al*. Biofortified, selenium enriched, fruit and cladode from three *Opuntia* cactus pear cultivars grown on agricultural drainage sediment for use in nutraceutical foods. *Food Chem* 2012;**135**:9–16.

165 Funes-Collado V, Morell-Garcia A, Rubio R, López-Sánchez JF. Study of selenocompounds from selenium-enriched culture of edible sprouts. *Food Chem* 2013;**141**:3738–3743.

166 Vale G, Rodrigues A, Rocha A *et al*. Ultrasonic assisted enzymatic digestion (USAED) coupled with high performance liquid chromatography and electrothermal atomic absorption spectrometry as a powerful tool for total selenium and selenium species control in Se-enriched food supplements. *Food Chem* 2010;**121**:268–274.

167 Kápolna E, Hillestrøm PR, Laursen KH, Husted S, Larsen EH. Effect of foliar application of selenium on its uptake and speciation in carrot. *Food Chem* 2009;**115**:1357–1363.

168 Hsieh YJ, Jiang SJ. Determination of selenium compounds in food supplements using reversed-phase liquid chromatography–inductively coupled plasma mass spectrometry. *Microchem J* 2013;**110**:1–7.

169 Maseko T, Callahan DL, Dunshea FR, Doronila A, Kolev SD, Ng K. Chemical characterisation and speciation of organic selenium in cultivated selenium-enriched *Agaricus bisporus*. *Food Chem* 2013;**141**:3681–3687.

170 Zhao YQ, Zheng JP, Yang MW, Yang GD, Wu YN, Fu FF. Speciation analysis of selenium in rice samples by using capillary electrophoresis-inductively coupled plasma mass spectrometry. *Talanta* 2011;**84**:983–988.

171 Wrobel K, Wrobel K, Caruso JA. Speciation small molecules. In: Beauchemin D, Matthews DE (eds) *Elemental and Isotope Ratio Mass Spectrometry*. Elsevier, Amsterdam, 2010, pp. 222–233.

172 Ramos SJ, Faquin V, Guilherme LRG *et al*. Selenium biofortification and antioxidant activity in lettuce plants fed with selenate and selenite. *Plant Soil Environ* 2010;**56**:584–588.

173 Zembrzuska J, Matusiewicz H, Polkowska-Motrenko H, Chajduk E. Simultaneous quantitation and identification of organic and inorganic selenium in diet supplements by liquid chromatography with tandem mass spectrometry. *Food Chem* 2014;**142**:178–187.

174 Quijano MA, Moreno P, Gutierrez AM, Perez Conde MC, Camara C. Selenium speciation in animal tissues after enzymatic digestion by high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry. *J Mass Spectrom* 2000;**35**:878–884.

175 Önning G. Speciation of soluble selenium compounds in different fish species. *Food Chem* 2000;**68**:133–139.

176 Huerta VD, Sánchez MLF, Sanz-Medel A. Quantitative selenium speciation in cod muscle by isotope dilution ICP-MS with a reaction cell: comparison of different reported extraction procedures. *J Anal Atom Spectrom* 2004;**19**:644–648.

177 Moreno P, Quijano MA, Gutierrez AM, Perez-Conde MC, Camara C. Study of selenium species distribution in biological tissues by size exclusion and ion exchange chromatagraphy inductively coupled plasma-mass spectrometry. *Anal Chim Acta* 2004;**524**:315–327.

178 Hinojosa-Reyes LH, Guzmán Mar JL, Mizanur Rahman GM, Seybert B, Fahrenholz T, Skip Kingston HM. Simultaneous determination of arsenic and selenium species in fish tissues using microwave-assisted enzymatic extraction and ion chromatography–inductively coupled plasma mass spectrometry. *Talanta* 2009;**78**:983–990.

179 Shah M, Wuilloud RG, Kannamkumarath SS, Caruso JA. Iodine speciation studies in commercially available seaweed by coupling different chromatographic techniques with UV and ICP-MS detection. *J Anal Atom Spectrom* 2005;**20**:176–182.

180 Romarís-Hortas V, Bermejo-Barrera P, Moreda-Pineiro A. Ultrasound-assisted enzymatic hydrolysis for iodinated amino acid extraction from edible seaweed before reversed-phase high performance liquid chromatography-inductively coupled plasma-mass spectrometry. *J Chromatogr A* 2013;**1309**:33–40.

181 Gómez-Jacinto V, Arias-Borrego A, García-Barrera T, Garbayo I, Vilchez C, Gómez-Ariza JL. Iodine speciation in iodine-enriched microalgae *Chlorella vulgaris*. *Pure Appl Chem* 2010;**82**:473–481.

182 Michalke B. Trace element speciation in human milk. *Pure Appl Chem* 2006;**78**:79–90.

183 Michalke B, Fernández-Sánchez ML, Sanz-Medel A. Elemental speciaton in human milk and substitute food for newborns. In: Caroli S (ed.) *The Determination of Chemical Elements in Food*. John Wiley & Sons, Hoboken, NJ, 2007, pp. 535–566.

184 Hoac T, Lundh T, Purup S, Önning G, Sejrsen K, Åkesson B. Separation of selenium, zinc, and copper compounds in bovine whey using size exclusion chromatography linked to inductively coupled plasma mass spectrometry. *J Agric Food Chem* 2007;**55**:4237–4243.

185 Fernández-Sánchez ML, St Remy RRF, González Iglesias H *et al*. Iron content and its speciation in human milk from mothers of preterm and full-term infants at early stages of lactation: a comparison with commercial infant milk formulas. *Microchem J* 2012;**105**:108–114.

186 Milacic R, Ajlec D, Zuliani T, Zigon D, Scancar J. Determination of Zn-citrate in human milk by CIM monolithic chromatography with atomic and mass spectrometry detection. *Talanta* 2012;**101**:203–210.

187 Malik J, Rankova A, Drabek O, Szakova J, Ash C, Kokoska L. Aluminium and other elements in selected herbal tea plant species and their infusions. *Food Chem* 2013;**139**:728–734.

188 Karak T, Bhagat RM. Trace elements in tea leaves, made tea and tea infusion: a review. *Food Res Int* 2010;**43**:2234–2252.

189 Costa LM, Gouveia ST, Nóbrega JA. Comparison of heating extraction procedures for Al, Ca, Mg and Mn in tea samples. *Anal Sci* 2002;**18**:313–318.

190 Pirker KF, Baratto MC, Basosi R, Goodman BA. Influence of pH on the speciation of copper(II) in reactions with the green tea polyphenols, epigallocatechin gallate and gallic acid. *J Inorg Biochem* 2012;**112**:10–16.

191 Wrobel K, Wrobel K, Colunga Urbina EM. Determination of total Al, Cr, Cu, Fe, Mn and Ni and their fractions leached to the infusions of black tea, green tea, *Hibiscus sabdariffa* and *Ilex paraguariensis* (mate) by ETA-AAS. *Biol Trace Elem Res* 2000;**78**:271–280.

192 Welna M, Szymczycha-Madeja A, Pohl P. A comparison of samples preparation strategies in the multi-elemental analysis of tea by spectrometric methods. *Food Res Int* 2013;**53**:922–930.

193 Odegard KE, Lund W. Multi-element speciation of tea infusion using cation-exchange separation and size exclusion chromatography in combination with inductively coupled plasma mass spectrometry. *J Anal Atom Spectrom* 1997;**12**:403–408.

194 Welna M, Szymczycha-Madeja A, Stelmach E, Pohl P. Speciation and fractionation of elements in tea infusions. *Crit Rev Anal Chem* 2012;**42**:349–365.

195 Scancar J, Zuliani T, Zigon D, Milacic R. Ni speciation in tea infusions by monolithic chromatography ICP-MS and Q-TOF-MS. *Anal Bioanal Chem* 2013;**405**:2041–2051.

196 Novotnik B, Zuliani T, Scancar J, Milacic R. Chromate in food samples: an artefact of wrongly applied analytical methodology? *J Anal Atom Spectrom* 2013;**28**:558–566.

197 Yu Z, Sun J, Jing M, Cao X, Lee F, Wang X. Determination of total tin and organotin compounds in shellfish by ICP-MS. *Food Chem* 2010;**119**:364–367.

198 Olivares D, Bravo M, Feldmann J, Raab A, Neaman A, Quiroz W. Development of an analytical method for antimony speciation in vegetables by HPLC-hydride generation-atomic fluorescence spectrometry. *J AOAC Int* 2012;**95**:1176–1182.

199 Naozuka J, Marana SR, Oliveira PV. Water-soluble Cu, Fe, Mn and Zn species in nuts and seeds. *J Food Compos Anal* 2010;**23**:78–85.

200 Easter RN, Caruso JA, Vonderheide AP. Recent developments and novel applications in GC-ICPMS. *J Anal Atom Spectrom* 2010;**25**:493–502.

201 Zachariadis GA. In situ derivatization of metals and organometallics using borate reagents in gas chromatographic speciation studies. *J Chromatogr A* 2013;**1296**:47–69.

202 Paterson AC, McAlister GC, Quarmby ST, Griep-Raming J, Coon JJ. Development and characterization of GC-enabled QLT-Orbitrap for high-resolution and high-mass accuracy GC/MS. *Anal Chem* 2010;**82**:8618–8628.

203 Kolachova K, Cajka T, Sandy C, Hajslova J, Pulkrabova J. High throughput sample preparation in combination with gas chromatography coupled to triple quadrupole tandem mass spectrometry (GC-MS/MS): a smart procedure for (ultra)trace analysis of brominated flame retardants in fish. *Talanta* 2013;**105**:109–116.

204 Pitarch E, Portolés T, Marín JM, Ibáñez M, Albarrán F, Hernández F. Analytical strategy based on the use of liquid chromatography and gas chromatography with triple-quadrupole and time-of-flight MS analyzers for investigating organic contaminants in wastewater. *Anal Bioanal Chem* 2010;**397**:2763–2776.

205 Leach AM, Heisterkamp FC, Adams FC, Hieftje GM. Gas chromatogrpahy–inductively coupled plasma time of flight mass spectrometry for the speciation analysis of organometallic compounds. *J Anal Atom Spectrom* 2000;**15**:151–155.

206 Szpunar J, Bouyssiere B, Lobinski R. Sample preparation techniques for elemental speciaiton studies. In: Caruso JA, Ackley KL (eds) *Comprehensive Analytical Chemistry*. Elsevier Science BV, Amsterdam, 2000, pp. 7–40.

207 Vas G, Vékey K. Solid-phase microextraction: a powerful sample preparation tool prior to mass spectromic analysis. *J Mass Spectrom* 2004;**39**:233–254.

208 Lord HL, Zhan W, Pawliszyn J. Fundamentals and applications of needle trap devices: a critical review. *Anal Chim Acta* 2010;**677**:3–18.

209 Sarafraz-Yazdi A, Amiri A. Liquid-phase microextraction. *Trends Anal Chem* 2010;**29**:1–14.

210 Viñas P, Campillo N, López-Gacía I, Hernández-Córdoba M. Dispersive liquid–liquid microextraction in food analysis. A critical review. *Anal Bioanal Chem* 2014;**406**:2067–2099.

211 Diez S, Bayona JM. Determination of Hg and organomercury species following SPME: a review. *Talanta* 2008;**77**:21–27.

212 Hirner AV. Speciation of alkylated metals and metalloids in the environment. *Anal Bioanal Chem* 2006;**385**:555–567.

213 Pereiro IR, Diaz AC. Speciation of mercury, tin, and lead compounds by gas chromatography with microwave-induced plasma and atomic-emission detection (GC-MIP-AED). *Anal Bioanal Chem* 2002;**372**:74–90.

214 Westöö G. Determination of methylmercury compounds in foodstuffs. I. Methylmercury compounds in fish, identification and determination. *Acta Chem Scand* 1966;**20**:2131–2137.

215 Bouyssiere B, Szpunar J, Lobinski R. Gas chromatography with inductively coupled plasma mass spectrometric detection in speciation analysis. *Spectrochim Acta B* 2002;**57**:805–828.

216 Carro AM, Mejuto MC. Application of chromatographic and electrophoretic methodology to the speciation of organomercury compounds in food analysis. *J Chromatogr A* 2000;**882**:283–307.

217 Krachler M, Emons H, Zheng J. Speciation of antimony for 21st century: promisses and pitfalls. *Trends Anal Chem* 2001;**20**:79–90.

218 Tseng CM, Amouroux D, Brindle ID, Donard OF. Field cryofocussing hydride generation applied to the simultaneous multi-elemental determination of alkyl-metal(loid) species in natural waters using ICP-MS detection. *J Environ Monit* 2000;**2**:603–612.

219 Voegborlo RB, Matsuyama A, Adimado AA, Akagi H. Determination of methylmercury in marine and freshwater fish in Ghana using a combined technique of dithizone extraction and gas–liquid chromatography with electron capture detection. *Food Chem* 2013;**124**:1244–1248.

220 Berzas Nevado JJ, Rodríguez Martín-Doimeadios RC, Krupp EM *et al*. Comparison of gas chromatographic hyphenated techniques for mercury speciation analysis. *J Chromatogr A* 2011;**1218**:4545– 4551.

221 Berzas Nevado JJ, Rodríguez Martín-Doimeadios RC, Guzmán Bernardo FJ, Rodríguez Fariñas N, Patiño Ropero MJ. Mercury speciation analysis in terrestrial animal tissues. *Talanta* 2012;**99**:859–864.

222 Kim YH, Kim KH, Yoon HO, Brown RJC. The application of gas chromatography–time-of-flight mass spectrometry to the analysis of monomethyl mercury at sub-picogram levels. *Microchem J* 2013;**110**:107–112.

223 Park JS, Lee JS, Kim GB *et al*. Mercury and methylmercury in freshwater fish and sediments in South Korea using newly adopted purge and trap GC-MS detection method. *Water Air Soil Pollut* 2010;**207**:391–401.

224 Chung SW, Chan BT. A reliable method to determine methylmercury and ethylmercury simultaneously in foods by gas chromatography with inductively coupled plasma mass spectrometry after enzymatic and acid digestion. *J Chromatogr A* 2011;**1218**:1260–1265.

225 Clémens S, Monperrus M, Donard OFX, Amouroux D, Guérin T. Mercury speciation in seafood using isotope dilution analysis: a review. *Talanta* 2012;**89**:12– 20.

226 Arroyo-Abada U, Mattusch J, Mothes S *et al*. Detection of arsenic-containing hydrocarbons in canned cod liver tissue. *Talanta* 2010;**82**:38–43.

227 Sele V, Amlund H, Berntssen MHG, Berntsen JA, Skov K, Sloth JJ. Detection of arsenic-containing hydrocarbons in a range of commercial fish oils by GC-ICPMS analysis. *Anal Bioanal Chem* 2013;**405**:5179–5190.

228 Meija J, Bryson JM, Vonderheide AP, Montes-Bayon M, Caruso JA. Studies of selenium-containing volatiles in roasted coffee. *J Agric Food Chem* 2003;**51**:5116–5122.

229 Meija J, Montes-Bayon M, Caruso JA, Leduc DL, Terry N. Speciation of volatile selenium species in plants using gas chromatography/inductively coupled plasma mass spectrometry. *Se Pu* 2004;**22**:16–19.

230 Meija J, Montes-Bayon M, Le Duc DL,Terry N, Caruso JA. Simultaneous monitoring of volatile selenium and sulfur species from Se accumulating plants (wild type and genetically modified) by GC/MS and GC/ICPMS using solid-phase microextraction for sample introduction. *Anal Chem* 2002;**74**:5837–5844.

231 Dietz C, Sanz Landaluze J, Ximénez-Embún P, Madrid-Albarran Y, Camara C. SPME-multicapillary GC coupled to different detection systems and applied to volatile organo-selenium speciation in yeast. *J Anal Atom Spectrom* 2004;**19**:260–266.

232 Amoako PO, Uden PC, Tyson JF. Speciation of selenium dietary supplements: formation of S-(methylseleno)cysteine and other selenium compounds. *Anal Chim Acta* 2009;**652**:315–323.

233 Ghasemi E, Sillanpää M, Najafia NM. Headspace hollow fiber protected liquid-phase microextraction combined with gas chromatography–mass spectroscopy for speciation and determination of volatile organic compounds of selenium in environmental and biological samples. *J Chromatogr A* 2011;**1218**:380–386.

234 Vonderheide AP, Montes-Bayon M, Caruso JA. Solid-phase microextraction as a sample preparation strategy for the analysis of seleno amino acids by gas chromatography-inductively coupled plasma mass spectrometry. *Analyst* 2002;**127**:49–53.

235 Zhang X, Yang L, Mester Z. Determination of amino acids in selenium-enriched yeast by gas chromatography–mass spectrometry after microwave assisted hydrolysis. *Anal Chim Acta* 2012;**744**:54–59.

236 Gionfriddo E, Naccarato A, Sindona G, Tagarelli A. A reliable solid phase microextraction–gas chromatography–triple quadrupole mass spectrometry method for the assay of selenomethionine and selenomethylselenocysteine in aqueous extracts: difference between selenized and not-enriched selenium potatoes. *Anal Chim Acta* 2012;**747**:58–66.

237 Dietz C, Sanz J, Sanz E, Munoz-Olivas R, Camara C. Current perspectives in analyte extraction strategies for tin and arsenic speciation. *J Chromatogr A* 2007;**1153**:114–129.

238 Abalos M, Bayona JM, Compano R, Granados M, Leal C, Prat MD. Analytical procedures for the determination of organotin compounds in sediment and biota: a critical review. *J Chromatogr A* 1997;**788**:1–49.

239 Tzollas NM, Zachariadis GA. Speciation of inorganic and tetramethyltin by headspace solid-phase microextraction and gas chromatography–mass spectrometry. *J Sep Sci* 2010;**33**:1610–1616.

240 Xiao Q, Hu B, He M. Speciation of butyltin compounds in environmental and biological samples using headspace single drop microextraction coupled with gas chromatography–inductively coupled plasma mass spectrometry. *J Chromatogr A* 2008;**1211**:135–141.

241 Campillo N, Viñas P, Peñalver R, Cacho JI, Hernández-Córdoba M. Solid-phase microextraction followed by gas chromatography for the speciation of organotin compounds in honey and wine samples: a comparison of atomic emission and mass spectrometry detectors. *J Food Compos Anal* 2012;**25**:66–73.

242 Cacho JL, Campillo N, Viñas P, Hernández-Córdoba M. Headspace sorptive extraction for the analysis of organotin compounds using thermal desorption and gas chromatography with mass spectrometry. *J Chromatogr A* 2013;**1279**:1–6.

243 Miravet R, Hernández-Nataren H, Sahuquillo A, Rubio R, López-Sánchez JF. Speciation of antimony in environmental matrices by coupled techniques. *Trends Anal Chem* 2010;**29**:28–39.

244 Maldonado Santoyo M, Landero Figueroa JA, Wrobel K, Wrobel K. Analytical speciation of mercury in fish tissues by reversed phase liquid chromatography–inductively coupled plasma mass spectrometry with Bi^{3+} as internal standard. *Talanta* 2009;**79**:706–711.

245 Hill SJ, Pitts LJ, Fisher AS. High-performance liquid chromatography isotope dilution inductively coupled plasma mass spectrometry for speciation studies: an overview. *Trends Anal Chem* 2000;**19**:120–126.

246 Heumann KG. Isotope-dilution ICP-MS for trace element determination and speciation: from a reference method to a routine method? *Anal Bioanal Chem* 2004;**378**:318–329.

247 Martone M, Mizamur Rahman GM, Pamuka M, Skip Kingston HM. Determination of chromium species in dietary supplements using speciated isotope dilution mass spectrometry with mass balance. *J Agric Food Chem* 2013;**61**:9966–9976.

248 Sturgeon RE, Francesconi KA. Enhancing reliability of elemental speciation results: quo vadis? *Environ Chem* 2009;**6**:294–297.

249 Quevauviller P. Traceability of environmental chemical analyses: can theory match practice? *Anal Bioanal Chem* 2005;**382**:1800–1807.

250 Meija J, Mester Z. Paradigms in isotope dilution mass spectrometry for elemental speciation analysis. *Anal Chim Acta* 2008;**607**:115–125.

251 Leermakers M, Baeyens W, Quevauviller P, Horvat M. Mercury in environmental samples: speciation, artifacts and validation. *Trends Anal Chem* 2005;**24**:383–393.

252 Quevauviller P. Accuracy and traceability concepts applied to environmental speciation analysis. *Environ Sci Pollut Res Int* 2001;**8**:251–255.

253 Rodríguez González P, García-Alonso IJ. Recent advances in isotope dilution analysis for elemental speciation. *J Anal Atom Spectrom* 2010;**25**:239–259.

254 Rodríguez González P, Marchante-Gayón JM, Garcia Alonso JI, Sanz-Medel A. Isotope dilution analysis for elemental speciation: a tutorial review. *Spectrochim Acta B* 2005;**60**:151–207.

CHAPTER 19
Drinking water

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Abstract: All our water comes from the water cycle and it is this process that controls our water resources. These resources are interrelated and have advantages and disadvantages as sources of drinking water. Nearly 70% of the earth's surface is covered with water, of which 97.2% is salt water and the remaining 2.8% is fresh water. Water contains a number of mineral elements in varying concentrations. It can be said that natural mineral waters are a source that can compensate for deficiencies in minerals and trace elements in humans. The reliability of drinking water depends on the chemical and physical quality of water. The World Health Organization (WHO) drinking water guidelines are health-based. The increase or decrease in the current guideline values for mineral elements in drinking water calls for the control of their content. The outcome of ongoing research might suggest reassessment of guideline values when correlated with health issues.

Keywords: water as a source for mineral elements, modes of purification, concentration and chemical species, As, F, Fe in drinking water, case studies, trends in the control of mineral content

19.1 Water as a source for mineral element

Nearly 70% of the earth's surface is covered with water, of which 97.2% is salt water and the remaining 2.8% is fresh water (Figure 19.1). This water is purified and distributed as drinking water. Be it fresh water or groundwater, it contains a number of mineral elements in varying concentrations. Studies have shown that minerals from water are easily absorbed by the human body. It can be said that natural mineral waters are a source that can compensate for deficiencies in minerals and trace elements in humans [1].

The hardness of water is caused by the presence of calcium and magnesium ions, and iron in water is very common. The removal of arsenic and fluoride from drinking water is a serious challenge to researchers, and there are numerous publications on the removal of heavy metals from tap water and well water. All the above state clearly that water is a source of mineral elements. However, studies on potable water have shown that when the concentration of mineral elements present in water is above the permissible limit, it affects human health and poses threat to the body. Table 19.1 gives a summary of mineral elements found in water [2], their guideline values recommended by the World Health Organization (WHO) and probable health risks.

Fresh water generally includes constituents and materials other than water. These may be naturally occurring or introduced by human activities and may include chemicals, microbial agents, radioactive species, and other agents. Where drinking water is supplied through public water supply systems, contaminants may also be introduced during delivery from water treatment facilities to consumers. For example, iron exists naturally in rivers, lakes and underground water as iron salts are easily soluble in water. It may also be released in water from natural deposits, industrial wastes, refining of iron ores, and corrosion of iron-containing metals. Dissolution of iron can occur as a result of oxidation and decrease in pH. Despite iron being the second most abundant metal in the earth's crust, its concentration in natural water is surprisingly low. This is because its solubility depends strongly on the oxidation intensity

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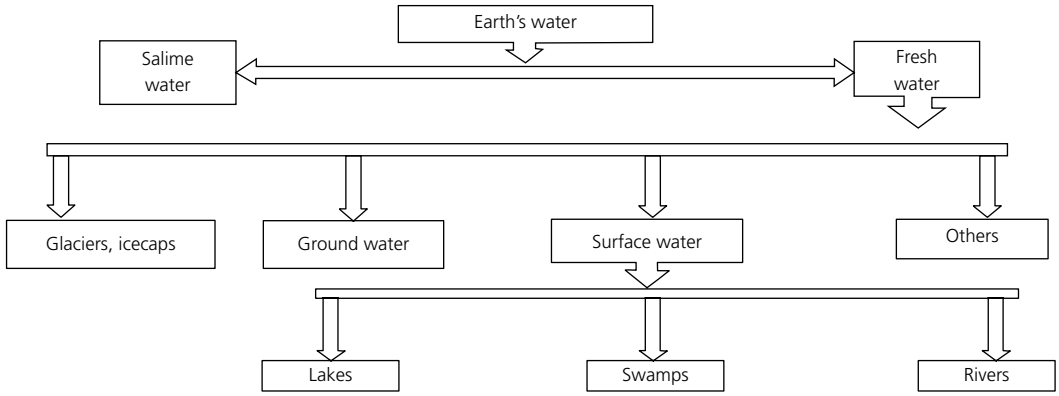


Figure 19.1 Water distribution on earth.

and the pH of the system. Fe(II) is the most common form found in groundwater. Studies reveal that both ferrous [3] and ferric [4] complexes exist in water, the latter being responsible for the yellow to brown colour. However, iron in water does not pose any threat to the human body.

Fluorine reduces risk of tooth decay by promoting stronger enamel. The permissible limit of fluoride in drinking water is 1.5 mg/L [2]. Although fluoride is necessary for the human body, it leads to health risks (fluorosis) if the concentration is more than the permissible limit. Fluoride is widely distributed in the geological environment. It is well known that granite aquifers contain fluoride-rich groundwater. It is generally released into the groundwater by slow dissolution of fluorine-containing rocks. Various minerals (e.g. fluorite, biotites, topaz, and their corresponding host rocks such as granite, basalt, syenite and shale) contain fluoride that can be released into groundwater, but fluorite is the most abundant of them. Dissolution of fluoride in groundwater takes place due to water–rock interactions. Studies show that during the weathering process at normal temperature and pressure, fluoride dissolution is favoured when there is pH range 7.6–8.6, high concentration of bicarbonate anion (350–450 mg/L) and specific conductivity of 750–1750 μS/cm [5].

Around the world, aquifers have been identified with high concentrations of arsenic. Despite the geographical differences there are also some similarities between these regions. The majority of the high-arsenic groundwater provinces are in young unconsolidated sediments, usually of Quaternary age, and often of Holocene age (<12,000 years ago). These aquifers are usually large inland closed basins in arid or semi-arid settings or large alluvial and deltaic plains. However, it is arsenic mobilization and transport in water that poses the actual threat of arsenic poisoning. The anaerobic reducing conditions that favour arsenic mobilization tend to occur in shallow aquifers in Quaternary strata underlying South and East Asia's large alluvial and deltaic plains. There are also some localized groundwater arsenic problems which relate to ore mineralization and mining activity. Recent hydrogeochemical investigations have improved our knowledge of the occurrence and distribution of arsenic in groundwater, though much uncertainty remains regarding the source, mobilization and transport of the element in aquifers [6].

Naturally occurring boron is present in groundwater primarily as a result of leaching from rocks and soils containing borates and borosilicates. The borate content of surface water can be increased as a result of wastewater discharges. Barium occurs naturally in trace amounts in most surface waters and groundwater from their exposure to barium-containing minerals. Cadmium contamination in drinking water may be caused by impurities in the zinc of galvanized pipes and solders and some metal fittings in addition to its release in wastewaters from anthropogenic sources. Chloride in drinking water originates from natural sources, sewage and industrial effluents, urban runoff containing de-icing salt and saline intrusion. Excessive chloride concentrations increase rates of corrosion of metals in the distribution system, depending on the alkalinity of the water. This can lead to increased concentrations of metals in the supply. Chromium is widely distributed in

Table 19.1 Mineral elements in water and their characteristics at a glance.

Mineral element	Guideline value	LOD	Water treatment	Health risks	Comments
Al	0.2 mg/L	—	—	No health concerns	Drinking-water plants use Al-based coagulants in the concentration range 0.1–0.2 mg/L
Sb	0.02 mg/L	0.01 µg/L EAAS	Product control (plumbing)	Possible carcinogenicity	Exposure from drinking-water is very low compared with occupational exposure
As	0.01 mg/L	0.1 µg/L ICP-MS	Ion exchange	Increased risks of cancer in the skin, lungs, bladder and kidney, arsenicosis	Of greatest health concern in some natural waters
Ba	0.7 mg/L	0.1 µg/L ICP-MS	Ion exchange/ precipitation softening	No adverse effects observed	Study population was relatively small and limited
Be	Less than trace amount	—	—	—	Rarely found in drinking-water at concentrations of health concern
B	2.4 mg/L	0.15 µg/L ICP-MS	Ion exchange/ reverse osmosis	—	Developmental toxicity has been demonstrated in rats, mice and rabbits
BrO_3^-	0.01 mg/L	0.2 µg/L IC with UV/Vis	Appropriate control of disinfection conditions	No sufficient evidence for the carcinogenicity of bromate in humans	Bromate is not normally found in water; the main source in drinking water is its formation during ozonation when the bromide ion is present in water
Cd	0.003 mg/L	0.01 µg/L ICP-MS	Coagulation/ precipitation softening	The kidney is the main target organ for cadmium toxicity	Released to the environment in wastewater
Cl	5 mg/L	0.01 µg/L HPLC	Reduction	Not of health concern at levels found in drinking water	Most commonly used disinfectant and oxidant in drinking water treatment
Cr	0.05 mg/L	0.05–0.2 µg/L AAS	Coagulation	Carcinogenic	Data in terms of total chromium
Cu	2 mg/L	0.02–0.1 µg/L ICP-MS	Ion exchange	Gastrointestinal effects	Copper is both an essential nutrient and a drinking-water contaminant
Ca	—	—	Ion exchange	Not of health concern at levels found in drinking water	Responsible for hardness in water
F^-	1.5 mg/L	0.01 mg/L IC	Ion exchange	Skeletal fluorosis	Of greatest health concern in some natural waters
Fe	—	—	Ion exchange	Not of health concern at levels found in drinking water	May affect acceptability of drinking water
Pb	0.01 mg/L	1 µg/L AAS	Removal of plumbing and fittings containing lead	Neurodevelopmental effects, cardiovascular disease, impaired renal function, hypertension, fertility and adverse pregnancy outcomes	Lead is exceptional compared with other chemical hazards

(Continued)

Table 19.1 (Continued)

Mineral element	Guideline value	LOD	Water treatment	Health risks	Comments
Mg	—	—	Ion exchange	Not of health concern at levels found in drinking water	Responsible for hardness in water
Mn	0.4 mg/L	—	Ion exchange	Not of health concern at levels found in drinking water	May affect acceptability of drinking water
Hg	0.006 mg/L	0.05 μg/L CVAAS	Coagulation/ sedimentation/filtration, ion exchange	Kidney damage	Mercury is present in the inorganic form in surface water and groundwater at concentrations usually <0.5 μg/L
Ni	0.07 mg/L	0.1 μg/L ICP-MS	Coagulation	Concentration in drinking-water normally <0.02 mg/L	Drinking water is generally a minor contributor
NO_3^-	50 mg/L	0.01–1 mg/L spectrometry	Ion exchange	Methaemoglobinaemia in infants associated with simultaneous diarrhoeal disease	Drinking-water can be the major external source of exposure to nitrate
K	—	—	—	Not of health concern at levels found in drinking water	Occurs in drinking water as a consequence of water treatment
Se	0.04 mg/L	0.5 μg/L HGAAS	Activated alumina adsorption, ion exchange, reverse osmosis and nanofiltration	—	Most drinking-water contains concentrations of selenium that are much lower than 10 μg/L
Na	—	—	—	Not of health concern at levels found in drinking water	May affect acceptability of drinking water
U	0.03 mg/L	0.01 μg/L ICP-MS	Coagulation/ ion exchange	Intake through drinking water is normally extremely low	Where uranium is present in a drinking water source, the majority of intake can be through drinking water
Zn	—	—	Ion exchange	Not of health concern at levels found in drinking water	May affect acceptability of drinking water

Source: data from World Health Organization [2].

the earth's crust. It can exist in valences of +2 to +6. Copper is both an essential nutrient and a drinking-water contaminant. It is used to make pipes, valves and fittings and is present in alloys and coatings. Lead is rarely present in tap water as a result of its dissolution from natural sources; rather, its presence is primarily from corrosive water effects on household plumbing systems containing lead in pipes, solder, fittings or the service connections to homes. Manganese is naturally occurring in many surface waters and groundwater sources, particularly in anaerobic or low oxidation conditions, and this is the most important source for drinking water.

The elements found in water in different quantities are essential for health. Mineral elements like Ca, P, Mg, F and Si are beneficial for bone and membrane structures; Na, K and Cl for water electrolyte balance and Zn, Se and Mg for metabolic catalysis. There are some elements that help in hormonal balance [7].

19.2 Sources of drinking water

All our water comes from the water cycle and it is this process that controls our water resources. The principal resources within the water cycle are surface waters and groundwater. These resources are interrelated and have advantages and disadvantages as sources of drinking water.

The reliability of drinking water depends on the chemical and physical quality of water. Only a small fraction of fresh water is used for drinking purposes. Of the 37 million cubic kilometres of fresh water estimated to be present on the earth, about 22% exists as groundwater, which constitutes about 97% of all liquid fresh water potentially available for human use [8]. Groundwater is less risky than surface waters. Water sources contaminated by surface runoff pose higher risks. Sources of fresh water include surface water, under river flow, ground water, frozen water and desalination of sea water.

Groundwater chemistry is mainly controlled by natural as well as anthropogenic factors. The composition of water can reveal the various processes in groundwater. The chemical composition of geological formations affects the hydrochemical characteristics of groundwater during its circulation in the subsurface [9].

19.3 Modes of purification before supplying as drinking water

Water purification treatment includes some fundamental steps [10, 11], namely disinfection, removal of solid particles, coagulation, flocculation, filtration and hardness and Fe removal. The above treatments are meant to improve water quality but are not designed to remove mineral elements present in water.

The traditional water treatment chemicals include alum $Al_2(SO_4)_3$ and ferric chloride $FeCl_3$, which acts as coagulants; Cl_2 which acts as as disinfectant/oxidant; $CuSO_4$ which acts as an algaecide and fungicide; and F^- which is added for fluoridation. The very same chemicals may be retained by the treated water and require removal. Some purification methods, other than the ones mentioned above, are discussed below.

Several methods such as chelating resin, reverse osmosis, electrodialysis, coagulation, sedimentation and filtration, and lime softening are used for removal of aluminium from water [12]. Tekerlekopoulou *et al.* [13] offer a very attractive solution for iron removal from drinking water that involved the construction of a simple pilot-scale trickling filter. Biological and physicochemical iron oxidation is the basis for iron removal. Bio-oxidation improved filter efficiency by about 5–6%.

At high pH, banana rind, banana pseudo-stem, banana leaf, rice husk and bamboo ash enhance precipitation of iron. This low-cost simple iron removal system removes iron to below 0.3 ppm without increasing the pH above the acceptable WHO limit [14]. $NaHCO_3$ and $KHCO_3$ along with ash are more effective in iron removal. $KHCO_3$ when used alone, by formation of goethite, is the most efficient precipitant, removing iron ion by up to 99–100% and retaining pH at 6.8–7.7, well within the acceptable range [15]. Simultaneous removal of arsenic and iron ions from water has been studied using some common pH conditioners (lime, banana ash, Na_2CO_3, K_2CO_3, $NaHCO_3$, $KHCO_3$ and their binary mixture). The simultaneous removal of arsenate and iron by pH conditioners decreases in the order lime > $KHCO_3$ > $NaHCO_3$ > K_2CO_3 > Na_2CO_3 > ash. Lime requires post-treatment correction of highly alkaline pH [16].

Because of their large surface areas and high activities, caused by the size quantization effect, nanosized metal oxides (NMOs) act as adsorbents with high

capacity and selectivity for removal of heavy metals from water. Nanosized ferric oxides, manganese oxides, aluminium oxides, titanium oxides, magnesium oxides and cerium oxides are classified as promising candidates for heavy metal removal from aqueous systems. Cu(II), Cd(II), Co(II), Ni(II), Pb(II), Pd(II), Mn(II), Zn(II), Cr(III) and Cr(VI) are some heavy metals that can be removed from water using NMOs. The optimal pH for Cr(VI), Cu(II) and Ni(II) are 2.5, 6.5 and 8.5, respectively. Studies show that sorption preference follows the order Pb(II) > Cd(II) > Zn(II). Sorption capacities of NMOs depend on their shape, size and surface area. Some sorption capacities reported are as follows: 149.25 mg/g for Cu(II), 100 mg/g for Pb(II), 83.33 mg/g for Cd(II), 100 mg/g for Cr(III), 41.66 mg/g for Co(II), 18.18 mg/g for Ni(II), 6.289 mg/g for Mn(II) and 121.95 mg/g for Cr(VI) [17].

Alternative methods to replace chlorine as a treatment chemical are also being sought. Among the most promising chemical alternatives are chlorine dioxide and ozone. Activated carbon has been used to remove chlorine. Recently, carbon nanotubes (CNTs) have also been successfully used. Adsorption on activated charcoal, biosorption, ion exchange on zeolites, chelating resins and flotation–microfiltration are some treatment processes for the removal of copper from water. Among these methods, chemical precipitation is the most economic and commonly utilized procedure. At present, electrochemical methods like cathodic reduction and electrowinning, electrodialysis and electrodeionization contribute to the removal of toxic heavy metals such as copper.

Membrane techniques and adsorption are two common defluoridation techniques. Membrane techniques comprise reverse osmosis, nanofiltration, dialysis and electrodialysis. Activated and impregnated alumina, rare earth oxides, activated clay, impregnated silica, carbonaceous materials, alum sludge, solid industrial wastes like red mud, spent catalysts and fly ash, zeolites, and related ion exchangers comprise some of the variety of adsorbents used for defluoridation. However, a few adsorbents reduce the concentration of fluoride to, or below, the 1.0–1.5 mg/L level required for drinking water and for effluent water that could find its way into drinking water sources.

The versatility of CNTs has been applied in water treatment. In fact, recent advances have shown that these nanomaterials have exceptional adsorption capacity for various inorganic pollutants such as fluoride and heavy metals. CNTs can work as heterogeneous coagulants in the treatment of wastewaters. A number of studies have reported the use of CNTs for filtration and separation; they can also be regenerated for reuse [12].

19.4 Variation in drinking water standards

Setting standards for a drinking water contaminant is a very difficult process. Apart from scientific considerations, it is also affected by factors such as economic, political and social issues. Data relating human health effects to chemicals in drinking water are limited. Often, the presence of multiple chemicals that might influence the toxicity of a single element/chemical is not considered while setting the guideline values of drinking water. Again, it is difficult for scientists to predict the effects on human health due to consumption of trace amounts of elements/chemicals for many years. So these standard values are reviewed and reassessed from time to time to keep up with the latest findings and, if need be, to change the existing values. Drinking water standards are based on all the scientific knowledge available at the time.

The drinking water guidelines determined by the WHO are health-based. It provides national authorities with a basis for setting national standards on water quality but does not guarantee that water with a contaminant level below the standard is totally safe, nor do they indicate that water exceeding the limit will pose severe threats. The guidelines can only guarantee that drinking water does not represent any significant risk to health over a lifetime of consumption.

A perusal of Table 19.2 shows that national authorities have slightly different acceptable limits for the concentrations of some elements. These changes take into account the hydrogeology of the region, and political, economic and social issues. Sometimes, lack of adequate testing facilities for lower concentrations also influence the setting of higher permissible values [18].

19.5 Concentration levels and chemical species of the elements present in drinking water

Table 19.1 lists the concentrations of the elements present in water in the permissible range. As stated earlier, variance in the concentrations of elements can be

Table 19.2 WHO and US EPA guideline values and permissible limits in India, Pakistan, Egypt, Italy and the EU for drinking water [44–47].

Mineral element	IS:10500 1991 (mg/L)	Pak-EPA limits 2010 (mg/L)	Egyptian Ministry of Health 2007 (mg/L)	Italian Law DL 31/2001 (mg/L)	EU Directive 1998/83/EC (mg/L)	WHO 2011 (mg/L)	USEPA 2009 (mg/L)
Calcium	200	—	—	—	—	—	—
Magnesium	75	—	—	—	—	—	—
Copper	1.5	2	2	1	2	2	1.3
Manganese	0.3	≤0.05	0.4	0.05	0.05	0.4	—
Sulfate	400	—	250	250	250	500	—
Nitrate	100	≤50	—	50	50	50	10
Fluoride	1.5	≤1.5	—	1.5	1.5	1.5	4
Mercury	0.001	≤0.001	—	0.001	0.001	0.006	0.002
Cadmium	0.001	0.01	0.003	0.005	0.005	0.003	0.005
Selenium	0.01	0.01	—	0.01	0.01	0.04	0.01
Arsenic	0.05	≤0.05	—	0.01	0.01	0.01	0.01
Lead	0.05	≤0.05	0.01	0.01	0.01	0.01	0.015
Chromium(VI)	0.05	0.05	0.05	0.05	0.05	0.05	0.1
Aluminium	0.2	≤0.2	—	0.2	0.2	0.2	—
Boron	5	0.3	—	1	1	2.4	—

observed. Lithology, anthropogenic reasons, oxidizing or reducing conditions and pH dictate the concentration and the species of the element being formed. Studies reveal that As, Fe, Mn and Mo are more soluble under reducing conditions compared with Cu, Pb, Se, U and Zn which are found to be soluble under oxic conditions.

Based on aquated cations or hydroxylated aluminates, Al can form different monomeric and polymeric hydroxy species, colloidal polymeric solutions and gels, and precipitates. Al concentrations in water at near-neutral pH values usually range from 0.001 to 0.05 mg/L but rise to 0.5–1 mg/L under acidic conditions or in the presence of organic matter. Statistical analysis showed that the concentration of total Al in drinking water in Xi'an ranged from 0.051 to 0.417 mg/L and the concentration in about 24.7% of studied samples was higher than the current guideline value. Compared with poly aluminium chlorine and poly aluminium ferric chloride, water treatment with alum coagulant shows a higher monomeric aluminium concentration in water. Other than coagulant type, the presence of orthophosphate salts decreases Al dissolution in the pH range 6.5–8.2 [19]. It was found that the influence of fulvic acid and humic acid on aluminium speciation depended mainly on their molecular weight. Within the pH range 6.5–7.5, soluble aluminium increased notably in water with organic matter. Fulvic acid with molecular mass larger than 5000 Da and humic acid increased the concentration of soluble aluminium significantly, even at concentration below 0.5 mg/L. During drinking water treatment, reduction of fulvic and humic acid concentration and maintaining pH in the range 6.5–7.5 helps to keep Al concentration within the guideline value [20].

The most favoured form of Sb in water is the pentavalent oxoanion $Sb(OH_6)^-$. Antimony could be a natural co-contaminant with arsenic. Studies report that total dissolved Sb concentrations are usually less than 1.0 μg/L in natural water. Most studies report the dominance of Sb(V) under oxic conditions during redox speciation, though significant proportions of Sb(III) are sometimes detected. This is in direct contrast with thermodynamic equilibrium predictions and discrepancies are often attributed to biological activity or kinetic effects but proof is lacking. Similarly, the existence of Sb(V), although not thermodynamically predicted, is often reported under anoxic conditions. A few studies report low concentrations of methylated Sb species. Antimony is not considered to be a highly reactive element in oceans, where concentrations are in the order of 200 ng/L [21].

Depending on pH, As(III) may be found as arsenite and As(V) will be present as arsenate. As(V) is found to be the main species in oxygen-rich surface water and As(III) in groundwater [18]. Studies reveal that As(III) is usually more difficult to adsorb but desorbs more readily compared with As(V). Moreover, after treatment residual arsenite at trace levels is more toxic than arsenate [2]. Therefore, iron, hydrogen peroxide, chlorine, hypochlorite and manganese are used to oxidize As(III) other than naturally occurring oxidation. The range of As concentrations found in natural water is from less than 0.5 mg/L to over 5000 mg/L. These large-scale naturally occurring As levels in groundwater are found in two types of environment: inland or closed basins in arid or semi-arid areas and strongly reducing aquifers often derived from alluvium [18].

The water-soluble barium acetate, nitrate and halides from granite-like igneous rocks, alkaline igneous and volcanic rocks and manganese-rich sedimentary rocks are the natural sources of Ba in water. In a study of water supplies of cities in the USA, a median value of 43 μg/L was reported for Ba concentration [2].

Beryllium is the smallest of all the metal cations and is relatively immobile in natural waters at neutral pH but under acidic conditions it is mobile and is released into water, causing environmental problems and possible toxicity to fish and other aquatic organisms [22]. It can be also be released in water as a result of coal burning, Be-using industries and weathering of Be-containing rocks and soils. Because of the insolubility of beryllium oxides and hydroxides at the normal pH range, Be is not likely to be found in natural water above trace levels. Surface waters have been reported to contain beryllium at concentrations up to 1000 ng/L.

Although the range between deficiency and excess is narrow, boron is an important micronutrient for humans. In water at neutral pH boron is mainly present as boric acid mostly in undissociated form [23]. The majority of the earth's boron occurs in the oceans, with an average concentration of 4.5 mg/L. Concentrations of boron in groundwater throughout the world range widely from less than 0.3 to over 100 mg/L [11]. In natural water, cadmium is found mainly in bottom sediments and suspended particles. Cadmium concentrations in non-polluted natural waters are usually considerably lower than 1 μg/L.

In water, chlorine reacts to form hypochlorous acid and hypochlorites. All three species exist in equilibrium with each other, the relative amounts varying with pH. In dilute solutions and at pH above 4.0, very little molecular chlorine exists in solution. The concentrations of hypochlorous acid and the hypochlorite ion are approximately equal at pH 7.5 and 25°C. Chlorine can react with ammonia or amines in water to form chloramines. Chlorine is present in most disinfected drinking water at concentrations of 0.2–1 mg/L.

Chromium Cr(VI) usually occurs as CrO_4^{2-} or $HCrO_4^-$, whereas Cr(III) occurs as $Cr(OH)_n^{(3-n)+}$. Cr(III) is an essential nutrient while Cr(VI) is strongly carcinogenic. It has long been recognized that Cr(III) occurs naturally in most environmental media, while Cr(VI) has only recently been discovered to also occur naturally in groundwater. Total chromium content of surface water is found to be in the range 0.5–2 μg/L while dissolved chromium content is 0.02–0.3 μg/L.

Cu(II) ion is the major stable species in water up to pH 6 and forms complexes with hydroxide and carbonate ions. $CuCO_3$ is prevalent at pH 6–9.3 whereas $[Cu(CO_3)_2]^{2-}$ predominates at pH 9.3–10.7. Depending on pH, hardness and copper availability, Cu concentrations in drinking water vary widely. Copper levels in drinking water can range from 0.005 mg/L or less to more than 30 mg/L [24]. It can be said that calcium-based hardness is predominant, since in comparison with calcium the concentrations of magnesium are very low. Whereas Ca concentration is 100 mg/L or more in groundwater, Mg concentrations range from negligible to about 50 mg/L and rarely above 100 mg/L [2].

Fluoride in water contributes significantly to the total exposure of an individual to this element. Areas rich in fluoride-containing minerals have been reported to contain up to about 10 mg/L of fluoride. The concentration of fluoride in bottled drinking waters purchased from Riyadh supermarkets was found to vary between 0.50 and 0.83 mg/L. No imported brands had fluoride concentration below 0.6 mg/L, and only one of the twelve local brands tested had fluoride content below that level [25].

Iron-containing layers in the soil when in contact with oxygen affect the quality of both groundwater and surface water if the groundwater table is lowered or nitrate leaching takes place. Dissolution of iron can occur as a result of oxidation and decrease in pH. The median iron concentration in rivers has been reported to be 0.7 mg/L, whereas Fe(II) concentrations in anaerobic groundwater are in the range 0.5–10 mg/L though concentrations up to 50 mg/L can sometimes be found [26]. Use of iron salts as coagulating agents for water treatment or the use of cast iron, steel and galvanized iron pipes for water distribution manifest higher concentrations of iron in drinking water than the normal (<0.3 mg/L).

Both groundwater and surface water rarely contain Pb in excess of 10 μg/L, though lead content of drinking water may vary considerably. This can be attributed to the use of lead in old plumbing systems. For example, studies have shown a large variation in the lead concentration in tap water in Hamburg, Germany, from less than 5 up to 330 μg/L [24].

Inorganic mercury is predominantly the form found in water. Mercury concentrations in water resources are generally very low, between 0.005 and 0.1 μg/L, with an average concentration in drinking water of 0.025 μg/L [24]. Occasionally, concentrations above 2 μg/L are reported in shallow wells.

Drinking water rarely contains more than 20 μg/L of nickel, although elevated levels are possible in groundwaters when contaminated by natural nickel deposits, or when both surface water and groundwaters are contaminated by industrial sources [2].

The selenium concentration in drinking water depends on the geology but is normally less than 10 μg/L. Se mainly exists as water-soluble selenate and selenite [2]. Se content in surface water is influenced by pH. Levels vary widely in groundwater and surface water, from 0.06 to about 400 μg/L, and in some areas groundwater concentrations may reach 6000 μg/L [24]. Fluorimetrically determined total Se concentration in various waters from all over Greece revealed that most of the drinking water, either tap or bottled, was within the range 100–200 ng/L and showed no great seasonal fluctuations. Coastal surface water contains 100–250 ng/L, while higher concentrations are found at locations regarded as polluted [27].

Uranium in solution is likely to be present as a carbonate complex in highly alkaline and oxygenated water. With high concentrations of organic matter and low concentrations of inorganic ions at pH below 6, uranium is present as soluble organic complex [28]. A nationwide survey of uranium concentrations in groundwater and natural spring water in Switzerland showed that elevated concentrations of uranium in the

alpine regions may be attributed to the geology of the bedrock. Uranium content varied considerably, from below the LOD to 100 µg/L. Among 5548 samples, 99.7% were below the revised WHO provisional guideline value (2011) of 30 µg/L. Sources with high natural concentration along with areas warranting further investigation were identified [29].

In natural surface waters the concentration of zinc is usually below 10 µg/L and in groundwaters 10–40 µg/L [24]. Zinc concentrations in potable water ranged from 2.0 to 73.0 µg/L in southeastern Spain. Zn concentrations in waters from the zone were not affected by human activity [30].

19.6 Methodologies used for the determination of elements

Some of the methodologies used for the determination of elements are listed in Table 19.1. However, scientists are keen on developing more efficient and sophisticated techniques. Some novel methodologies are discussed in this section.

A kinetic–catalytic spectrophotometric method was suggested for the successive determination of Cu and Fe at nanogram levels based on their catalytic effects on the oxidative coupling of *p*-anisidine with *N*,*N*-dimethylaniline (DMA) in the presence of H_2O_2 at pH 3.2 to form a coloured compound (λ_{max} 740 nm). The selectivity improved in the presence of diphosphate as a masking agent. Table 19.3 gives the specifications of catalysis. The method was successfully applied to the analyses of copper and iron in tap, well, river and pond waters [31].

Veríssimo *et al*. [32] developed a new methodology based on an inexpensive aluminium acoustic wave sensor. The methodology developed with the acoustic wave sensor allowed aluminium quantitation above 0.07 mg/L. Several water samples from Portugal were analysed using the acoustic wave sensor, as well as by UV/visible spectrophotometry. Results obtained with both methodologies matched statistically ($\alpha = 0.05$), both in terms of accuracy and precision. This new methodology proved to be adequate for aluminium quantitation in drinking water and was faster with less reagent consumption than the UV spectrophotometric methodology.

Ni, Cd and Pb in drinking water have been accurately determined by adopting exact-matching isotope dilution mass spectrometry (IDMS). When compared with international standards high accuracy results were obtained that were in excellent agreement with the key comparison reference value. The measurement uncertainty of the method was rigorously investigated and values with less than 2% combined uncertainty ($k = 2$) were achieved for all analytes. An expanded uncertainty with less than 2% combined uncertainty ($k = 2$) was achieved for all analytes [33].

A multi-element detection system involving no chemical treatment and instrumental neutron activation analysis and proton-induced X-ray emission allowed the concentration of 40 elements to be determined in drinking water in Rome and Florence. The study showed that concentrations of potentially toxic elements like Cr, Hg, Ni and Sb and essential elements like I and Se were all within the permissible range except for Fe (Rome) and Al (Florence). In Rome, Fe is the only element in drinking water that had a concentration considered critical. Because of water treatment involving aluminium salts, Al showed a concentration of 103–267 µg/L in Florence [34].

A biological aerated filter was used in a drinking water treatment plant for Mn(II) removal. The experimental design comprised face centred-central composite design with three operational variables: chemical oxygen demand (COD), aeration rate (AR) and hydraulic retention time (HRT). Optimum conditions for maximum Mn^{2+} removal were determined through response surface methodology, where COD load was set as the

Table 19.3 Specifications for determination of Cu and Fe at nanogram levels.

Element	Activator	Determinable range (µg/L)	Concentration (µg/L)	RSD (%)
Cu	2,9-Dimethyl-1,10-phenanthroline (neocuproine)	0.16–10	10	1.1
Fe	1,10-Phenanthroline (phen)	1–100	40	0.97

maximum while AR and HRT were minimized. The optimum conditions were found to be COD load of 0.90 kg/m^3, AR of 0.30 L/min and HRT of 7.47 hours with Mn(II) removal at 94.8% [35].

A highly selective, low-cost extractive spectrofluorometric method has been developed for determination of trace concentrations of Cr(III) and Cr(VI) in water samples using the fluorescent reagent tetraphenylphosphonium bromide ($TPP^+{\cdot}Br^-$). The ion associate [$TPP^+{\cdot}CrO_3Cl^-$] was extracted and fluorescence quenching of $TPP^+{\cdot}Br^-$ in chloroform was measured at $\lambda_{ex/em}$ 242/305 nm. The fluorescence intensity of $TPP^+{\cdot}Br^-$ decreased linearly on increasing the Cr(VI) concentration in the range 1–114 μg/L. The LOD and LOQ of Cr(VI) were 0.43 and 1.42 μg/L, respectively. Cr(III) was determined after its oxidation to Cr(VI). Chemical speciation of Cr(III) and Cr(VI) at trace levels was achieved. The method was applied for analysis of Cr in certified reference material (IAEA Soil-7) and in tap water and wastewater samples and compared successfully (>95%) with the results from inductively coupled plasma mass spectrometry (ICP-MS) [36].

Cloud point extraction (CPE) methodology has been successfully employed for the pre-concentration of ultratrace arsenic species in aqueous samples prior to hydride generation atomic absorption spectrometry (HGAAS). This method has the lowest detection limit compared with similar CPE studies present in the literature. In this study, As(III) forms an ion-pairing complex with pyronin B in the presence of sodium dodecyl sulfate (SDS) at pH 10.0 and is extracted into the non-ionic surfactant polyethylene glycol tert-octylphenyl ether (Triton X-114). The surfactant-rich phase diluted with 2 mL of 1 M HCl and 0.5 mL of 3.0% (w/v) Antifoam A was used to extract As(III). A calibration curve in the range 0.03–4.00 μg/L was obtained under the optimized conditions with a pre-concentration factor of 60, LOD 0.008 μg/L and a correlation coefficient of 0.9918. The procedure was successfully applied to the determination of As(III) ions in certified standard water samples, namely TMDA-53.3 and NIST 1643e (a low-level fortified standard for trace elements) and samples including natural drinking water and tap water. The CPE/HGAAS system for arsenic determination and speciation in real samples has been applied for the first time [37].

A unique hierarchical multi-shell sensor was created by surface-coating of mesoporous core/double-shell silica platforms using a cationic surfactant with double alkyl tails followed by dicarboxylate 1,5-diphenyl-3-thiocarbazone signalling probe to selectively detect and remove metals ions from drinking water. In this novel sensing system, colour changes and reflectance intensity of the multi-shelled sensor for Cu(II), Co(II), Cd(II) and Hg(II) ions were recorded at 448, 431, 436 and 512 nm, using 20 mg/L sensor at pH 2, 8, 11.5 and 9.5, respectively. The multi-shelled sensor is added to enable accessibility for continuous monitoring of several different toxic metal ions and efficient multi-ion sensing and removal capabilities with respect to reversibility, selectivity and signal stability [38].

A simple CPE method followed by inductively coupled plasma optical emission spectrometry (ICP-OES) was developed for simultaneous pre-concentration and determination of trace amounts of U(VI), Th(IV), Zr(IV) and Hf(IV) ions in aqueous samples. The metal ions containing 0.1 M sodium acetate (pH 6.0) formed complexes with dibenzoylmethane (DBM) followed by the addition of Triton X-114 (0.2%, w/v). A phase separation occurred at 50°C. The surfactant-rich phase was diluted with 1.0 mL of 20 : 80 (v/v) of methanol/1 M HNO_3. The metal ions were then determined using ICP-OES. Under optimized conditions, enhancement factors in the range 37.0–43.6 were obtained. The calibration curve was linear in the range 2.5–1240 μg/L for U with correlation coefficients better than 0.9926. The detection limits were between 0.1 and 1.0 μg/L and the relative standard deviation (RSD) for seven replicates was lower than 6.1% [39].

19.7 Level and significance of the presence of arsenic, fluorine and iron in drinking water

As and F are now recognized as the most serious inorganic contaminants in drinking water on a worldwide basis. Millions of people in the world are chronically exposed to inorganic arsenic via drinking water. A number of large aquifers in various parts of the world have been identified with As concentrations well above 50 mg/L. The most affected areas are parts of Argentina, Bangladesh, Chile, China, Hungary, India (West Bengal), Mexico, Romania, Taiwan, Vietnam and many parts of the USA. Arsenic occurs in the environment in –3, 0, +3 and +5 oxidation states but in natural waters mostly inorganic As oxyanions of arsenite [As(III)] or arsenate

[As(V)] are found. Compared with the other oxyanion-forming elements, As is one of the most threatening elements in the environment because of its relative mobility over a wide range of redox conditions. Recent hydrogeochemical investigations have improved our knowledge of the occurrence and distribution of arsenic in groundwater, though much uncertainty remains regarding the source, mobilization and transport of the element in aquifers. Depending on the geochemical environment, concentrations of As in fresh water vary widely. Under natural conditions, the greatest range and the highest concentrations of As are found in groundwaters as a result of the strong influence of water–rock interactions and the greater tendency in aquifers for the physical and geochemical conditions to be favourable for As mobilization and accumulation [18]. In oxidizing environments, the principal attenuation mechanism of As migration is its adsorption on Fe(III) oxide and hydroxides. The adsorption affinity is higher for As(V) under lower pH conditions and for As(III) under higher pH conditions [40]. Over recent decades researchers have been motivated to lower the concentration of arsenic in drinking water and develop low-cost techniques of wide applicability for arsenic removal in water. Several iron- and aluminium-based adsorbents [41] and emulsion liquid membranes are new developments in the field of As removal. Using an emulsion liquid membrane, Mousavi *et al*. [42] demonstrated 60% As(V) extraction at the following optimum conditions: stirring speed, 500 rpm; concentration of sulfuric acid in the feed, 1.5 g mol/L; concentration of reagent in internal phase, 1.5 g mol/L; and concentration of carrier in 3-mL kerosene which is added to the membrane phase, 0.1 g mol/L.

Elevated fluoride concentrations in the groundwater occur in various parts of the world. Fluoride concentrations in water are limited by fluorite solubility and thus the absence of dissolved calcium indicates higher fluoride solubility in the groundwater of areas where fluoride-bearing minerals are common and vice versa [43]. Fluorine intake usually occurs in the form of fluoride consumption. When fluoride is ingested, some of it is taken up by both hard and soft body tissues of the body, with long-term deposition in teeth and bones. Besides affecting teeth and bones, other risks have been suggested by researchers in association with fluoride ingestion. These include effects on the immune system, kidney and gastrointestinal tract [18]. The bioavailability of fluoride is reduced in the presence of Ca(II), Mg(II) and Al(II) ions present in water but only at concentrations above 1 ppm of fluoride [24]. Ion exchange, reverse osmosis and electrodialysis have been studied for the removal of excess amounts of fluoride from drinking water. Bhatnagar *et al*. [5] reviewed fluoride removal by adsorption using a range of sorbents, including alumina/activated alumina, iron, calcium, carbon-based adsorbents, natural materials, biosorbents, agricultural and industrial wastes as sorbents, layered double hydroxides, building materials and nanosorbents. Depending on pH, initial fluoride concentration, temperature, contact time and adsorbent surface charge, adsorption capacity varies widely from 0.19 to 163.3 mg/g.

Iron occurs naturally in soil and sediments and is present in many types of rocks. When water flows through the soil, sediment or rock beds it dissolves all soluble salts. Iron may also be released into water from industrial wastes, refining of iron ores, and corrosion of iron-containing metals. Iron in water can be present in two forms, the clear and colourless soluble Fe(II) or the reddish brown insoluble Fe(III). Water with Fe(II) under aerobic conditions turns cloudy due to the precipitate of Fe(III). Iron is an essential trace element in living organisms. The largest fraction of body iron is present in haemoglobin, myoglobin and haem-containing enzymes while the remaining fraction is stored in the body as ferritin and haemosiderin, mainly in the spleen, liver, bone marrow and striated muscle [26]. Taste is not usually noticeable at iron concentrations below 0.3 mg/L, although turbidity and colour may develop in piped systems at levels above 0.05–0.1 mg/L. Available iron concentrations in water do not affect human health.

19.8 Case studies on quality of drinking water

The Chandrapur district in India, situated on the Wainganga and Wardha river basin and covered by Deccan basalts and Quaternary alluvium, holds coal mines, a thermal power station, and cement, ferro alloys and paper industries. Tambekar *et al*. [44] carried out a study on drinking water quality in the vicinity of Chandrapur city by taking samples of drinking water from seven sites (surface water and groundwater). The pH was measured and analysis of dissolved oxygen, COD, alkalinity, chlorides, fluorides, total hardness, total solids,

turbidity and iron content was carried out. The measured pH values of 6.5–8.5 were well within the range given in the WHO recommendations and the permissible limit of pH in drinking water, but showed high alkalinity (225–608 mg/L) owing to the occurrence of limestone in the region surrounding the sampling stations. The water samples contained a permissible amount of hardness but less fluoride and chloride ion concentrations compared with permissible limits. The iron content of different sites in Chandrapur district was in the range 0.15–0.20 mg/L, which is well below standard values. Total dissolved solids (TDS) at different locations in Chandrapur district varied from 390 to 1418 mg/L. Compared with WHO recommended limits, sites S1, S2 and S3 showed high concentrations of TDS compared with other stations. It can be concluded that though most of the sampling stations showed a permissible range of concentrations, some of them were highly polluted. Efforts to reduce the concentration of those parameters with levels above the permissible limits could allow these sources of water to be used for domestic purposes in the region.

Mandour [45] performed a study with 30 drinking water samples in Egypt. The samples comprised both surface water and groundwater. Trace element analysis on drinking water samples was done for Pb, Zn, Cu, Co, Cd, Ni, Cr, Mo, Fe and Mn with pre-concentration of heavy metals in water samples to be within the detection limit of atomic absorption spectrometry (AAS). One water sample in Aga district showed chloride to be higher than the permissible limits of the Egyptian Ministry of Health (EMH) and the WHO, which may be attributed to leaching from various rocks into water by weathering. Drinking water contaminated mainly with lead and cadmium in samples from Mansoura, Talka, Aga and Mit-ghamr district was compatible with a corresponding analysis of urine samples. Lead and cadmium values in water and urine samples exceeded the permissible limits prescribed by EMH and WHO. Patients suffered from renal failure due to consumption of water containing lead and cadmium in excess of the guideline values. Toxicity of Ni is enhanced by the presence of other metals such as Co, Cu, Fe and Zn in drinking water. This study reports for the first time the relationship between Ni and hair loss, as shown by the water and urine samples from Mansoura and Aga districts. Table 19.4 displays the relation between trace elements present in excess in drinking water and corresponding urine samples in the same affected area.

In Charsadda district, Pakistan, different heavy metals were detected in drinking water and some of them exceeded their respective permissible limits [46]. On the basis of findings it was concluded that drinking water in the study areas was contaminated with SO_4^{2-}, NO_3^-, Pb, Cd, Fe, Ni and Zn. The concentrations of Pb in drinking water samples ranged from 1.59 to 372.2 mg/L and some of these sources exceeded the permissible limit set by Pak-EPA and the WHO. The minimum concentration of Pb was found in the sample collected from village of Mira khel (tehsil Charsadda), while the maximum concentration was found in the sample from the village of Dora khel (tehsil Tangi). Cd concentrations in two water sources each from tehsil Tangi and Shabqadar and three water sources from tehsil Charsadda exceeded the permissible limit. However, Cr and Cu concentrations were

Table 19.4 Trace elements present in drinking water and urine samples in excess of guideline values (Dakahlyia Governorate, Egypt).

District	Sample no.	Water source	Elements (mg/L) in water	WHO/EMH limits (mg/L)	Elements (μg/L) in urine	Iyengar median reference (μg/L)
Mansoura	2	Surface	Cd 0.005	0.003	Cd 0.87	0.8
Mansoura	4	Surface	Cd 0.004	0.003	Cd 0.85	0.8
			Pb 0.016	0.01	Pb 11.5	11
Mansoura	1	Surface	Ni 0.022	0.07/0.02	Ni 2.6	2.5
Talka	1	Surface	Cd 0.004	0.003	Cd 0.83	0.8
Talka	4	Surface	Pb 0.014	0.01	Pb 11.3	11
Aga	1	Ground	Cd 0.008	0.003	Cd 0.81	0.8
Aga	2	Ground	Ni 0.023	0.07/0.02	Ni 2.51	2.5
Mit-ghamr	1	Ground	Pb 0.015	0.01	Pb 13	11
Mit-ghamr	4	Ground	Pb 0.017	0.01	Pb 15	11

Source: data from Mandour [45].

within the permissible limits. Except for water samples from the villages of Karewar and Bariband kali (tehsil Tangi), Zn concentrations in drinking water samples from the district of Charsadda were within the permissible limit. Nearly all water sources had concentrations of Ni within permissible limits, except in water samples collected from the villages of Dora khel and Dosehra. The concentrations of Fe in drinking water samples fell within the permissible limits, except in water samples collected from the villages of Hakim abad and Bahram dheri in tehsil Tangi, Akhon dheri in tehsil Charsadda and Kangra and Mir zai in tehsil Shabqadar. Several research studies have demonstrated the fact that changes in the quality of drinking water cause health problems. The health survey carried out in the study area gives a clear picture of how the presence of some mineral elements in excess affects human health. Figure 19.2 portrays the reported ailments of the population under study. Further research work was suggested for water treatment to improve the quality of drinking water so as to protect the inhabitants from chronic health problems.

Dinelli *et al.* [47] collected 157 tap-water samples from all over Italy and tested them for 69 elements: Ag, Al, As, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Hf, Hg, Ho, I, K, La, Li, Lu, Mg, Mn, Mo, Na, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Sr, Ta, Tb, Te, Th, Ti, Tl, Tm, U, V, W, Y, Yb, Zn, Zr, Br^-, HCO_3^-, Cl^-, F^-, NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, SO_4^{2-} and SiO_2. The samples showed homogeneity regardless of their varied sources. Of the 157 samples, most had low mineral concentrations, with a few showing intermediate levels and only one with very low and one with very high mineral concentrations. Aluminium concentrations in Italian tap waters range from 0.3 to 454 µg/L. The aquifer located in Mt Peloritani, which comprises peraluminous granitic lithologies, is the reason that the tap water from Messina province exhibited Al concentrations above 200 µg/L.

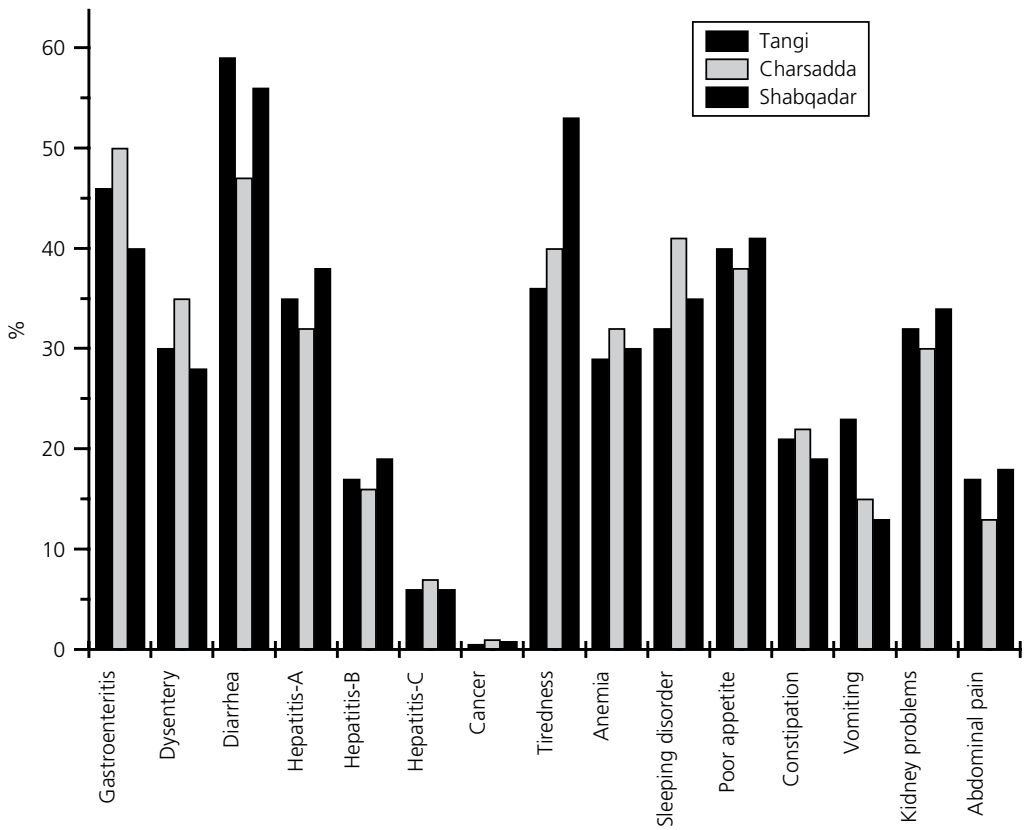


Figure 19.2 Disease reported during health survey in Charsadda district, Pakistan.

As concentrations in Italian tap waters ranged from 0.016 to 27.2 μg/L. In 95% of the samples As concentrations were within the permissible range, but some samples recorded high As concentrations due to the non-homogeneous geographic distribution of As, forcing regional authorities to increase the threshold value to 50 μg/L. F^- concentrations did not cross the threshold value of the Italian legislation. The highest concentration of fluoride (1.43 mg/L) were found in Latium, Viterbo Province, where the permitted value has been raised because of high F^- background values in surface waters. With regard to the concentration of rare earth elements (REE) in surface waters, there are no reference data but the Forum for European Geological Surveys (FOREGS) indicates a median for European surface water of 192 ng/L, higher than the Italian value of 64 ng/L. The relatively high REE concentrations are associated with acidic plutonic volcanic rocks, alkaline volcanic rocks and metamorphic rocks. Although U is a non-essential element, its consumption through drinking water is six times as harmful as its radioactivity. The highest U concentration (18.1 μg/L), which exceeds the FAO guideline value (15 μg/L), was found in one sample from Bolzano (Trentino Alto Adige, central Alps), which may be due to uranium ore deposits. In central-southern Italy with volcanic regions and rich in geothermal spring waters, tap-water samples recorded high Rb concentrations. Levels of B, Cr, Se and V do not exceed the threshold of the Italian and EU drinking water directive. The majority of the analysed tap waters had concentrations that do not exceed the guideline values as per Italian Law DL 31/2001, EU Directive 1998/83/EC, US EPA (2003, 2009), WHO (1996, 2008) and FAO codex 108-1981. Despite some regional government authorities issuing enhanced thresholds for several elements because of high geological background, several samples had NO_3^-, NH_4^+, Fe, Al, Mn, As and Pb concentrations exceeding EU and Italian thresholds. High values of elements like As, Rb, U, V and F could be directly related to interaction with alkaline volcanic rocks.

19.9 Trends in the control of mineral content of drinking water

Water can be termed a calorie-free drink which helps to meet the body's needs and provides us with the necessary minerals. Any increase or decrease in the current guideline values for mineral elements in drinking water calls for the control of their content. The outcome of ongoing research might suggest reassessment of guideline values when correlated with health issues.

Recent epidemiological studies suggest the need to reassess the safe upper limit in drinking water of selenium, a metalloid with both toxicological and nutritional properties. Depending on its biological activity for different species, selenium consumed by humans through drinking water may cause toxicity at much lower levels than previously surmised. Studies indicate that health risks may occur at exposures below the current EU and WHO upper limit and guideline of 10 and 40 μg/L, respectively, and suggest reduction to 1 μg/L in order to adequately protect human health [48].

Norway has a high incidence of hip fractures, and the variation may reflect the variable concentrations of Ca(II) and Mg(II) in municipal drinking water. A trace metal survey of drinking water catering to 64% of the Norwegian population was linked to hip fractures from hospitals. Poisson regression models were fitted, adjusting for age, degree of urbanization, region of residence, type of water source, and pH. The concentrations of Ca(II) and Mg(II) in drinking water were generally low. An inverse correlation was found between concentration of Mg(II) and risk of hip fracture in both genders but no such correlation between Ca(II) and hip fracture risk was observed. The correlation between Mg(II) and hip fracture did not explain the variation in hip fracture risk between city and rural areas. Magnesium in drinking water may have a protective role against hip fractures. However, the authors suggest further investigation in this matter [49]. Further, it has been suggested that Ca(II) and Mg(II) in drinking water play a role in cardiovascular disease (CVD). Several studies have reported lower heart disease risk in areas with high levels of Ca(II) and Mg(II), the principal components of water hardness and essential nutrients. The available information supports the hypothesis that intake of hard water, especially rich in magnesium, decreases the risk of CVD, although there are as yet no international regulations on either minimum or maximum limits for Ca(II), Mg(II) or water hardness [50].

Abbreviations

AAS: Atomic absorption spectrometry
AR: Aeration rate
CNT: Carbon nanotube
COD: Chemical oxygen demand
CPE: Cloud point extraction
CVD: Cardiovascular disease
DBM: Dibenzoylmethane
DMA: *N*,*N*-Dimethylaniline
EMH: Egyptian Ministry of Health
EPA: Environmental Protection Agency
FAO: Food and Agriculture Organization
FOREGS: Forum of European Geological Surveys
HGAAS: Hydride generation atomic absorption spectrometry
HRT: Hydraulic retention time
ICP-MS: Inductively coupled plasma mass spectrometry
ICP-OES: Inductively coupled plasma optical emission spectrometry
IDMS: Isotope dilution mass spectrometry
LOD: Limit of detection
LOQ: Limit of quantitation
NMO: Nanosized metal oxide
REE: Rare earth elements
RSD: Relative standard deviation
SDS: Sodium dodecyl sulfate
TDS: Total dissolved solids
TPP^+: Tetraphenylphosphonium ion
WHO: World Health Organization

References

1 Gundermann G, Gutenbrunner C, Karagulle O. Are medicinal mineral waters suitable for mineral substitution? *Ernährung Medizin* 2004;**19**:63–68.
2 World Health Organization. *Guidelines for Drinking-water Quality*, 4th edn. WHO, Geneva, 2011.
3 Theis TL, Singer PC. Complexation of iron (II) by organic matter and its effect on iron (II) oxygenation. *Environ Sci Technol* 1974;**8**:569–573.
4 Stumm W, Morgan JJ. *Aquatic Chemistry*. Wiley-Interscience, New York, 1970.
5 Bhatnagar A, Kumar E, Sillanpää M. Fluoride removal from water by adsorption: a review. *Chem Eng J* 2011;**171**:811–840.
6 The World Bank. Arsenic contamination of groundwater in South and East Asian countries. Vol. II. Technical Report. Water and Sanitation Program, The World Bank, 2005.
7 CREDOC (Centre de Recherche pour l'Étude et l'Observation des Conditions de Vie). L'eau: hydratation et santé (Enquête). Danone Nutritopics, 31 December 2004.
8 Foster S. Groundwater assessing vulnerability and promotion protection of a threatened resource. In: *Water: The Key to Socio-economic Development and Quality of Life. Proceedings of the 8th Stockholm Water Symposium, 10–13 August 1998*. Stockholm International Water Institute, Sweden, 1998, pp. 79–90.
9 Elango L, Kannan R, Senthilkumar M. Major ion chemistry and identification of hydrogeochemical processes of groundwater in a part of Kancheepuram district, Tamil Nadu, India. *J Environ Geosci* 2003;**10**:157–166.
10 De Zuane J. *Handbook of Drinking Water Quality*, 2nd edn. John Wiley & Sons, Inc., New York, 1997.
11 Gray NF. *Drinking Water Quality: Problems and Solutions*, 2nd edn. Cambridge University Press, Cambridge, 2008.
12 Simate GS, Iyuke SE, Ndlovu S, Heydenrych M, Walubita LF. Human health effects of residual carbon nanotubes and traditional water treatment chemicals in drinking water. *Environ Int* 2012;**39**:38–49.
13 Tekerlekopoulou AG, Vasiliadou IA, Vayenas DV. Physicochemical and biological iron removal from potable water. *Biochem Eng J* 2006;**31**:74–83.
14 Das B, Hazarika P, Saikia G *et al*. Removal of iron from groundwater by ash: a systematic study of a traditional method. *J Hazard Mater* 2007;**141**:834–841.
15 Bordoloi S, Nath SK, Dutta RK. Iron ion removal from groundwater using banana ash, carbonates and bicarbonates of Na and K, and their mixtures. *Desalination* 2011;**281**:190–198.
16 Bordoloi S, Nath M, Dutta RK. pH-conditioning for simultaneous removal of arsenic and iron ions from groundwater. *Process Saf Environ Prot* 2014;**91**:405–414.
17 Hua M, Zhang S, Pan Bi, Zhang W, Lv L, Zhang Q. Heavy metal removal from water/wastewater by nanosized metal oxides: a review. *J Hazard Mater* 2012;211– 212:317–331.
18 Smedley PL, Kinniburgh DG. A review of the source, behaviour and distribution of arsenic in natural waters. *Appl Geochem* 2002;**17**:517–568.
19 Wang W, Li H, Wang X, Liu Y. Spatial variations of aluminum species in drinking water supplies in Xi'an studied applying geographic information system. *J Environ Sci China* 2010;**22**:519–525.
20 Wang W, Yang Hi, Wang X, Jiang J, Zhu W. Effects of fulvic acid and humic acid on aluminum speciation in drinking water. *J Environ Sci China* 2010;**22**:211–217.
21 Filella M, Belzile N, Chen Y-W. Antimony in the environment: a review focused on natural waters: I. *Occurrence. Earth Sci Rev* 2002;**57**:25–176.
22 Edmunds WM. Beryllium: environmental geochemistry and health effects. In: Nriagu JO (ed.) *Encyclopedia of Environmental Health*. Elsevier, Amsterdam, 2011, pp. 293–301.
23 Geffen N, Semiat R, Eisen MS, Balazs Y, Katz I, Dosoretz CG. Boron removal from water by complexation to polyol compounds. *J Membrane Sci* 2006;**286**:45–51.

24 Nordberg GF, Fowler BA, Nordberg M, Friberg L (eds) *Handbook on the Toxicology of Metals*, 3rd edn. Academic Press, Burlington, MA, 2007.

25 Aldrees AM, Al-Manea SM. Fluoride content of bottled drinking waters available in Riyadh, Saudi Arabia. *Saudi Dent J* 2010;**22**:189–193.

26 National Research Council. *Iron*. University Park Press, Baltimore, MD, 1979.

27 Bratakos MS, Zarifopoulos ThF, Siskos PA, Ioannou PV. Total selenium concentration in tap and bottled drinking water and coastal waters of Greece. *Sci Total Environ* 1988;**76**:49–54.

28 Brunskill GJ, Wilkinson P. Annual supply of ^{238}U, ^{234}U, ^{230}Th, ^{226}Ra, ^{210}Pb, ^{210}Po, and ^{232}Th to Lake 239 (Experimental Lakes Area, Ontario) from terrestrial and atmospheric sources. *Can J Fish Aquat Sci* 1987;**44**:215–230.

29 Stalder E, Blanc A, Haldimann M, Dudle V. Occurrence of uranium in Swiss drinking water. *Chemosphere* 2012;**86**:672–679.

30 Terrés-Martos C, Navarro-Alarcón M, Martín-Lagos F, Giménez-Martínez R, De La Serrana HL-G, López-Martíne MC. Determination of zinc levels in waters from south-eastern Spain by electrothermal atomic absorption spectrometry: relationship with industrial activity. *Water Res* 2002;**36**:1912–1916.

31 Ohno S, Teshima N, Zhang H, Sakai T. Utilization of activating and masking effects by ligands for highly selective catalytic spectrophotometric determination of copper and iron in natural waters. *Talanta* 2003;**60**:1177–1185.

32 Veríssimo MIS, Teresa M, Gomes SR. The quality of our drinking water: aluminium determination with an acoustic wave sensor. *Anal Chim Acta* 2008;**617**:162–166.

33 Nig SY, Zou A, Sim LP *et al*. High accuracy measurements of trace elements in drinking water by exact matching isotope dilution mass spectrometry (IDMS). *Int J Mass Spectrom* 2012;**321**–322:19–24.

34 Avino P, Capannesi G, Rosada A. Ultra-trace nutritional and toxicological elements in Rome and Florence drinking waters determined by instrumental neutron activation analysis. *Microchem J* 2011;**97**:144–153.

35 Hasan HA, Abdullah SRS, Kamarudin SK, Kofli NT. Response surface methodology for optimization of simultaneous COD, $NH4^{+}$–N and Mn^{2+} removal from drinking water by biological aerated filter. *Desalination* 2011;**275**:50–61.

36 El-Shahawi MS, Al-Saidi HM, Bashammakh AS, Al-Sibaai AA, Abdelfadeel MA. Spectrofluorometric determination and chemical speciation of trace concentrations of chromium (III and VI) species in water using the ion pairing reagent tetraphenyl-phosphonium bromide. *Talanta* 2011;**84**:175–179.

37 Ulusoy Hİ, Akçay M, Ulusoy S, Gürkan R. Determination of ultra trace arsenic species in water samples by hydride generation atomic absorption spectrometry after cloud point extraction. *Anal Chim Acta* 2011;**703**:137–144.

38 Shenashen MA, El-Safty SA, Elshehy EA. Architecture of optical sensor for recognition of multiple toxic metal ions from water. *J Hazard Mater* 2013;**260**:833–843.

39 Shariati S, Yamini Y, Zanjani MK. Simultaneous preconcentration and determination of U(VI), Th(IV), Zr(IV) and Hf(IV) ions in aqueous samples using micelle-mediated extraction coupled to inductively coupled plasma-optical emission spectrometry. *J Hazard Mater* 2008;**156**:583–590.

40 Sracek O, Bhattacharya P, Jacks G, Gustafsson J-P, von Brömssen M. Behavior of arsenic and geochemical modeling of arsenic enrichment in aqueous environments. *Appl Geochem* 2004;**19**:169–180.

41 Giles DE, Mohapatra M, Issa TB, Anand S, Singh P. Iron and aluminium based adsorption strategies for removing arsenic from water. *J Environ Manage* 2011;**92**:3011–3022.

42 Mousavi SM, Kiani S, Farmad MR, Hemati A, Abbasi B. Extraction of arsenic(V) from water using emulsion liquid membrane. *J Disper Sci Technol* 2012;**33**:123–129.

43 Edmunds WM, Smedley PL. Groundwater geochemistry and health: an overview. In: Appleton JD, Fuge R, McCall GJH (eds) *Environmental Geochemistry and Health: With Special Reference to Developing Countries*. Geological Society Special Publication 113. Geological Society, London, 1996, pp. 91–105.

44 Tambekar P, Morey P, Batra RJ, Weginwar RG. Quality assessment of drinking water: a case study of Chandrapur District (M.S.). *J Chem Pharm Res* 2012;**4**:2564–2570.

45 Mandour RA. Human health impacts of drinking water (surface and ground) pollution Dakahlyia Governorate, Egypt. *Appl Water Sci* 2012;**2**:157–163.

46 Khan S, Shahnaz M, Jehan N, Rehman S, Shah MT, Din I. Drinking water quality and human health risk in Charsadda district, Pakistan. *J Clean Prod* 2012;**60**:93–101.

47 Dinelli E, Lima A, Albanese S *et al*. Major and trace elements in tap water from Italy. *J Geochem Explor* 2012;**112**:54–75.

48 Vinceti M, Crespi CM, Bonvicini F *et al*. The need for a reassessment of the safe upper limit of selenium in drinking water. *Sci Total Environ* 2013;**443**:633–642.

49 Dahl C, Søgaard AJ, Tell GS *et al*. Nationwide data on municipal drinking water and hip fracture: could calcium and magnesium be protective? A NOREPOS study. *Bone* 2013;**57**:84–91.

50 Anne K. Magnesium and calcium in drinking water and heart diseases. In: Nriagu JO (ed.) *Encyclopedia of Environmental Health*. Elsevier, Amsterdam, 2011, pp. 535–544.

CHAPTER 20

Elemental composition in grapes and wine: role, analytical methods and their use

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Abstract: The analysis of elements or minerals (macro and micro) in wine is used to determine legal limits for export purposes, to monitor trace contaminants, to monitor the content of certain metal salts (e.g. calcium and potassium), for authenticity purposes and as an indication of potential changes in colour and oxidation, as well as to promote its health benefits, among other uses. This chapter presents an overview on the origin and role of elements in grape and wine, the analytical methods and techniques currently used to determine elements and the recent applications of such techniques. The effects of elements on quality, authentication and fermentation are also discussed.

Keywords: grape, wine, authentication, quality, fermentation

20.1 Overview of elements and minerals in grapes and wine

The analysis of elements or minerals (macro and micro) in wine is used to determine legal limits for export purposes, to monitor trace contaminants, to monitor the content of certain metal salts (e.g. calcium and potassium), for authenticity purposes and as an indication of potential changes in colour and oxidation, as well as to promote its health benefits [1–8].

Precise analytical measurements are required at all stages of the winemaking process, starting in the vineyard, during the fermentation and maturation stages, and at bottling, certification and marketing of the final product. These measurements are required for various reasons. For instance, analytical methods are used to provide information required by law for the production and marketing of these products [1–8]. Consumption of high-quality wines is increasing worldwide, as well as demands from the consumer about the origin and characteristics of wine [1–8].

The concentration of some minerals in wine is also important because of their health impacts as well as their role in the stability of wine, the possibility of toxicological risks and issues associated with food regulations [9, 10]. In recent years, the mineral profile of wines has been used as a fingerprint in order to characterize wines based on their varietal or geographical origin [2, 3, 11–18].

The mineral content of grapes and wine is influenced by several factors. Natural source plays the main role in the total metal contribution in grapes and wine, reflecting soil geochemistry [6, 7]. The concentrations of these metals are characteristic and influenced by maturity of the grapes, their variety, the type of soil in the vineyard, and the climatic conditions during their growth [6, 9]. The content of metals of a secondary origin is associated with external contamination of the vine during growth or during different stages in winemaking (from harvesting to bottling and cellaring) [6, 9]. During grape growth and development in the vineyard, contamination comes from the soil, from fertilizing and plant protection practices, or from environmental pollution [6, 9]. The second source of contamination comes from anthropogenic source such as the application of pesticides, fungicides and fertilizers during the growing period of grapes. All these management practices lead to an increase in the concentrations of elements such as As, Cd, Cu, Mn, Pb

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and Zn [6, 19]. Contamination may also occur during the process of wine production, for instance the long contact time between wine and the materials comprising the various winemaking machinery parts, pipes, casks and barrels may lead to an increase in amounts of Al, Cd, Cr, Cu, Fe and Zn [6–19].

This chapter presents an overview of the origin and role of elements in grape and wine, the analytical methods and techniques used to determine elements, as well as recent applications and effects on quality, authentication and fermentation.

20.2 Origin and role of elements in grape and wine

Mineral elements are not metabolized or transformed during the oenological processes and they remain almost unaltered from musts to wines. The classification of samples according to geographical regions or denomination of origins and the detection of certain adulterations might rely on elemental composition profiling [20–25]. Some winemaking practices could affect the content of some trace elements.

In the 1990s, the determination of trace elements in wines was based mainly on flame atomic spectroscopy (FAS). The absorption mode was, in general, sufficiently sensitive to quantify transition metals (e.g. Fe, Cu, Ca, Co, Mn), often present in low concentrations (mg/L). Similarly, FAS was utilized for determining some alkaline metals (e.g. Li, Na, K) and alkaline earth metals (e.g. Ca, Sr) [24].

In recent years, the introduction of highly sensitive atomic spectroscopies has expanded the variety of elements that can be quantified in grapes and wine [22–24]. Although trace elements are essential for humans, many of them (e.g. As, Sb, Bi, Pb, Sn, Se, Te) have unknown biological function and are regarded as toxic because they accumulate in the human organism [22–24]. It is well known that some trace elements play a relevant role in winemaking, for example Zn is essential at low concentrations for the correct development of alcoholic fermentation, while Cu, Fe and Mn have organoleptic effects at increased levels, contributing to the haze and taste of wines [22]. Iron is found in substantial quantities in all grape and wine varieties. The iron concentration of grapes and wines depends on several factors, the most important being the ground where the grapes are produced. The iron derived from the soil reaches wine in two ways: it either covers the grape berries as part of the dust (this is the reason for the detected high iron content in rinsing samples) or it is absorbed by the plants through the roots [22]. Excessive amounts of iron (10–20 mg/L or more) can cause stabilization problems in wines, since it may be oxidized to a ferric form that causes precipitation of pigmented materials ('blue haze') or with orthophosphate ions ('white haze') [22].

Ajtony *et al*. [25] have stated that the concern about human exposure to trace metals in beverages and food products, including wines, has started to attract considerable attention, since the consumption of wines, especially in large volumes, may significantly contribute to the daily dietary trace element intake by humans.

As described in the previous section, the mineral content of wines is determined by many factors such as type of soil, variety of grape, weather conditions, pollution, and viticultural practices. In fact, the regionally varying trace metal content of wines can also be used for source identification purposes [13, 24]. The secondary mineral contamination is derived from the use of pesticides and fertilizers or acquired during winemaking (despite the overall reduction in mineral content during this process) [13, 24]. It has been shown, for example, that while the origin of Cu in wines is associated with copper-based vineyard sprays, the content of As, Cd and Pb reflects differences in grape variety and environmental factors such as soil, rain and other environmental characteristics [13, 24]. The concentration of copper in wines may be of exogenous or endogenous origin. Most of the exogenous copper is due to copper sulfate used for spraying the vines to prevent mildew.

Sodium arsenite is employed in viticulture as a fungicide against a grapevine necrotic disease known as eutypa dieback, caused by the fungus *Eutypa lata*. Arsenic is a suspected carcinogen and little is known about its chronic sublethal effects [26]. The concentration of As in wines mainly depends on factors such as soil composition, grape variety, climatic conditions, use of pesticides, winemaking technology and storage conditions [26]. The Office International de la Vigne et du Vin (OIV) has established the maximum limit of As in wine at 200 pg/μL, but in general the presence of only a few picograms per microlitre of As in uncontaminated wines is accepted [26, 37].

In the case of lead, its presence in wine could be the consequence of airbone particulate matter on grapes

and/or by grapevine uptake from groundwater and soil. The metal may also be released from bronze tanks, taps, pumps and tubing containers used in winemaking [27]. Lead adversely affects multiple enzyme systems of the body, as any ligand with sulfhydryl groups can be vulnerable. Detection of lead in wine is usually focused on the determination of the total lead Pb^{2+} [27].

It has been reported that the structurally complex pectic polysaccharide rhamnogalacturonan II (RG-II), an anionic biomolecule released from the grape during winemaking, can form coordination complexes with specific cations such as lead [28]. The toxic effects of cadmium are due to the inactivation of enzymes containing sulfhydryl groups and the uncoupling of oxidative phosphorylation in mitochondria. Cadmium may also compete with other metals such as zinc and selenium for inclusion into metalloenzymes and it may compete with calcium for binding sites on regulatory proteins such as calmodulin [28]. The OIV establishes the maximum concentrations of lead and cadmium in wine at 150 and 10 μg/L, respectively [28, 37]. The multiple sources responsible for the content of metal trace elements in wines make the establishment of appropriate strategies to prevent or avoid their occurrence in wines rather difficult. Obviously, an adequate soil for each grape variety and environmental conditions, regulation in the use of fertilizers, fungicides and other pesticides, and adequate winemaking equipment could greatly reduce the amount of these elements in wines. Recently, La Pera *et al*. [30, 31] in a study performed over two consecutive vintages have shown that treatments with the fungicides mancozeb, zoxamine and copper oxychloride significantly increased the levels of Mn, Zn, Cu, Pb and Cd of wines, grapes, marc and grape stalks.

Some strategies that could be effective for the control of trace heavy metals imply the use of some polymers from natural sources [32]. Chitin, chitosan and other derivatives are non-toxic biodegradable polymers that can remove metals and organic contaminants from food products [32]. Chitin exists widely in the cell walls of fungi, moulds and yeasts but also in the exoskeletons of some invertebrates such as molluscs and insects [32]. Chitosan is found only in a few species of fungi. Chitin and chitosan consist of repeating units of 2-acetamido-2-deoxy-β-D-glucose and 2-amido-2-deoxy-β-D-glucose, respectively. It was shown that the addition of chitosan, chitin, chitin–glucan and chitin–glucan hydrolysate of fungal origin (up to 2 g/L) to wine can reduce the amount of Fe by 32–91%, Cd by 11–57% and Pb by 33–84%, depending on the type of wine [32].

Furthermore, based on the parietal adsorption activity of yeasts, the use of inactive yeast or yeast derivatives in wines could also be considered in the future as a mechanism for controlling trace metal compounds in order to improve wine safety [33]. As described above, metals in grape and wine can originate from both natural and anthropogenic sources, and their concentrations can be a significant parameter affecting consumption and conservation of wine. The trace and ultratrace mineral element composition of wines seems to be an excellent source of information to be exploited in characterization studies [33–35]. Grapes receive trace elements from the soil, so that their elemental composition is reasonably correlated with the geological characteristics of the producing areas [13].

Metallic ions also have an important role in oxidative–reductive reactions resulting in wine browning, turbidity, cloudiness and astringency, so wine quality depends greatly on its metal composition. Some metals in wine may also affect human health. Consumption of wine may contribute to the daily dietary intake of essential metals (e.g. Cu, Fe and Zn) but can also have potentially toxic effects if metal concentrations are not kept under allowable limits. Therefore, strict analytical control of metal concentration is required during the whole process of wine production. Some of the metals that have an impact on wine quality and human health are Al, Ar, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn [33–35].

20.3 Analytical and data mining methods

Taking into consideration the importance of these elements with regard to their potential toxicity and technological features, it is worth noting the great amount of scientific work that has been focused on developing adequate analytical tools that can offer sufficient selectivity, sensitivity and robustness for the analysis of these compounds in wines [24, 33–37]. As a consequence, there are many analytical techniques for quantification of trace metal in wines, such as ion chromatography (IC), stripping potentiometry, inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma atomic emission spectrometry (ICP-AES), FAS and

graphite furnace atomic absorption spectrometry (GFAAS). All have been extensively reviewed elsewhere [24, 33–37]. Various methods have been developed to analyse elements in wine. Atomic absorption spectrometry (AAS), multi-element GFAAS and FAS are the most commonly used instruments for routine analysis [33–37]. Apart from atomic spectroscopies, X-ray fluorescence and electrophoresis have been used to determine the elemental composition of wines [33–37].

For the determination of mineral content of wines, atomic spectroscopic techniques are most often used. Application of flame AAS and electrothermal AAS for metal analysis in wine has been reported [33–37]. Aside from regulatory analyses, geographical authenticity of wines may be established by a combination of multi-element analysis of wines and their provenance soils and multivariate statistical methods [13, 17, 18].

Many minerals are found in wine and, in most instances, these reflect uptake characteristics of the rootstock and climatic influences on the rate of transpiration. Since heavy metals typically precipitate during fermentation, their elevated occurrence in finished wine is usually associated with contamination after fermentation [37]. Heavy metals are determined in wine with spectrophotometric and spectroscopic techniques. Because of the low maximum levels that are typically enforced for toxic elements, specialized techniques such as GFAAS (for Pb and Cd) and hydride generation AAS (for As and Hg) are prescribed by the OIV [37]. Flame AAS methods are used for elements such as Cu, Fe and Sn, for which relatively high maximum levels are typically enforced [33–36].

Onianwa *et al*. [38] successfully applied flame AAS for the determination of various metals, including Pb and Cd, in non-alcoholic wines using suitable sample mineralization and pre-concentration techniques. Inductively coupled plasma emission spectroscopy (using both optical and mass spectrometric detection) may also be used for multi-element analysis.

Dessuy *et al*. [39] developed and validated a method for the determination of Pb in wine using electrothermal AAS. The use of various chemical modifiers was investigated and palladium was found to produce optimal stabilization of Pb during pyrolysis. The optimized procedure enabled the determination of Pb in wine without any sample preparation with a limit of detection of 0.5 g/L. Since this procedure is fully automated and sufficiently sensitive, it is suited for routine regulatory determination of Pb in wines.

Different research groups have reported on ICP-MS as well as improvements in sampling accessories to analyse elements in wine. For the analysis of Chinese wine, a microfluidic sub-microliter sample was developed for use with ICP-MS [40]. The system consisted of a microfluidic chip integrating variable-volume sampling channels (0.1–0.8 μL), an eight-way multifunctional valve used in flow injection analysis (FIA), a syringe pump and a peristaltic pump of the ICP-MS instrument. The experimental results reported by the authors showed that neither alteration of plasma stability nor carbon deposition was observed when the sampling volume of 20% ethanol containing 100 g/L glucose was downscaled to 0.8 μL. Further reducing the sampling volume to 0.4 μL produced no significant difference between the intensities of multi-element standard prepared in three simulated Chinese rice wine matrices and those in aqueous solution. The authors indicated no negative effect of Chinese rice wine matrix on the ICP-MS intensities [40].

In recent years correlative methods based on near infrared (NIR) spectroscopy have been reported as a method for predicting elements in grapes and wines [41–45]. However, the determination of inorganic compounds using NIR spectroscopy is more challenging compared with organic compounds owing to the lack of direct relation between the element involved and the spectra obtained [41–45]. NIR spectroscopy could be used for determining mineral concentration, due to the association between minerals and organic functional groups in the food matrix and the effect on O–H bonding [41–45]. The estimation of mineral elements by NIR spectroscopy is therefore generally dependent on the presence of those elements in mixtures of organic or hydrated compounds and salts (cations and anions) [41–45]. Despite the reports of many satisfactory calibrations for mineral elements for a particular material, it is difficult to apply the results from one food to another because the form of the organic molecule/mineral compounds can vary seasonally and/or among species, type of food and processing methods [41–45]. This may lead to unstable calibrations, inconsistent results and difficulty expanding calibrations beyond a well-characterized population. Therefore, the development of universal calibrations for minerals is difficult. For example, phosphorus occurs in plants mainly as ionic organic molecules of phytate, phospholipids and nucleic acids, and the proportion of total P in these forms varies among food, plant species and seasonally [41–45]. Thus, in order to

develop a NIR spectroscopy calibration model for minerals in wines, it would be necessary to include a wide range of varieties [44]. Sauvage *et al*. [45] reported for the first time the use of NIR spectroscopy to measure minerals in wine. These authors attempted to determine six elements (K, Na, Mg, Ca, Fe and Cu) using only 24 white wine samples.

20.4 Applications

20.4.1 Origin and authentication

The first successful attempts to use multi-element analysis for classification of wines according to their provenance or country of origin can be dated as early as 1979 [13, 46]. However, only comparatively abundant elements were measured at that time (Rb, Mn, K, Li, Mg, Ca, Na, Fe and Sr) as they were the most cited for their capacity to discriminate wines according to their geographical origin [2, 7, 8, 13, 46]. For example, Coetzee *et al*. [47] described a fingerprinting technique for classification of South African wines according to geographical origin based on elemental composition (Table 20.1). The method uses the assumption that provenance soil is a primary contributor to the trace element composition of wines [2, 7, 8, 13, 46, 47]. Several other reports can be found in the literature that have determined the content of inorganic elements in wines samples, measured by ICP-MS, with the goal of classifying samples according to their origin [13, 47–49]. Various multivariate methods and chemometrics are used to interpret and extract information from complex data obtained by instrumental techniques in the analysis of foods and wine [50]. Results from other studies show that correct discrimination and classification of wines depend not only on sample variability but also on the variables used for discrimination classification. For instance, organic compounds can be very useful for discrimination between varieties, but may not be good predictors for provenance discrimination [51].

The application of chemometric methods to high-resolution ICP-MS data has been investigated as a means of differentiating wine samples on the basis of trace element content [13]. As the number of trace elements in wines can be affected by winemaking technique or anthropogenic influence, and therefore become less useful for discrimination purposes, studies based on this type of approach are a step towards the optimal choice of measured elements/variables and statistical techniques used with respect to the ultimate goal of the research [13]. The

Table 20.1 Concentration (mg/L) of elements in grapes, juice and wine from different countries and regions.

	K	Ca	Fe	Na	Mg	Reference
Wine						
Argentina						
Cordoba	488	50	3.7	36	70	[84]
La Rioja	236	31	2.6	138	111	[84]
San Juan	1637	286	2.8	58	107	[84]
Spain						
Tenerife	1099	76	2.66	70	85.3	[93]
La Palma	1158	74.9	2.75	34	70.7	[93]
Lanzarote	1243	69.2	5.16	168	89.6	[93]
Gran Canaria	1236	75.3	3.65	89	94.7	[93]
South Africa						
Stellenbosch		50.4	1.3	146	163	[47]
Robertson		49.7	1.00	153	237	[47]
Swatland		52.4	0.75	140	241	[47]
Grape juice						
Barossa Valley				54		[94]
Clare Valley				59		[94]
Coonawarra				66		[94]
Riverland				48		[94]

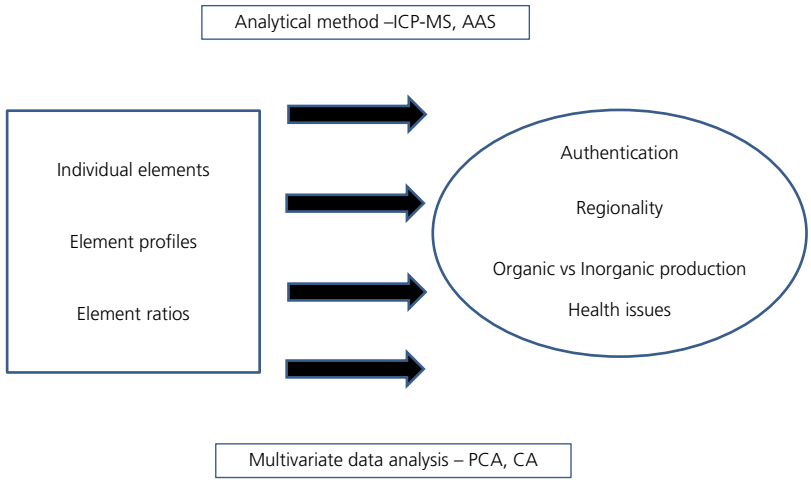


Figure 20.1 Combination of analytical element analysis and multivariate data methods for characterizing wines.

application of multivariate analysis (MVA) methods such as principal components analysis (PCA), analysis of variance (ANOVA), cluster analysis (CA) or linear discriminant analysis (LDA) provides the means to use and understand the data generated by instrumental techniques based on the overall properties of the sample and to perform a classification without the need for additional compositional chemical data [50] (Figure 20.1). Multivariate statistical techniques also allow verification of the contribution of each variable to the model, and its capacity to discriminate one category from another.

The determination of element profiles in wines is one of the most common methods used to determine the origin of wines. Several examples can be found in the literature that report the analysis of elements combined with MVA to classify and discriminate wines from different origins [52–63]. Analysis of trace elements and ratios of stable isotopes have been shown to be a valuable tool for discriminating wines according to their region of origin. Several factors, such as environmental contamination, agricultural practices, climatic changes and vinification processes, may change markedly the multi-element composition of the wine and may endanger the relationship between wine and soil composition [52–63].

One of the most challenging analytical issues in terms of wine authenticity is the identification or determination of geographical origin [13]. This question becomes more and more important in the current context of a global wine market. Single parameters are often not sufficient on their own to determine product identity with respect to wine labelling. Recent developments have shown that control is improved by application of MVA methods to a combination of compositional and isotopic data [64–70]. In past years, the official European wine control bodies have been confronted with wines that have had to be judged as suspicious in terms of their sophisticated authenticity (i.e. falsifications that are not readily identifiable), especially as regards grape variety and geographical origin. To improve the measures of authenticity control, a project with the title 'Establishing of a wine data bank for analytical parameters for wines from third countries (WINE-DB project, G6RD-CT-2001-00646)' was initiated in 2002 and funded by the European Commission within the programme Competitive and Sustainable Growth [64–70]. The project consortium of this multinational project was composed of official and private laboratories from the EU, from new European member states and from a group of university partners for the statistical evaluation [64–70]. Within the framework of the WINE-DB project, commercial and authentic wines originating from new European member states (Czech Republic, Hungary and Romania) and from overseas countries (South Africa and Australia) are also collected and analysed. More than 1800 wines from three vintages were analysed. While SIMCA modelling was performed using all variables, UNEQ requires a preliminary variable selection, leading to only four variables (Cu, Zn, anthocyanins and SO_2) being included in the models [64–70].

Both techniques provided highly sensitive and specific category models and also resulted in a very reliable classification of samples (only one or two samples misclassified in SIMCA and UNEQ, respectively) [64–70].

Lead and its isotope ratios (IRs) in 83 Italian wines from 42 different administrative provinces produced on laboratory scale in 2000 were measured by ICP-MS [64]. Lead had a median of 19.0 μg/L, ranging from 10.0 up to 149 μg/L, and was higher in the samples from north-western Italy. The values of the IRs were (mean standard deviation) 1.171 ± 0.011 for $^{206}Pb/^{207}Pb$, 2.071 ± 0.017 for $^{208}Pb/^{206}Pb$, 2.425 ± 0.014 for $^{208}Pb/^{207}Pb$, 17.84 ± 0.32 for $^{206}Pb/^{204}Pb$, 15.24 ± 0.31 for $^{207}Pb/^{204}Pb$, and 37.31 ± 0.52 for $^{208}Pb/^{204}Pb$ [64]. These ratios agree with those in the literature for several European regions. $^{206}Pb/^{207}Pb$ distinguished the north-western from the north-eastern and southern Italian wines, and the southern samples were distinguishable only from the north-western ones. $^{208}Pb/^{206}Pb$ distinguished the north-western from the southern Italian samples [64].

Wines produced from Slovak vineyard regions were analysed to determine their geographical origin and authentication [65]. A model for the prediction of geographical origin of wines combining specific element and discriminant analysis was proposed on the basis of geochemical characterization of soil from six Slovak vineyard regions, namely Small Carpathian, South Slovakian, Nitra region, Central Slovakian, Eastern Slovakian and Tokaj regions. The classification model was verified on an independent set of grape samples and soils from these vineyard regions. According to the authors, for geographical authentication, elements such as rubidium, lithium, barium, strontium, arsenic, calcium, cobalt, chromium, magnesium, tin and vanadium were selected due to their variability in the soil. Differentiation of vineyard regions was performed using PCA analysis. The selected elements were analysed using AAS and ICP-MS [65].

Wines from three different south Brazilian winemaking regions were analysed in order to differentiate them by geographical origin of the grapes [66]. Brazil's wide territory and climate diversity allow grape cultivation and winemaking in many regions with different and unique characteristics. Wine grape cultivation for winemaking is concentrated in the South Region (Rio Grande do Sul), which is responsible for 90% of domestic wine production. However, in recent years, two new production regions have developed: the Campanha, the plains to the south, and the Serra do Sudeste, the hills to the south-east of the state. Analysis of IRs of $^{18}O/^{16}O$ of wine water, $^{13}C/^{12}C$ of ethanol, and of minerals were used to characterize wines from different regions. The isotope analysis of $\delta^{18}O$ of wine water and minerals Mg and Rb were the most efficient for differentiating the regions. By using isotope and mineral analysis, and discrimination analysis, it was possible to classify the wines from south Brazil [66].

A robust protocol was proposed for determination of the authenticity of white wines from four German winegrowing regions (Baden, Rheingau, Rheinhessen and Pfalz) based on their content of major, trace and an ultratrace element [71]. In this study, 127 white wine samples possessing a certificate of origin, all of the 2000 vintage, were analysed. The concentrations of 13 elements (Li, B, Mg, Ca, V, Mn, Co, Fe, Zn, Rb, Sr, Cs and Pb) were determined in wine diluted 1 : 20 by sector field (SF/ICP-MS) using indium as internal standard. Supervised pattern recognition techniques such as discriminant analysis (DA) and classification trees were applied for interpretation of the data. Quadratic discriminant analysis (QDA) allowed the four regions to be discriminated with 83% accuracy when using only eight variables (Li, B, Mg, Fe, Zn, Sr, Cs and Pb), and predictive ability for classifying new samples was 76%. By use of a second method, a decision tree, the classification of samples coming from the four regions could be performed with an accuracy of 84% when only four elements were used: Li (very low in samples from Baden), Zn (abnormally low in the samples from the Rheingau), and Mg and Sr (both important for the differentiation between Pfalz and Rheinhessen samples). For this method, predictive ability was only 74% in the identification of unknown samples. The robustness of the QDA model was not good enough, and therefore the tree is better recommended for the classification of new wine samples from these areas of German wine production [71].

Wines collected from four countries (Hungary, Czech Republic, Romania and South Africa) and three different vintages were analysed for 63 chemical parameters in the context of the European WINE-DB project [17]. The aim of the project was to apply MVA classification techniques to determine the country of origin of wines based on their chemical content . Moreover, it was important to evaluate whether models built for authentic samples can be used to discriminate commercial samples. The results indicated that South African wines are

easily discriminated from the other countries on the basis of a single IR such as Ethanol$(D/H)_1$. Discrimination of the European countries, and especially the separation between Hungarian and Czech wines, is less straightforward [17].

Several types of compound were determined as suitable for characterizing wines made from botrytized grapes and for determining their origin and authenticity [72]. Amines, acids, macroelements and microelements of botrytized sweet wine specialities, coming from Hungary and different countries, were analysed. Measured values of 21 Tokaji aszu wines and 23 foreign botrytized wines were compared by multivariate statistical methods. Characterizing the effect of *Botrytis cinerea* and the winemaking technology, amines were the most suitable components for determination of authenticity and origin of wines from the three types of compounds studied. However, for acids and elemental composition, differentiation of wine samples by PCA was not complete but a trend can be observed for separation according to origin. Knowledge of composition of acids and elements can support the results of amine analysis in determining the origin of wines [72].

The content of five metals (Cu, Mn, Fe, Cd and Pb) in several red and white wines originating from different regions of Serbia were determined by name and GFAAS [73]. The data were processed using chemometric techniques such as PCA and factor analysis. Application of cluster analysis (CA) to the studied wines indicated differentiation of the samples according to origin. No discrimination between red and white wines was found by the authors [73].

Fifty wines from the denomination of origin (DO) of Condado de Huelva were analysed for mineral content by measuring 12 elements (Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, Sr and Zn) using ICP-OES [74]. Samples were previously digested by heating with H_2O_2/HNO_3 mixture. The results obtained showed that metal datasets were non-normally distributed and accordingly non-parametric statistics were applied. The average levels (medians) of these elements found in the samples are as follows (in mg/L): 2.54 (Al); 0.06 (Ba); 82.58 (Ca); 0.21 (Cu); 3.53 (Fe); 865.34 (K); 68.87 (Mg); 0.71 (Mn); 32.77 (Na); 71.61 (P): 0.48 (Sr); and 0.56 (Zn). The interrelationship of metals was studied via the Spearman non-parametric sample correlation, Fe/Al, P/Mg and Zn/Ba being the most important correlations established. As a result of this study, the authors suggest that the contribution to the safety intake limits (per week) of the studied elements by wine consumption is not significant. Actually, they range between 0.1% in Fe and 11.9% in Mg, for normal drinkers [74].

Spanish white wines from four production areas protected by appellation control laws have been analysed by ICP-OES to determine the contents of aluminium, barium, boron, calcium, chromium, copper, iron, magnesium, manganese, nickel, phosphorus, potassium, silicon, sodium, strontium, sulfur and zinc [75]. These elements were used as chemical descriptors in order to differentiate wines from different brands certified of origin. The Kruskal–Wallis test was applied to highlight significant differences between the four considered classes and pattern recognition methods were applied to construct classification models. In this way, PCA was used to visualize data trends and backward stepwise LDA was applied in order to reduce the number of input variables. The concentrations of chromium, manganese, silicon, sodium and strontium were used to construct a support vector machine classification model, obtaining a 100% classification performance [75].

A technique to determine the geographic origin of wine was examined using 99 samples from Japan and four other countries [76]. The contents of 21 elements (Al, B, Ba, Ca, Cd, Cr, Cs, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sr, V and Zn) were determined by ICP-OES. LDA models were developed to identify wine from Japan and other countries. With these parameters, 99% correct classification was achieved. The LDA models calibrated with the analytical results of five elements (B, Ca, Cr, K and Mg) chosen by a backward stepwise procedure correctly identified the origin of wines as Japan with 91% certainty. These results suggested that discriminating by inorganic element composition may be useful for identifying Japanese wine for import clearance [76].

The Casablanca valley of Chile is one of the youngest wine-producing areas of the country [77]. This valley is of interest to national and international wine producers and consumers because of its unique climate. However, no systematic studies have been performed to evaluate differences in the climate inside the valley. In this work, the chemometric zoning of the Casablanca valley was assessed for the first time using samples of Pinot Noir produced in two sub-zones. The wines were chemically characterized by UV/Vis spectroscopy, anthocyanin profiles and mineral element profiles. Data were analysed

using PCA, LDA, SIMCA and PLS-DA. The best results were obtained by combining all chemical data (R^2 >98%). The authors have reported that no differences were detected among the applied statistical procedures. Finally, the results of this preliminary survey yield promising scientific evidence of two existing sub-zones within the Casablanca valley, the Zona Alta and the Zona Baja, in agreement with the previous opinion of oenologists [77].

Levels of arsenic and other trace elements (Al, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Sb, Se, Sn, Sr, Ti, Tl, U, V and Zn) in nine brands of wine from Slavonia (eastern Croatia) were investigated. Wines from two other viticulture areas, Istria (western Croatia) and Vojvodina (northern Serbia), were used for comparison [78]. Element concentrations were determined by high-resolution ICP-MS and multivariate statistical methods were applied for the interpretation of obtained data. Regional differences were identified based on a set of geogenic elements (Al, Li, Be, Ti). Elements As and Pb were found to be of predominantly natural origin. Significant differences between the obtained values for As in wines from Slavonia and Vojvodina, compared with wines from Istria, indicate that the geochemical As anomalies present in these regions contribute to some extent to higher As content in analysed wines. Cd, Ni, Zn, Fe and Cr in wine were found to be primarily of anthropogenic origin [78].

An ICP-MS methodology was validated and used for accurately determining the concentration of 17 elements (Al, As, B, Ba, Ca, Co, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Sr, Zn) in Portuguese wines [79]. This study determined the relationships between element concentration and two variables: wine type (white vs. red) and appellation of origin (Alentejo, Bairrada, Dao, or Vinho Verde). Boron made it possible to distinguish among red wines from different producing regions. Factor analysis produced four components (C) with different elements main loading: C1 (the lithophile elements Ba, Mg and Mn): C2 (B, K and P, elements related to fertilization practices and/or winemaking procedures); C3 (Zn, a metal mainly derived from the use of phytosanitary products and/or stainless steel or brass materials); and C4 (As, mainly derived from phytosanitary products). In total, these components explained 78.1% of the variance between wine type and appellation. Red wines showed higher scores of C2 than white samples. With respect to the appellation, Bairrada had the highest scores for C2 while Alentejo wines, followed by Dao, showed highest loadings for C1. DA also achieved a high recognition percentage (98.2%) for the classification of wine samples according to appellation of origin. These results highlight the usefulness of ICP-MS element analysis for categorization of wine origin and show that this is a promising technique regarding the authentication of wine certified brand of origin (CBO) [79].

The element composition of red wines from two important wine-producing Spanish regions (Ribera del Guadiana and Mentrida) was used in a multivariate statistical analysis to discriminate the wines according to their geographical origin [80]. A total of 19 elements were analysed by ICP-AES. By using the element concentrations as chemical descriptors, both classes of certified red wine were perfectly discriminated, when applying pattern recognition techniques such as PCA and LDA. Specifically, LDA using As, Cd, Cu, Li, Pb and Sr led to 97% and 88% recognition ability, respectively, for Ribera del Guadiana and Mentrida red wines [80].

Italic Riesling is one of the most important white wine grape cultivars (*Vitis vinifera*) grown in the Serra Gaucha viticultural region (Rio Grande do Sul, Brazil) [81]. It has acquired economic importance in this region due to the production of varietal white wine and for its use in the production of sparkling wine. Because of its importance to the wine industry, the physicochemical composition of this wine was evaluated to develop a databank and to establish its varietal identity and regional characterization. Italic Riesling wines (65 samples) from the 2002–2004 vintages, made by 28 wineries, were analysed. Classical variables were performed by physicochemical methods, volatile compounds by gas chromatography, and mineral elements by AAS [82]. Results showed that varietal Italic Riesling wines had parameters according to Brazilian legislation [81].

Low values of dry extract, reduced dry extract, proline, pH, volatile acidity and ethyl acetate; medium of alcohol, sum of higher alcohols, and Mg; and high K, Ca and P were detected in the elemental composition of 2002 Cabernet Sauvignon wines from four different regions of Rio Grande do Sul (Brazil) determined using particle-induced X-ray emission (PIXE) [83]. In total, wines from 13 different vineyards were evaluated. The authors gave particular attention to wines from Vale dos Vinhedos, one of the most important wine-producing regions in Brazil. Typical PIXE spectra consisted of elements with atomic number between 11 and 38 such as P, S, K, Ti, Mn, Fe, Cu, Zn and Rb. Physicochemical

variables such as volatile acidity, alcohol, pH and dry extract were also determined for some wines. Variations in element concentrations among wines from Vale dos Vinhedos and from different regions were observed. With respect to European wines, results were characterized by relatively low concentrations of Cu and Zn and high concentration of Rb. Traceability of wines requires knowledge of their characteristics, which are associated with the geographical origin of grape, soil, water, climate as well as the winery techniques [83].

Wines and soil from three production areas of Argentina were classified according to multi-element data [84]. The influence of the provenance soil on wine element composition was also investigated. Eleven elements were determined in 31 wine samples and 137 soil samples from regions under study. Stepwise DA allowed correct classification of 100% of the wines analysed. Wines were classified according to regions using only seven parameters (K, Fe, Ca, Cr, Mg, Zn and Mn) and 92% correct classification for soils using seven variables (Ca, Cr, K, Fe, Cu, Zn and, Mg). Canonical analysis between soils and wines datasets showed that 85% of variability observed among wines could be attributed to the soil in which the vines were cultivated. The authors concluded that the analysis of element concentrations in the wines and soils, in combination with chemometric methods, provides a powerful tool for verifying the geographical origin of wines [84].

20.4.2 Inorganic and organic wine

In recent years element or mineral analysis or profiles have been used to characterize organic and inorganic wines or grape production [85–88]. Organic viticulture is currently growing in many countries. In EU countries, protection laws are firmly applied to guarantee the provenance of the grapes and wines. Converting from conventional viticulture to organic viticulture is a dynamic process of creating a sustainable and self-regulating system. Measurement of minerals has an important role in organic practice: rational fertilization of plantations; avoidance of nitrogen excess, which causes raising vine diseases and pest attack; avoidance of deficiencies or excess of potassium; timely tillage; elimination of many sources of infection; and determination of forecasts and warnings of economic pest thresholds [85–88]. The benefits of alternatives for biological control of pests and diseases include avoiding the use of polluting products, using low-phytotoxicity products, obtaining crops without chemical treatments, preventing resistance to pesticides, and ensuring human and other creatures health by using cleaner control methods [85–88].

Sugar content is an element quality for wine grapes and its accumulation is a feature typical of the variety or the variety cultivated and is determined by climatic and technological factors. Blidariu and Sala [88] evaluated the comparative influence of two systems of fertilization, organic and mineral, on the sugar content of the grape variety Italian Riesling. Organic fertilizer was provided by manure in doses of 30, 40 and 50 t/ha. Mineral fertilizer was the NPK(S) type, fertilizer with zinc [15:15:15(+3+Zn)] applied in doses of NPK 50, NPK 100 and NPK 150 kg active substance (a.s.) per hectare. Sugar content accumulated under the influence of the two types of fertilization ranged from 221.5 ± 4.27 g/L in the control variant to 262.14 ± 5.38 g/L in the variant with NPK 100 kg a.s./ha, organic fertilization providing a content of 255.30 g/L when applying 40 t/ha [88].

20.4.3 Quality, composition and storage

The monitoring of wine ageing is of great commercial and scientific interest, as wine quality and value are closely related to the organoleptic characteristics developed during the ageing process [89]. In this study, Chinese rice wines from three well-known wineries (Guyuelongshan, Kuaijishan and Pagoda) were analysed for 21 chemical parameters, including six conventional parameters, five sugars, lactic acid and nine macroelements. Then the experimental data were subjected to multivariate statistical analysis to predict and classify samples of different ageing status (3, 9, 15, 21 and 33 months). Systematic differences between samples were revealed by a two-way ANOVA and PCA. Discrimination model built by forward stepwise LDA based on the 16 selected parameters achieved 88.5% accuracy in leave-one-out (LOO) cross-validation. The most five discriminant variables were Zn, Mn, alcohol, Cu and Al, respectively. When the discrimination was performed on the samples from each winery, the classification accuracy in LOO cross-validation was 97.7%, 91.1% and 78.0%, respectively. The results demonstrated that these chemical parameters have the potential to enable the authentication of ageing status of rice wine [89].

Three sour cherry and three table grape cultivars were analysed using ICP-OES [90]. The elements Na, K, Ca, Mg, Fe, Cu, Zn, Mn, Cr, Cd, Co, Pb and Ni were

detected in all samples; four elements were very abundant (K, Na, Ca and Mg) and four not abundant (Cu, Fe, Mn and Zn). Five of them are trace elements (Cr, Cd, Co, Pb and Ni) at levels of less than 0.1 mg/kg. Among the 13 elements analysed, potassium was the most abundant element distributed throughout all categories of fruits. Iron was the predominant minor element constituent. Manganese concentration was the highest in table grape cultivars. The accuracy of the results was evaluated by spike recovery tests. Analysis of variance was used by the authors to establish the metals with significant difference in mean content between the cultivars from sour cherries and between table grapes. PCA was used to evaluate the distribution of metals, in particular the determination of the 14 naturally occurring elements of the lanthanide series in red wine [90].

Ultrasound (US) was used for sample preparation prior to lanthanide determination using ICP-MS [91]. Flow injection and pneumatic nebulization/aerosol desolvation were used for nebulization of aliquots of 50 μL of sample and its subsequent transportation to plasma. Sample preparation procedures, matrix interference and time of sonication were evaluated. Better results for lanthanides in red wine were obtained by sonication with US probe for 90 s and sample 10-fold diluted. The limits of detection of La, Ce, Nd, Sm, Gd, Pr, Eu, Tb, Dy, Ho, Er, Tm, Lu and Yb were 6.57, 10.8, 9.97, 9.38, 2.71, 1.29, 1.22, 0.52, 2.35, 0.96, 2.30, 0.45. 0.24 and 1.35 ng/L, respectively. Red wines of different varieties from three countries in South America were discriminated according to the country of origin by means of MVA of lanthanide concentration [91].

20.4.4 Fermentation

The composition of elements (in particular metals) in wine during fermentation is not stable. During fermentation, the concentration of some metals is lower than those in the grape and must be due to the precipitation with tartrate (e.g. K and Ca) or other insoluble precipitations (e.g. Al, Cu, Fe, Mn). These changes can be accentuated by the changes in pH due to alcohol production [19].

The variation in mineral profiles in the vinification process was reported by Cheng and Liang [92]. These authors determined the concentrations of 10 mineral elements (Al, Cu, Fe, Zn, Mn, Ca, K, Mg, Na and Sr) in grape juice, must and wine, analysed by ICP-OES. Cabernet Sauvignon grapes from two districts, Yinchuan and Changli, were used in two vinification batches. The results indicated that there were significant differences ($P < 0.01$) of the contents of each element among individual vinification steps for two batches. The content of Fe in the batch using grapes from Yinchuan was 6.66 mg/L, which was close to the maximum level of 8 mg/L in Chinese GB 15037-2006. Some measures should be taken in practice to control contamination. In addition, strong correlations ($P < 0.01$) of the mineral profiles between the wines and origin grapes were observed in both batches. The profile of the 10 mineral elements in a wine can be tentatively used as a fingerprint to distinguish one wine origin from another. The results will guide the winery to control and enhance the quality of the wine. In addition, a mineral profile of a wine was potentially taken as the fingerprint of the wine origin [92].

20.5 Concluding remarks

This chapter has demonstrated that the analysis of elements or minerals (macro and micro) have been used extensively by the grape and wine industry. Applications such as determination of legal limits for export purposes, monitoring of trace contaminants and the content of certain metal salts (e.g. calcium and potassium), authentication, traceability and an indication of potential changes in colour and oxidation are the most reported uses by the wine industry worldwide. Among them, the determination of wine authenticity or regionality by combining element profiles or isotopic analysis with different MVA methods is the most attractive application in the modern wine industry. In recent years, the incorporation of new techniques (e.g. X-ray) has opened new possibilities for the analysis of elements in grapes and wine.

Abbreviations

AAS: Atomic absorption spectrophotometry
ANOVA: Analysis of variance
CA: Cluster analysis
CBO: Certified brand of origin
DA: Discriminant analysis
DO: Denomination of origin
FAS: Flame atomic spectroscopy

FIA: Flow injection analysis
FI: Flow injection
GFAAS: Graphite furnace atomic absorption spectrometry
IC: Ion chromatography
ICP-AES: Inductively coupled plasma atomic emission spectrometry
ICP-MS: Inductively coupled plasma mass spectrometry
ICP-OES: Inductively coupled plasma optical emission spectrometry
IR: Isotope ratios
LDA: Linear discriminant analysis
LOO: Leave-one-out cross-validation
MVA: Multivariate analysis
NIR: Near infrared
OIV: Office International de la Vigne et du Vin
PCA: Principal components analysis
PIXE: Particle-induced X-ray emission
PLS-DA: Partial least squares discriminant analysis
QDA: Quadratic discriminant analysis
SIMCA: Soft independent of class analogy
US: Ultrasound

References

1 Suhaj M, Korenovska M. Application of elemental analysis for identification of wine origin. A review. *Acta Aliment* 2005;**34**:393–401.
2 Arvantoyannis I, Katsota MN, Psarra P, Soufleros E, Kallinthraka S. Application of quality control methods for assessing wine authenticity: use of multivariate analysis (chemometrics). *Trends Food Sci Technol* 1999;**10**:321–336.
3 Van Leeuwen C, Seguin G. The concept of terroir in viticulture. *J Wine Res* 2006;**17**:1–10.
4 Kelly S, Heaton K, Hoogewerff J. Tracing the geographical origin of food: the application of multi-element and multiple isotope analysis. *Trends Food Sci Technol* 2005;**16**: 555–567.
5 Buglass AJ (ed.) *Handbook of Alcoholic Beverages: Technical, Analytical and Nutritional Aspects*, Vol. **2**. John Wiley & Sons Ltd, Chichester, 2011.
6 Grembecka M, Kalis A, Szefer P. What do metals tell us about wine? In: *3rd International IUPAC Symposium on Trace Elements in Food*, Rome, 2009, p. 130.
7 Serapinas P, Venskutonis PR, Aninkevicius V, Ezerinskis Z, Galdikas A, Juzikiene V. Step by step approach to multi-element data analysis in testing the provenance of wines. *Food Chem* 2008;**107**:1652–1660.
8 de Villiers A, Alberts P, Tredoux AGJ, Nieuwoudt HH. Analytical techniques for wine analysis: an African perspective. *A review. Anal Chim Acta* 2012;**730**:2– 23.
9 Fiket Ž, Mikac N, Kniewald G. Arsenic and other trace elements in wines of eastern Croatia. *Food Chem* 2011;**126**:941–947.
10 Lachenmeier DW, Przybylski MC, Rehm J. Comparative risk assessment of carcinogens in alcoholic beverages using the margin of exposure approach. *Int J Cancer* 2012;**131**: E995–E1003.
11 Frias S, Conde JE, Rodriguez-Bencomo JJ, Garcia-Montelongo F, Perz-Trijillo JP. Classification of commercial wines from Canary Islands (Spain) by chemometric techniques using metallic contents. *Talanta* 2003;**59**:335–344.
12 Taylor VF, Longerich HP, Greenough JD. Multi-element analysis of Canadian wines by inductively coupled plasma mass spectrometry (ICP-MS) and multivariate statistics. *J Agric Food Chem* 2003;**51**:856–860.
13 Arvantoyannis I. Wine authenticity, traceability and safety monitoring. In: Reynolds AG (ed.) *Managing Wine Quality. Volume 1. Viticulture and Wine Quality*. Woodhead Publishing Ltd, Cambridge, 2010, pp. 218–270.
14 Kment P, Mihaljevic M, Ettler V, Sebek O, Strnad L, Rohlova L. Differentiation of Czech wines using multi-element composition: a comparison with vineyard soil. *Food Chem* 2005;**91**:157–165.
15 Saurina J. Characterization of wines using compositional profiles and chemometrics. *Trends Anal Chem* 2010;**29**:234–245.
16 Suhaj M, Korenovska M. Application of elemental analysis for identification of wine origin: a review. *Acta Aliment* 2005;**34**:393–401.
17 Smeyers-Verbeke J, Jaeger H, Lanteri S *et al*. Characterization and determination of the geographical origin of wines. Part II: descriptive and inductive univariate statistics. *Eur Food Res Technol* 2009;**230**:15–29.
18 Thiel G, Geisler G, Blechschmidt I, Danzer K. Determination of trace elements in wines and classification according to their provenance. *Anal Bioanal Chem* 2004;**378**:1630–1636.
19 Pohl P. What do metals tell us about wine? *Trends Anal Chem* 2007;**26**:941–949.
20 Alvarez M, Moreno IM, Pichardo S, Cameán AM, Gustavo González A. Mineral profile of 'fino' wines using inductively coupled plasma optical emission spectrometry methods. *Food Chem* 2012;**135**:309–313.
21 Lara R, Cerutti S, Salonia JA, Olsina RA, Martinez LD. Trace element determination of Argentine wines using ETAAS and USN-ICP-OES. *Food Chem Toxicol* 2005;**43**:293–297.
22 Galani-Nikolakaki S, Kallithrakas-Kontos N, Katsanos AA. Trace element analysis of Cretan wines and wine products. *Sci Total Environ* 2002;**285**:155–163.
23 Janja K, Marjan V, Metka S. The contents of Cu, Mn, Zn, Cd, Cr and Pb at different stages of the winemaking process. *Acta Chim Slov* 2003;**50**:123–136.
24 Pyrzynska K. Analytical methods for the determination of trace metals in wine. *Crit Rev Anal Chem* 2004;**34**:69–83.
25 Ajtony Z, Szoboszlai N, Susko EK, Mezei P, Gyoergy K, Bencs L. Direct sample introduction of wines in graphite furnace atomic absorption spectrometry for the simultaneous

determination of arsenic, cadmium, copper and lead content. *Talanta* 2008;**76**:627–634.
26 Bertoldi D, Villegas TR, Larcher R, Santato A, Nicolini G. Arsenic present in the soil–vine–wine chain in vineyards situated in an old mining area in Trentino, Italy. *Environ Toxicol Chem* 2013;**32**:773–779.
27 McKinnon AJ, Scollary GR. Size fractionation of metals in wine using ultrafiltration. *Talanta* 1994;**44**:1649–1658.
28 Tahiri M, Pellerin P, Tressol JC *et al*. The rhamnogalacturonan-II dimer decreases intestinal absorption and tissue accumulation of lead in rats. *J Nutr* 2000 ;**130**:249–253.
29 Teissedre PL, Lobinski R, Cabanis MT, Szpunar-Lobinska J, Cabanis JC, Adams FC. On the origin of organo-lead compounds in wine. *Sci Total Environ* 1994;**153**:247–252.
30 La Pera L, Dugo G, Rando R, Di Bella G, Maisano R, Salvo F. Statistical study of the influence of fungicide treatments (mancozeb, zoxamide and copper oxychloride) on heavy metal concentrations in Sicilian red wine. *Food Addit Contam* 2008;**25**:302–313.
31 La Pera L, Saitta M, Di Bella G, Dugo G. Simultaneous determination of Cd(II), Cu(II), Pb(II), and Zn(II) in citrus essential oils by derivative potentiometric stripping analysis. *J Agric Food Chem* 2003;**51**:1125–1129.
32 Bornet A, Teissedre PL. Chitosan, chitin–glucan and chitin effects on 20 minerals (iron, lead, cadmium) and organic (ochratoxin A) contaminants in wines. *Eur Food Res Technol* 2008;**226**:681–689.
33 Tariba B. Metals in wine: impact on wine quality and health outcomes. *Biol Trace Elem Res* 2011;**144**:143–156.
34 Santos S, Lapa N, Alves A, Morais J, Mendes B. Analytical methods and validation for determining trace elements in red wines. *J Environ Sci Health* 2013;**48**:364–375.
35 Grindlay G, Mora J, Luis G, Loos-Vollebregt M. Atomic spectrometry methods for wine analysis: a critical evaluation and discussion of recent applications. *Anal Chim Acta* 2011;**691**:18–32.
36 Stafilov T, Karadjova I. Atomic absorption spectrometry in wine analysis: a review. *Maced J Chem Chem Eng* 2009;**28**:17–31.
37 Office International de la Vigne et du Vin (OIV). *Compendium of International Methods of Wine and Must Analysis*. OIV, Paris, 2002.
38 Onianwa PC, Adeyemo AO, Idowu OE, Ogabiel EE. Copper and zinc contents of Nigerian foods and estimates of the adult dietary intakes. *Food Chem* 2001;**72**:89–95.
39 Dessuy B, Vale R, Souza S, Ferreira C, Welz B, Katskov A. Method development for the determination of lead in wine using electrothermal atomic absorption spectrometry comparing platform and filter furnace atomizers and different chemical modifiers. *Talanta* 2007;**74**:1321–1329.
40 Cheng H, Liu J, Xu Z, Yin X. A micro-fluidic sub-microliter sample introduction system for direct analysis of Chinese rice wine by inductively coupled plasma mass spectrometry using external aqueous calibration. *Spectrochim Acta A* 2012;**73**:55–61.
41 Cozzolino D, Shah N, Cynkar W, Smith P. Technical solutions for analysis of grape juice, must and wine: the role of infrared spectroscopy and chemometrics. *Anal Bioanal Chem* 2011;**401**:1475–1484.
42 Cozzolino D, Cynkar WU, Dambergs RG, Gishen M. Analysis of grape and wine by near infrared spectroscopy: a review. *J Near Infrared Spectrosc* 2006;**14**:279–289.
43 Cozzolino D. Recent trends on the use of infrared spectroscopy to trace and authenticate natural and agricultural food products. *Appl Spectrosc Rev* 2012;**47**:518–530.
44 Cozzolino D, Kwiatkowski MJ, Dambergs RG *et al*. Analysis of elements in wine using near infrared spectroscopy and partial least squares regression. *Talanta* 2008;**74**:711–716.
45 Sauvage L, Frank D, Stearne J, Millikan MB. Trace metal studies of selected white wines: an alternative approach. *Anal Chim Acta* 2002;**458**:223–230.
46 Kwan W-O, Kowalski BR, Skogerboe RK. Pattern recognition analysis of elemental data. Wines of *Vitis vinifera* cv. Pinot Noir from France and the United States. *J Agric Food Chem* 1979;**27**:1321–1326.
47 Coetzee PP, Steffens FE, Eiselen RJ, Augustyn OP, Balcaen L, Vanhaecke F. Multi-element analysis of South African wines by ICP-MS and their classification according to geographical origin. *J Agric Food Chem* 2005;**53**:5060–5066.
48 Geana I, Iordache A, Ionete R, Marinescu A, Ranca A, Culea M. Geographical origin identification of Romanian wines by ICP-MS elemental analysis. *Food Chem* 2013;**138**:1125–1134.
49 Šperková J, Suchánek M. Multivariate classification of wines from different Bochemian regions (Czech Republic). *Food Chem* 2005;**93**:659–663.
50 Cozzolino D, Shah N, Cynkar W, Smith P. A practical overview of multivariate data analysis applied to spectroscopy. *Food Res Int* 2011;**44**:1888–1896.
51 Villagra E, Santos LS, Gontilo Vaz B, Eberlin MN, Laurie VF. Varietal discrimination of Chilean wines by direct injection mass spectrometry analysis combined with multivariate statistics. *Food Chem* 2012;**131**:692–697.
52 Perez-Trujillo J-P, Barbaste M, Medina B. Chemometric study of bottled wines with denomination of origin from the Canary Islands (Spain) based on ultra-trace elemental content determined by ICP-MS. *Anal Lett* 2003;**36**: 679–697.
53 Gomez MMC, Feldman I, Jakubowski N, Andersson JT. Classification of German white wines with certified brand of origin by multi-element quantitation and pattern recognition techniques. *J Agric Food Chem* 2004;**52**:2962–2974.
54 Greenough JD, Mallory-Greenough LM, Fryer BJ. Geology and wine 9: regional trace element fingerprinting of Canadian wines. *Geosci Can* 2005;**32**:129–137.
55 Capron X, Smeyers-Verbeke J, Massart DL. Multivariate determination of the geographical origin of wines from four different countries. *Food Chem* 2007;**101**:1585–1597.
56 Marengo E, Aceto M. Statistical investigation of the differences in the distribution of metals in Nebbiolo-based wines. *Food Chem* 2003;**81**:621–630.

57 Gonzalvez A, Llorens A, Cervera ML, Armenta S, de la Guardia M. Elemental fingerprint of wines from the protected designation of origin Valencia. *Food Chem* 2009;**112**:26–34.

58 Razic S, Onjia A. Trace element analysis and pattern recognition techniques in classification of wine from central Balkan countries. *Am J Enol Viticult* 2010;**61**:506–511.

59 Rossano EC, Szilagyi Z, Malorni A, Pocsfalvi G. Influence of winemaking practices on the concentration of rare earth elements in white wines studied by inductively coupled plasma mass spectrometry. *J Agric Food Chem* 2007;**55**: 311–317.

60 Bentlin FRS, Pulgati FH, Dressler VL, Pozebon D. Elemental analysis of wines from South America and their classification according to country. *J Brazil Chem Soc* 2011;**22**:327–336.

61 Thiel G, Geisler G, Blechschmidt I, Danzer K. Determination of trace elements in wines and classification according to their provenance. *Anal Bioanal Chem* 2004;**378**:1630–1636.

62 Trujillo JPP, Conde JE, Pont MLP, Camara J, Marques JC. Content in metallic ions of wines from the Madeira and Azores archipelagos. *Food Chem* 2011;**124**:533–537.

63 Van Der Linde G, Fischer JL, Coetzee PP. Multi-element analysis of South African wines and their provenance soils by ICP-MS and their classification according to geographical origin using multivariate statistics. *S Afr J Enol Viticult* 2010;**31**:143–153.

64 Larcher R, Nicolini G, Pangrazzi P. Isotope ratios of lead in Italian wines by inductively coupled plasma mass spectrometry. *J Agric Food Chem* 2003;**51**:5956–5961.

65 Schlesier K, Fauhl-Hassek C, Forina M *et al*. Characterization and determination of the geographical origin of wines. Part I: Overview. *Eur Food Res Technol* 2009;**230**:1–13.

66 Dutra SV, Adami L, Marcon AR *et al*. Determination of the geographical origin of Brazilian wines by isotope and mineral analysis. *Anal Bioanal Chem* 2011;**401**:1571–1576.

67 Medina B. Food authentication. In: Ashurst PR, Dennis HJ (eds) *Wine Authenticity*. Blackie Academic & Professional, London, 1996, pp. 60–106.

68 Giaccio M, Vicentini A. Determination of the geographical origin of wines by means of the mineral content and the stable isotope ratios: a review. *J Commodity Sci Technol Quality* 2008;**47**:267–284.

69 Christoph N, Rossmann A, Schlicht C, Voerkelius S. Wine authentication using stable isotope ratio analysis: significance of geographic origin, climate, and viticultural parameters. In: Ebeler SE, Takeoka GR, Winterhalter P (eds) *Authentication of Food and Wine*. Oxford University Press, New York, 2007, pp. 166–179.

70 Guyon F, Gaillard L, Salagoity M-H, Medina B. Intrinsic ratios of glucose, fructose, glycerol and ethanol C-13/C-12 isotopic ratio determined by HPLC-co-IRMS: toward determining constants for wine authentication. *Anal Bioanal Chem* 2011;**401**:1551–1558.

71 Gomez MDM, Feldmann I, Jakubowski N, Andersson JT. Classification of German white wines with certified brand of origin by multielement quantitation and pattern recognition techniques. *J Agric Food Chem* 2005;**52**:2962–2974.

72 Sass-Kiss A, Kiss J, Havadi B, Adanyi N. Multivariate statistical analysis of botrytised wines of different origin. *Food Chem* 2008;**110**:742–750.

73 Razic S, Cokesa D, Sremac S. Multivariate data visualization methods based on elemental analysis of wines by atomic absorption spectrometry. *J Serb Chem Soc* 2007;**72**: 1487–1492

74 Alvarez M, Moreno IM, Pichardo S, Camean AM, Gustavo Gonzalez A. Mineral profile of 'fino' wines using inductively coupled plasma optical emission spectrometry methods. *Food Chem* 2012;**135**:309–313.

75 Marcos Jurado J, Alcazar A, Palacios-Morillo A, de Pablos F. Classification of Spanish DO white wines according to their elemental profile by means of support vector machines. *Food Chem* 2012;**135**:898–903.

76 Horii S, Hashiguchi T, Izu H, Sudo S. Analysis of element composition of japanese and other wine and their classification. *J Jpn Soc Hort Sci* 2011;**80**:506–511.

77 Saavedra J, Fuentealba C, Yanez L *et al*. Chemometric approaches for the zoning of Pinot Noir wines from the Casablanca valley, Chile. *Food Chem* 2011;**127**:1842–1847.

78 Fiket Z, Mikac N, Kniewald G. Arsenic and other trace elements in wines of eastern Croatia. *Food Chem* 2011;**126**: 941–947.

79 Rodrigues SM, Otero M, Alves AA *et al*. Elemental analysis for categorization of wines and authentication of their certified brand of origin. *J Food Compos Anal* 2011;**24**:548–562.

80 Garcia-Rodriguez G, Hernandez-Moreno D, Soler F, Perez-Lopez M. Characterization of 'Ribera del Guadiana' and 'Mentrida' Spanish red wines by chemometric techniques based on their mineral contents. *J Food Nutr Res* 2011;**50**:41–49.

81 Rizzon LA, Miele A, Scopel G. Analytical characteristics of Italic Riesling wines from the Serra Gaucha region. *Rev Brasil Agrocien* 2011;**17**:151–156.

82 Boschetti W, Rampazzo RT, Dessuy MB *et al*. Detection of the origin of Brazilian wines based on the determination of only four elements using high-resolution continuum source flame AAS. *Talanta* 2013;**111**:147–155.

83 Iochims dos Santos CE, Manfredi da Silva LR, Boufleur LA *et al*. Elemental characterisation of Cabernet Sauvignon wines using particle-induced X-ray emission (PIXE). *Food Chem* 2010;**121**:244–250.

84 Fabani MP, Arrua RC, Vazquez F, Diaz MP, Baroni MV, Wunderlin DA. Evaluation of elemental profile coupled to chemometrics to assess the geographical origin of Argentinean wines. *Food Chem* 2010;**119**:372–379.

85 Sala F, Blidariu C. Macro- and micronutrient content in grapevine cordons under the influence of organic and mineral fertilization. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Horticulture* 2012;**69**: 317–324.

86 Pozo-Bayón MA, Monagas M, Bartolomé B, Moreno-Arribas MV. Wine features related to safety and consumer health: an integrated perspective. *Crit Rev Food Sci Nutr* 2012;**52**:31–54.

87 Bara E, Barbu SF. Converting from conventional to organic viticulture in Minis-Maderat vineyard. *Res J Agric Sci* 2010;**42**:408–411.

88 Blidariu C, Sala F. Influence of organic and mineral fertilization on sugar content in Italian Riesling grape variety. *J Hortic Forest Biotechnol* 2012;**16**:251–254.

89 Shen F, Li F, Liu D, Xu H, Ying Y, Li B. Ageing status characterization of Chinese rice wines using chemical descriptors combined with multivariate data analysis. *Food Control* 2012;**25**:458–463.

90 Mitic SS, Obradovic MV, Mitic MN *et al*. Elemental composition of various sour cherry and table grape cultivars using inductively coupled plasma atomic emission spectrometry method (ICP-OES). *Food Anal Method* 2012;**5**: 279–286.

91 Bentlin FRS, dos Santos CMM, Flores EMM, Pozebon DRI, Flores E. Lanthanides determination in red wine using ultrasound assisted extraction, flow injection, aerosol desolvation and ICP-MS. *Anal Chim Acta* 2012;**710**:33–39.

92 Cheng J, Liang Ch. The variation of mineral profiles from grape juice to monovarietal Cabernet Sauvignon wine in the vinification process. *J Food Process Preserv* 2012;**36**:262–266.

93 Diaz C, Conde JE, Estevez D, Perez-Olivero SJ, Perez-Trujillo JP. Application of multivariate analysis and artificial neural networks for the differentiation of red wines from the Camry Islands according to the Island of origin. *J Agric Food Chem* 2003;**51**:4303–4307.

94 Leske PA, Sas A, Coulter A, Sockley CS, Lee TH. The composition of Australian grape juice: chloride, sodium and sulphate ions. *Aus J Grape Wine Res* 1997;**3**:26–30.

CHAPTER 21
Vegetables and fruits

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Abstract: Fruits and vegetables, because of their higher fibre content and beneficial phytochemicals, protect against various chronic diseases such as cardiac disease, type 2 diabetes, obesity and several forms of cancer. These foods are not only the source of various dietary macronutrients and micronutrients but may also represent a source of exposure to several toxic elements. Indeed, environmental geochemistry, sources of irrigation and atmospheric deposition have a direct effect on the element composition of fruits and vegetables as they are the products of primary production. This chapter examines publicly accessible national databases (US FDA, West African, Tanzanian, Danish, Finnish, Japanese, Malaysian and Australian) to determine what elements are generally analysed in fruits and vegetables. These elements include macronutrients such as potassium, magnesium, calcium and phosphorus as well as essential trace elements such as zinc, iron and copper. In addition to this, research articles have been reviewed to examine other datasets that included these and other elements. As many databases do not include toxic elements, this was the primary source of data for the toxic elements arsenic, cadmium, lead and mercury. An assessment of the data tabulated indicates that for macronutrients there is no significant variation among more common fruits and vegetables. For trace nutrients, some fruits and vegetables, whether raw or in processed form, are significant sources of elements such as manganese and chromium. There are also indications that contaminated soils and irrigation lead to enrichment of elements in fruits and vegetables.

Keywords: fruits, vegetables, toxic elements, macronutrients, neutron activation analysis, total reflection X-ray fluorescence, post-harvest management

21.1 Introduction

Consumption of fruits and vegetables is encouraged as part of a healthy diet. In fact the low consumption of fruits and vegetables is considered a contributing factor for ischaemic heart disease and stroke worldwide [1]. Evaluation of trace elements, heavy metals and potentially toxic elements has become increasingly popular for nutritional studies of fruits and vegetables. This trend in the publication of studies has not as yet translated into national food composition tables. Most publicly accessible food databases focus on the elements that are macronutrients for human nutrition. For this reason these databases share many common elements, such as calcium, magnesium, phosphorus and potassium (Table 21.1). The United States Food and Drug Administration (FDA) [2] food composition tables and the elements published in the UK Total Diet Study are notable exceptions. All mineral elements if taken in sufficient quantities and for long enough can be toxic [3]. This is more so for essential trace elements that have a narrow range before essentiality becomes toxicity and for this reason several publications assess the concentrations of elements such as chromium, copper, manganese and zinc, which are also essential.

Vegetables and fruits play a critical role in human mineral nutrition, being the source of many important trace elements. Some elements may show variation depending on where they are grown [4] and so many countries keep their own food composition databases, reflecting not only what is grown and/or eaten in that country but in many cases foods and food combinations unique to that country. However, this is fraught with challenges, particularly in developing countries, and as a result many countries use

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Table 21.1 Elements reported in online databases.

Database	Elements reported
Australia	Ca, Fe, I, K, Mg, Na, P, Zn
Denmark	Ca, Cu, Cr, Fe, I, K, Mg, Mn, Na, Ni, P, Se, Zn
Finland	Ca, Fe, I, K, Mg, Na, P, Se, Zn
Japan	Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn
Malaysia	Ca, Cu, Fe, K, Mg, Na, P, Zn
Tanzania	Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn
USA (FDA)	As, Ca, Cd, Cu, Fe, Hg, I, K, Mg, Mn, Na, Ni, P, Pb, Se, Zn
West Africa	Ca, Cu, Fe, I, K, Mg, Na, P, Se, Zn

data from larger, more mature databases from developed countries such as the USA, which may not be quite suited to their needs and likely would not include native fruits and vegetables or indigenous varieties.

Higher incomes allow a larger share of the household diet to come from non-staples, including fruits and vegetables [5]; however, in some cases policies have made processed foods more available and cheaper than fresh fruits and vegetables. As a result, processed fruits and vegetables, especially canned products, must also be considered. Issues of post-harvest management, perishability and limited seasonal availability also make it necessary to consider the contribution of processed fruits and vegetables to mineral nutrition. It must also be noted that sales of processed and packaged foods are growing rapidly in developing countries [5].

Many food databases do not include data for potentially toxic elements (As, Cd, Hg, Pb) and tend to focus on nutritionally essential or beneficial elements. Data for these potentially toxic elements are more commonly found in peer-reviewed publications. However, the importance of reporting data for these elements should not be discounted. While some fruits and vegetables would not normally be expected to be significant dietary sources of these elements, local farming practices, including pesticide and fertilizer selection, water treatment practices or other factors, may introduce these into the food chain. Additionally, some such as leafy vegetables and root crops may take up these elements in areas with high soil concentrations, and could be significant contributors to dietary exposure depending on local consumption.

21.2 Overview of available data

Among the most well known of food composition databases are those maintained by the US FDA and several European countries. The databases are extensive in the variety of foods and number of elements which are included. They are updated periodically and, in addition to fresh fruits and vegetables, include cooked and processed fruits and vegetables as well as some restaurant and fast food offerings in some instances. There have, however, been increased efforts to produce food databases in other countries, and particularly in developing countries, with the Food and Agriculture Organization (FAO) being central to these efforts with its INFOODS (International Network of Food Data Systems) initiative.

Of course it would be impractical, and indeed impossible, to include every available food in databases. When country-specific databases are being developed, food frequency questionnaires (FFQs) may be used to determine which foods may be included. Nutrition-related disease patterns and retail statistics may also be taken into consideration, as well as dietary habits, environmental factors, fortification, variation between cultivars, and maturity/ripeness, particularly for vegetables and fruits [6].

Processed fruits and vegetables may be dried, frozen or canned. Processing can preserve nutrients, as well as make timely use of fruits and vegetables that may be highly perishable. Additionally, fruits and vegetables may be made into value-added products such as preserves/jams, juices and sauces. While there has been recent backlash against processed foods, processed fruits and vegetables can play an important role in nutrition. From this standpoint processing is an important, and perhaps essential, facet of post-harvest management. Processing can also play an important role in food security for populations where fresh fruits and vegetables are not readily available.

There is no real consensus on the minimum number of a particular food type or item to be analysed, and so this becomes a function of both availability (and in the case of many fresh fruits and vegetables, seasonality) and the needs of the end users of a database. When market basket surveys are done, one may find that $n = 1$, particularly for a processed fruit or vegetable product that has been produced in large batches or lots.

For long-term or widespread study, *n* may number up to the hundreds.

The databases referenced here are from the USA (FDA), West Africa [7], Tanzania [8], Australia [9], Malaysia [10], Denmark [11], Finland [12] and Japan [13]. The databases and the elements included in each are shown in Table 21.1. The 2006 UK Total Diet Study [14] and several peer-reviewed publications are also referenced throughout.

21.3 Foods considered

A selection of foods considered from each database is shown in Table 21.2. These include raw fruits and vegetables, cooked vegetables, and processed fruit and vegetable products. Concentrations of toxic elements in foods from the US FDA database are shown in Table 21.3, and data on Jamaican-grown crops are shown in Table 21.4. The databases also include local foods which are not common across databases; examples of these foods are listed in Table 21.5.

21.4 Methodology

21.4.1 Methods of data collection

While data are perhaps most commonly generated from analysis of actual collected samples, other methods may be used to generate data. In the case of processed fruits and vegetables some data may be obtained from food labels, or by calculation from a recipe (i.e. the sum of constituent ingredients). For some databases the data may indeed have originated from another food composition database, such as in countries which adopt the US FDA or another well-known and established database.

Selection of analytical techniques should be carefully considered for analysis of elements. Some elements are better analysed by a particular technique, and the same is true for different sample types. In some cases the limit of detection/quantification may be the determining factor. Commonly used analytical techniques include spectrophotometric techniques such as atomic absorption spectrophotometry (AAS), flame photometry/atomic emission spectrophotometry, inductively coupled plasma optical emission spectroscopy (ICP-OES) and inductively coupled plasma mass spectroscopy (ICP-MS), and nuclear analytical techniques such as neutron activation analysis (NAA) and X-ray fluorescence (XRF).

21.4.2 Spectrophotometric techniques

Samples for analysis using spectrophotometric techniques generally must be subjected to mineral decomposition and dissolution via acid digestion prior to analysis. This may be done using open vessels, and for large numbers of samples heating block digesters are convenient. These may require special Pyrex tubes or polyethylene/polycarbonate vessels which fit into the heating block. More recently, however, microwave digestion systems have grown in popularity, and indeed are more efficient and result in better recovery for some sample types, particularly those with an appreciable fat content. Samples may be dried prior to digestion, or may be analysed as wet weight, but in many instances drying is preferred so as to preserve the sample in instances where it cannot be analysed immediately on receipt at the laboratory, or to improve limits of detection. In cases where the sample has been dried prior to digestion and analysis, the data reported should specify whether it is dry or wet weight.

While most of the more abundant mineral elements (Ca, Mg, Na, K) may be determined using flame atomic absorption spectrophotometry (FAAS), many of the trace elements are better determined using graphite furnace atomic absorption spectrophotometry (GFAAS) as they would likely fall below FAAS limit of detection (LOD). There are some exceptions, as in the case of zinc for example which is better determined by FAAS, and arsenic, mercury and selenium which require hydride generation prior to analysis. The downside of AAS is that in general it is a single element technique, although some AAS instruments do have multi-element determination capabilities.

ICP-OES and ICP-MS are multi-element techniques. While this may offer some advantages in terms of total analysis time, there are also considerations of detection limits (particularly for ICP-OES) and interferences which may not be an issue if using AAS.

Table 21.2 Essential elements in raw fruits and vegetables, cooked vegetables, and raw fruit and vegetable products.

Food	Ca (mg/kg)								Cr (μg/kg)							
	USA	West Africa	Tanzania	Australia	Malaysia	Denmark	Finland	Japan	USA	West Africa	Tanzania	Australia	Malaysia	Denmark	Finland	Japan
Raw																
Apple	43	NA	60	40–50	50	38.5	30–40	30	NA	NA	NA	NA	NA	7	NA	NA
Apricot	NA	NA	NA	150	NA	142	310	90	NA	NA	NA	NA	NA	NA	NA	NA
Asparagus	NA	NA	NA	110	120	166–228	270	190	NA	NA	NA	NA	NA	NA	NA	NA
Avocado	122	160	120	120–140	210	121	150	90	NA	NA	NA	NA	NA	7	NA	NA
Banana	51	80	50	50	110	44	70	60	NA	NA	NA	NA	NA	1.7	NA	NA
Broccoli	NA	NA	NA	330	400	442	480	380	NA	NA	NA	NA	NA	10	NA	NA
Cabbage	NA	NA	400	350	400	337–509	420	430	NA	NA	NA	NA	NA	17	NA	NA
Carrot	404	350	330	300	1380	360	290	280	NA	NA	NA	NA	NA	8	NA	NA
Cassava	NA	460	160	200	NA	160	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cauliflower	NA	NA	220	180	250	245	240	240	NA	NA	NA	NA	NA	11	NA	NA
Cucumber	114	NA	160	130–180	140	207	260	260	NA	NA	NA	NA	NA	17	NA	NA
Eggplant	NA	NA	90	220	150	84	70	NA	NA	NA	NA	NA	NA	7	NA	NA
Grape	93	NA	70	130	210	135	100	60	NA	NA	NA	NA	NA	1.7	NA	NA
Grapefruit	266	NA	120	240	210	283	330	NA	NA	NA	NA	NA	NA	1	NA	NA
Lettuce	168–338	NA	190	200	500	162–360	NA	190–660	NA	NA	NA	NA	NA	2.0–4	NA	NA
Mango	NA	170	100	70	190	140	100	150	NA	NA	NA	NA	NA	NA	NA	NA
Mushrooms	35	NA	30	30	20	90.5	88	NA	NA	NA	NA	NA	NA	21	NA	NA
Okra	NA	1030	810	NA	770	810	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Onion	215	270	230	250	380	229	330	190	NA	NA	NA	NA	NA	7	NA	NA
Orange	367	320	400	250–290	400	354	389	NA	NA	NA	NA	NA	NA	2	NA	NA
Papaya	NA	200	161	280	110	245	NA	200	NA	NA	NA	NA	NA	NA	NA	NA
Peach	42	NA	60	70	50	64.7	80	40	NA	NA	NA	NA	NA	3	NA	NA
Pear	78	NA	90	60	80	97.3	97	20–50	NA	NA	NA	NA	NA	18	NA	NA
Pineapple	NA	220	70	200	240	189	120	100	NA	NA	NA	NA	NA	110	NA	NA
Potato	NA	100	300	40	200	67.6	56	30	NA	NA	NA	NA	NA	6	NA	NA
Pumpkin	NA	NA	NA	180–410	210	201	250	200	NA	NA	NA	NA	NA	20	NA	NA
Sweet potato	NA	450	200–280	150–270	160	450	300	400	NA	NA	NA	NA	NA	NA	NA	NA
Tomato	77	120	50	90	120	88.7	90	70	NA	NA	NA	NA	NA	15	NA	NA
Turnip	NA	NA	NA	210	400	500	350	240	NA	NA	NA	NA	NA	5	NA	NA
Watermelon	67	70	70	NA	60	43.8	70	NA	NA	NA	NA	NA	NA	0	NA	NA

Cooked																
Broccoli	299	NA	NA	330	NA	400	NA	330	NA	NA	NA	NA	NA	NA	NA	NA
Cabbage	317	NA	200	350	NA	NA	483	400	NA	NA	NA	NA	NA	NA	NA	NA
Carrot	278	350	NA	320	NA	NA	296	300	NA	NA	NA	NA	NA	NA	NA	NA
Cassava	NA	460	190	180–300	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cauliflower	148	NA	230	170–200	NA	NA	NA	230	NA	NA	NA	NA	NA	NA	NA	NA
Okra	820	NA	223	780	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Potato	45–109	100	50	90	NA	NA	63	20	NA	NA	NA	NA	NA	NA	NA	NA
Sweet potato	NA	310–420	80–280	150	NA	NA	300–326	470	NA	NA	NA	NA	NA	NA	NA	NA
Turnip	356	NA	NA	210	NA	NA	NA	280	NA	NA	NA	NA	NA	NA	NA	NA
Processed																
Apple juice	95	NA	20	50	NA	90	90	20	NA	NA	NA	NA	NA	26	NA	NA
Cassava flour/starch	NA	1170	460	NA	NA	NA	NA	280	NA	NA	NA	NA	NA	NA	NA	NA
Fruit cocktail/ mixed fruit, canned	95	NA	NA	50	60	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Grape juice	71	NA	25	80	NA	90	100	30	NA	NA	NA	NA	NA	0	NA	NA
Grapefruit juice	259	NA	NA	90	NA	90	99	NA	NA	NA	NA	NA	NA	6	NA	NA
Orange juice	310	NA	400	80	NA	171	100	NA	NA	NA	NA	NA	NA	4	NA	NA
Peach, canned	33	NA	NA	20	NA	53.2	20	NA	NA	NA	NA	NA	NA	3	NA	NA
Pear, canned	47	NA	NA	50	NA	62.6	NA	NA	NA	NA	NA	NA	NA	18	NA	NA
Pineapples, canned	109	NA	NA	40	NA	92.8	200	70	NA	NA	NA	NA	NA	110	NA	NA
Raisins	535	NA	NA	NA	NA	451	540	650	NA	NA	NA	NA	NA	88	NA	NA
Tomato, sauce/relish/ canned	155	NA	100	220–290	NA	199	186	90–190	NA	NA	NA	NA	NA	13	NA	NA

(Continued)

Table 21.2 (Continued)

Food	Cu (mg/kg)								Fe (μg/kg)							
	USA	West Africa	Tanzania	Australia	Malaysia	Denmark	Finland	Japan	USA	West Africa	Tanzania	Australia	Malaysia	Denmark	Finland	Japan
Raw																
Apple	0.3	NA	0	NA	NA	0.28	NA	0.4	1	NA	1	1.1–1.6	2	1.24	<1.0	trace
Apricot	NA	NA	NA	NA	NA	0.78	NA	0.4	NA	NA	NA	3	NA	3.9	4	3
Asparagus	NA	NA	NA	NA	0.7	0.55	NA	1	0	NA	NA	10	6	NA	7	7
Avocado	2.6	2.3	2.2	NA	NA	1.9	NA	2.4	5.7	11	6	6.4–6.7	23	4	5	7
Banana	1.1	0.6	1	NA	NA	1.1	NA	0.9	2.6	10	3	2.9	6	2.4	4	3
Broccoli	NA	NA	NA	NA	0.4	0.7	NA	0.8	NA	NA	NA	8.6	7	7.1	11	10
Cabbage	NA	0.2	0	NA	NA	0.19–0.7	NA	0.2	NA	6	5	5.1–6	6	3.05–4.5	4	3
Carrot	0.4	0.6	0	NA	0.7	0.36	NA	0.4	1.7	9	3	2.8	8	2.4–4	5	2
Cassava	NA	0.5	1	NA	NA	1	NA	NA	NA	17	3	8	NA	2.7	NA	NA
Cauliflower	NA	NA	0	NA	NA	0.42	NA	0.5	NA	NA	4	4.8	9	4.02	6	6
Cucumber	0.3	NA	0	NA	NA	0.28	NA	1.1	1.5	NA	3	3	2	2.2	2	3
Eggplant	NA	NA	1	NA	0.8	0.8	NA	NA	NA	NA	4	1.9	6	4	3	NA
Grape	1.2	NA	0	NA	NA	1.5	NA	0.5	3.7	NA	2	3.1–4.2	5	2	2	1
Grapefruit	0.4	NA	0.5	NA	NA	0.42	NA	NA	1.1	NA	0.6	2.2	5	1.3	1	NA
Lettuce	0.2–0.5	NA	0	NA	NA	0.25–0.48	NA	0.3–0.6	2.8–15.2	NA	50	6.7	15	2.7–9.7	7	3.0–24
Mango	NA	0.4	1	NA	NA	1.1	NA	0.8	NA	10	1	3	2	2.4	NA	2
Mushrooms	2.5	NA	3	NA	0.3	4.2	NA	NA	1.9	NA	5	2.7	10	3.1	33	NA
Okra	NA	0.9	1	NA	0.6	0.94	NA	NA	NA	9	8	NA	15	8	NA	NA
Onion	0.4	0.4	0	NA	1	0.61	NA	0.4	1.6	5	2	3.1–3.7	46	3.3	5	3
Orange	0.5	0.5	1	NA	NA	0.7	NA	NA	1.2	1	1	3.7	21	1.38	1	NA
Papaya	NA	0.2	0	NA	NA	0.16	NA	0.5	NA	7	1	5	7	7	NA	2
Peach	0.6	NA	1	NA	NA	1.3	NA	0.5	1.5	NA	2	3	10	2.2	5	1
Pear	0.8	NA	1	NA	NA	0.71	NA	0.6–1.2	1	NA	2	1.7–2	5	1	2	1
Pineapple	NA	3.3	1	NA	NA	0.9	NA	1.1	NA	5	4	2.5	14	2	2	2
Potato	NA	0.9	4	NA	NA	0.52	NA	1	NA	8	32	5.5–6	14	10.4	7	4
Pumpkin	NA	NA	NA	NA	NA	0.8	NA	0.8	NA	NA	NA	2.4–3.2	7	2.7	3	5
Sweet potato	NA	2	2	NA	NA	1.51	NA	1.8	NA	19	4.0–5	5	6	9	6	7
Tomato	0.4	1.2	1	NA	0.5	0.39	NA	0.4	2	9	5	2.8	8	2.3	3	2
Turnip	NA	NA	NA	NA	NA	0.4	NA	0.3	NA	NA	NA	3	4	5	3	2
Watermelon	0.4	0.3	0	NA	0	0.3	NA	NA	2.2	2	2	NA	2	3	5	NA

Cooked															
Broccoli	0.3	NA	NA	NA	NA	0.61	NA	0.6	4.2	NA	NA	8.5	NA	6.7	NA	7
Cabbage	0	NA	0	NA	NA	NA	NA	0.2	4.8	NA	1	5.1–5.9	NA	NA	5	2
Carrot	0.4	0.6	NA	NA	NA	NA	NA	0.4	1.9	7	3	2.8–3.4	NA	NA	5	2
Cassava	NA	0.5	1	NA	NA	NA	NA	NA	NA	14	8	6.1	NA	NA	NA	NA
Cauliflower	0	NA	0	NA	NA	NA	NA	0.3	2.6	NA	3	4.7	NA	NA	NA	7
Okra	0.7	NA	1	NA	NA	NA	NA	NA	2.6	NA	6	10	NA	NA	NA	NA
Potato	0.7–0.9	0.8	2	NA	NA	NA	NA	0.8	3–7.9	8	4	5.3	NA	NA	7	4
Sweet potato	NA	1.8	2	NA	NA	NA	NA	1.7	NA	17	8	5.1	NA	NA	6	6
Turnip	0.1	NA	NA	NA	NA	NA	NA	0.2	2.1	NA	NA	3.1	NA	NA	NA	2
Processed																
Apple juice	0	NA	0	NA	NA	0.06	NA	0.3	0.2	NA	5	0.5	NA	3	1	4
Cassava flour/starch	NA	2.2	1	NA	NA	NA	NA	0.3	NA	46	19	NA	NA	NA	NA	3
Fruit cocktail/ mixed fruit, canned	0.5	NA	NA	NA	NA	NA	NA	NA	1.7	NA	NA	3.2	3	NA	NA	NA
Grape juice	0.1	NA	0	NA	NA	0.28	NA	0.2	1.2	NA	1	1.9	NA	2.4	2	1
Grapefruit juice	0.3	NA	NA	NA	NA	0.38	NA	NA	0.9	NA	NA	1	NA	1	1	NA
Orange juice	0.4	NA	1	NA	NA	0.38	NA	NA	0.9	NA	1	0.1	NA	1.8	1	NA
Peach, canned	0.3	NA	NA	NA	NA	0.43	NA	NA	1.6	NA	NA	3.4	NA	1.5	3	NA
Pear, canned	0.4	NA	NA	NA	NA	0.71	NA	NA	2	NA	NA	3	NA	1.8	NA	NA
Pineapples, canned	0.5	NA	NA	NA	NA	0.91	NA	0.7	2.9	NA	NA	4	5	3.1	3	3
Raisins	3.3	NA	NA	NA	NA	3.2	NA	3.9	19.8	NA	NA	NA	35	24	24	23
Tomato, sauce/relish/ canned	1.4	NA	1	NA	NA	1.1	NA	0.6–1.9	7.4	NA	5	2	NA	9.7	5	8

(Continued)

Table 21.2 (Continued)

Food	I (μg/kg)								K (μg/kg)							
	USA	West Africa	Tanzania	Australia	Malaysia	Denmark	Finland	Japan	USA	West Africa	Tanzania	Australia	Malaysia	Denmark	Finland	Japan
Raw																
Apple	0	NA	NA	5	NA	2	11	NA	984	NA	1070	950–1150	730–830	1200	1200	1100
Apricot	NA	NA	NA	22	NA	NA	10	NA	NA	NA	NA	3350	NA	2590	1600	2000
Asparagus	0	NA	NA	5	NA	1.5	NA	NA	NA	NA	NA	3200	1670	2020	2600	2700
Avocado	0	20	NA	5	NA	NA	14	NA	5179	5630	5990	5200	4670	4500	4000	7200
Banana	0	20	NA	5	NA	4	10	NA	3571	3770	3580	3480	2410	3530	3600	3600
Broccoli	NA	NA	NA	5	NA	8	10	NA	NA	NA	NA	3450	2260	3930	4000	3600
Cabbage	NA	NA	NA	5	NA	1.0–7	10	NA	NA	10	1700	2930–4500	1030	2400–2920	3200	2000
Carrot	7	190	NA	5	NA	30	10	NA	2180	2710	3200	2790	1360	2570–3030	3900	2800
Cassava	NA	NA	NA	5	NA	NA	NA	NA	NA	2710	2710	2700–2900	NA	2710	NA	NA
Cauliflower	NA	NA	NA	5	NA	8	10	NA	NA	NA	3030	2970	2140	2860	3700	4100
Cucumber	2	NA	NA	5	NA	3	10	NA	1374	NA	1470	970	760	1360	2400	2000
Eggplant	NA	10	NA	5	NA	1.5	0	NA	NA	2350	2300	1600	550	2400	2170	NA
Grape	0	NA	NA	5	NA	4	10	NA	1989	NA	1070	2210–2700	1180	1520	2300	1300
Grapefruit	0	NA	NA	5	NA	1	10	NA	1452	NA	1480	1350	1180	1450	1600	NA
Lettuce	0–5	NA	NA	23	NA	6	10	NA	1623–2764	NA	1580	2080	1390	1870–2470	3600	2000–4900
Mango	NA	20	NA	5	NA	3.2	NA	NA	NA	1720	1560	1970	1090	1050	NA	1700
Mushrooms	2	NA	NA	5	NA	10	235	NA	3358	NA	3180	3100	1950	3630	4138	NA
Okra	NA	NA	NA	NA	NA	NA	NA	NA	NA	220	3030	NA	800	3030	NA	NA
Onion	0	20	NA	5	NA	9	10	NA	1318	1470	1460	1830	110	1930	2200	1500
Orange	0	10	NA	5	NA	3	4	NA	1628	1770	1810	1470	1020	1530	1080	NA
Papaya	NA	NA	NA	5	NA	NA	NA	NA	NA	2060	2570	1400	390	1970	NA	2100
Peach	0	NA	NA	22	NA	3	10	NA	1893	NA	1900	2410	1240	1760	2000	1800
Pear	3	NA	NA	5	NA	4	10	NA	1194	NA	1190	1000	780	1160	1500	1400
Pineapple	NA	trace	NA	5	NA	14	14	NA	NA	2150	1130	1510	970	1740	1740	1500
Potato	NA	20	NA	5	NA	12	10	NA	NA	4710	4130	4420–4590	2030	4140	5000	4100
Pumpkin	NA	NA	NA	5	NA	1.5	10	NA	NA	NA	NA	3400–3820	1130	2430	2430	4000
Sweet potato	NA	20	NA	5	NA	NA	20	NA	NA	2450–3300	2510–3480	2150–2500	2100	4080	3370	4700
Tomato	0	10	NA	6	NA	2	10	NA	2326	2280	2220	2160	1290	2160	2900	2100
Turnip	NA	NA	NA	5	NA	5	10	NA	NA	NA	NA	3200	1260	2830	3000	2500
Watermelon	0	trace	NA	NA	NA	1.5	10	NA	1305	1130	1120	NA	780	1460	1000	NA

Cooked																
Broccoli	2	NA	NA	4	NA	NA	NA	NA	1624	NA	NA	3050	NA	2930	NA	1800
Cabbage	0	NA	NA	4	NA	NA	11	NA	1115	NA	610	2750–4220	NA	NA	3678	920
Carrot	0	200	NA	5	NA	NA	10	NA	2089	1290	NA	2700	NA	NA	3980	2400
Cassava	NA	NA	NA	5	NA	NA	NA	NA	NA	1280	2430	2810	NA	NA	NA	NA
Cauliflower	0	NA	NA	4.0–7	NA	NA	NA	NA	1565	NA	1210	2810	NA	NA	NA	2200
Okra	8	NA	NA	5	NA	NA	NA	NA	1192	NA	NA	2500	NA	NA	NA	NA
Potato	0–5	20	NA	5	NA	NA	119	NA	2713–4468	3770	3910	4940	NA	NA	3400	3400
Sweet potato	NA	20	NA	5	NA	NA	20	NA	NA	2640–2760	3030	3300	NA	NA	3370	4900
Turnip	0	NA	NA	5	NA	NA	NA	NA	1961	NA	NA	3100	NA	NA	NA	2500
Processed																
Apple juice	5	NA	NA	40	NA	7	10	NA	984	NA	900	1020	NA	800	1100	770
Cassava flour/starch	NA	NA	NA	NA	NA	NA	NA	NA	NA	5940	5830	NA	NA	NA	NA	480
Fruit cocktail/ mixed fruit, canned	61	NA	NA	8	NA	NA	NA	NA	1050	NA	NA	920	500	NA	NA	NA
Grape juice	0	NA	NA	40	NA	NA	10	NA	324	NA	359	850	NA	1320	2300	300
Grapefruit juice	3	NA	NA	31	NA	6	10	NA	1411	NA	NA	1000	NA	1120	1900	NA
Orange juice	0	NA	NA	13	NA	4	10	NA	1813	NA	1810	1430	NA	1680	1900	NA
Peach, canned	0	NA	NA	6	NA	18	10	NA	1113	NA	NA	1060	NA	1500	1000	NA
Pear, canned	6	NA	NA	9	NA	5	NA	NA	673	NA	NA	810	NA	660	NA	NA
Pineapples, canned	41	NA	NA	6	NA	14	10	NA	1199	NA	NA	800	180	940	1200	1200
Raisins	0	NA	NA	NA	NA	20	30	NA	7521	NA	NA	NA	6320	7850	6500	7400
Tomato, sauce/relish/ canned	14	NA	NA	11	NA	2	140	NA	3354	NA	2500	1910	NA	1880	3043	2400

(Continued)

Table 21.2 (Continued)

Food	Mg (mg/kg)								Mn (μg/kg)							
	USA	West Africa	Tanzania	Australia	Malaysia	Denmark	Finland	Japan	USA	West Africa	Tanzania	Australia	Malaysia	Denmark	Finland	Japan
Raw																
Apple	49	NA	50	40–50	NA	44	35	30	0.2	NA	0	NA	NA	0.47	NA	0.3
Apricot	NA	NA	NA	90	NA	100	88	80	NA	NA	NA	NA	NA	0.77	NA	2.1
Asparagus	NA	NA	NA	150	300	89	130	90	NA	NA	NA	NA	NA	NA	NA	1.9
Avocado	300	320	290	270	NA	250	290	330	1.6	NA	1	NA	NA	1.42	NA	1.8
Banana	298	340	270	300	NA	277	330	320	2.4	NA	3	NA	NA	2.4	NA	2.6
Broccoli	NA	NA	NA	220	300	207	240	260	NA	NA	NA	NA	NA	5.8	NA	2.2
Cabbage	NA	100	120	140–150	NA	90–140	140	140	NA	NA	2	NA	NA	0–2.4	NA	1.5
Carrot	96	120	120	120	170	96–120	140	100	0.9	NA	1	NA	NA	3.6	NA	1
Cassava	NA	NA	210	90–110	NA	210	NA	NA	NA	NA	1	NA	NA	3.84	NA	NA
Cauliflower	NA	NA	150	150	NA	132	150	180	NA	NA	2	NA	NA	2.3	NA	2.2
Cucumber	103	NA	130	90	NA	92.3	170	150	0.8	NA	1	NA	NA	1	NA	0.7
Eggplant	NA	120	140	80	300	100	140	NA	NA	NA	3	NA	NA	2.5	NA	NA
Grape	71	NA	50	80–120	NA	70	90	60	0.7	NA	1	NA	NA	0.85	NA	1.2
Grapefruit	93	NA	90	90	NA	100	110	NA	0.2	NA	0.1	NA	NA	0.36	NA	NA
Lettuce	75–137	NA	90	120	NA	74–140	210	80–150	1.2–2.4	NA	2	NA	NA	1.25–2.5	NA	1.3–4.3
Mango	NA	120	90	80	NA	90	NA	120	NA	NA	0	NA	NA	0.27	NA	1
Mushrooms	92	NA	90	100	110	105	114	NA	0.4	NA	0	NA	NA	0.83	NA	NA
Okra	NA	130	570	NA	520	570	NA	NA	NA	NA	10	NA	NA	9.9	NA	NA
Onion	92	70	100	100	400	96	110	90	1.1	NA	1	NA	NA	2.3	NA	1.4
Orange	111	110	100	110	NA	104	94	NA	0.2	NA	0	NA	NA	0.42	NA	NA
Papaya	NA	190	100	140	NA	220	NA	260	NA	NA	0	NA	NA	0.11	NA	0.4
Peach	78	NA	90	90	NA	60	119	70	0.5	NA	1	NA	NA	0.7	NA	0.4
Pear	65	NA	2	70	NA	63.2	70	40–50	0.4	NA	0	NA	NA	0.5	NA	0.3–0.4
Pineapple	NA	90	140	160	NA	140	140	140	NA	NA	17	NA	NA	15	NA	7.6
Potato	NA	270	230	200	NA	204	240	200	NA	NA	6	NA	NA	2.3	NA	1.1
Pumpkin	NA	NA	NA	140–170	NA	112	112	150	NA	NA	NA	NA	NA	0.4	NA	1
Sweet potato	NA	160–170	140–200	80–140	NA	150	250	250	NA	NA	5.6–40	NA	NA	2.58	NA	4.4
Tomato	92	120	110	70	180	90	110	90	1	NA	1	NA	NA	1.1	NA	0.8
Turnip	NA	NA	NA	80	NA	80	200	80	NA	NA	NA	NA	NA	0.68	NA	0.5
Watermelon	120	100	100	NA	0	84	80	NA	0.5	NA	0	NA	NA	0.38	NA	NA

Cooked																
Broccoli	118	NA	NA	210	NA	210	NA	170	1.6	NA	NA	NA	NA	0	NA	1.7
Cabbage	82	NA	50	140–150	NA	NA	161	90	1.3	NA	1	NA	NA	NA	NA	3.4
Carrot	89	80	NA	120	NA	NA	143	90	1.1	NA	NA	NA	NA	NA	NA	2.2
Cassava	NA	130	100	90	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA
Cauliflower	97	NA	70	150	NA	NA	NA	130	1.2	NA	1	NA	NA	NA	NA	1.7
Okra	340	NA	NA	460	NA	NA	NA	NA	2.6	NA	NA	NA	NA	NA	NA	NA
Potato	150	250	250	190–200	NA	NA	240	180	1.2	NA	2	NA	NA	NA	NA	1
Sweet potato	NA	140–150	260	80	NA	NA	250	190	NA	NA	2	NA	NA	NA	NA	5
Turnip	97	NA	NA	80	NA	NA	NA	90	1	NA	NA	NA	NA	NA	NA	0.7
Processed																
Apple juice	46	NA	50	50	NA	45	50	30	0.3	NA	2	NA	NA	0.56	NA	0.3
Cassava flour/starch	NA	460	240	NA	NA	NA	NA	50	NA	NA	3	NA	NA	NA	NA	0.9
Fruit cocktail/ mixed fruit, canned	60	NA	NA	50	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA
Grape juice	62	NA	17	50	NA	100	90	140	1	NA	0	NA	NA	3.6	NA	1.3
Grapefruit juice	97	NA	NA	70	NA	80	100	NA	0.02	NA	NA	NA	NA	0.36	NA	NA
Orange juice	109	NA	100	80	NA	100	90	NA	0.2	NA	0	NA	NA	0.42	NA	NA
Peach, canned	54	NA	NA	40	NA	55	60	NA	0.2	NA	NA	NA	NA	0.5	NA	NA
Pear, canned	45	NA	NA	50	NA	40	NA	NA	0.1	NA	NA	NA	NA	0	NA	NA
Pineapples, canned	144	NA	NA	110	NA	80	170	90	11.5	NA	NA	NA	NA	15	NA	15.8
Raisins	329	NA	NA	NA	NA	350	300	310	3	NA	NA	NA	NA	3.1	NA	2
Tomato, sauce/relish/ canned	160	NA	130	110	NA	110	119	130	1.1	NA	3	NA	NA	1.1	NA	0.9

(Continued)

Table 21.2 (Continued)

Food	Na (mg/kg)								Ni (μg/kg)							
	USA	West Africa	Tanzania	Australia	Malaysia	Denmark	Finland	Japan	USA	West Africa	Tanzania	Australia	Malaysia	Denmark	Finland	Japan
Raw																
Apple	16	NA	10	10.0–20	30–50	30	8.0–9	trace	5	NA	NA	NA	NA	8.18–8.95	NA	NA
Apricot	NA	NA	NA	20	NA	10	20	20	NA	NA	NA	NA	NA	NA	NA	NA
Asparagus	NA	NA	NA	20	130	64	10	20	NA	NA	NA	NA	NA	180	NA	NA
Avocado	32	50	70	40	80	60	60	70	301	NA	NA	NA	NA	597	NA	NA
Banana	1	20	10	0	290	20	3	trace	39	NA	NA	NA	NA	40.1	NA	NA
Broccoli	NA	NA	NA	220	120	80	69	200	NA	NA	NA	NA	NA	86	NA	NA
Cabbage	NA	130	180	150–230	160	60–100	50	50	NA	NA	NA	NA	NA	27–100	NA	NA
Carrot	690	390	690	400	1190	660	190	240	45	NA	NA	NA	NA	34.3–64.6	NA	NA
Cassava	NA	140	140	20	NA	140	NA	NA	NA	NA	NA	NA	NA	0	NA	NA
Cauliflower	NA	NA	300	310	570	170	250	80	NA	NA	NA	NA	NA	11.1	NA	NA
Cucumber	19	NA	20	210	130	100	60	10	26	NA	NA	NA	NA	6	NA	NA
Eggplant	NA	30	20	50	90	30	20	NA	NA	NA	NA	NA	NA	3.95	NA	NA
Grape	23	NA	0	40–50	90	30	9	10	8	NA	NA	NA	NA	8.95	NA	NA
Grapefruit	10	NA	0	40	90	20	20	NA	43	NA	NA	NA	NA	30.6	NA	NA
Lettuce	137–235	NA	90	240	100	80–280	230	20–60	65–135	NA	NA	NA	NA	41	NA	NA
Mango	NA	50	20	10	70	20	NA	10	NA	NA	NA	NA	NA	NA	NA	NA
Mushrooms	86	NA	50	80	60	80	4124	NA	2	NA	NA	NA	NA	3	NA	NA
Okra	NA	NA	6	NA	300	80	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Onion	42	60	40	120	140	29	37	20	22	NA	NA	NA	NA	35.5	NA	NA
Orange	8	30	0	30	50	20	12	NA	26	NA	NA	NA	NA	31.8	NA	NA
Papaya	NA	30	30	70	30	40	NA	60	NA	NA	NA	NA	NA	30	NA	NA
Peach	11	NA	0	20	20	30	10	10	93	NA	NA	NA	NA	151	NA	NA
Pear	4	NA	10	20	20	20	12	trace-10	43	NA	NA	NA	NA	89.9	NA	NA
Pineapple	NA	20	10	60	310	40	40	trace	NA	NA	NA	NA	NA	500	NA	NA
Potato	NA	50	100	40–50	330	70	10	10	NA	NA	NA	NA	NA	57.6	NA	NA
Pumpkin	NA	NA	NA	10	500	20	20	10	NA	NA	NA	NA	NA	50	NA	NA
Sweet potato	NA	110–370	70–100	100–120	360	330	550	40	NA	NA	NA	NA	NA	NA	NA	NA
Tomato	28	60	90	80	160	108–134	25	30	19	NA	NA	NA	NA	7.0–32	NA	NA
Turnip	NA	NA	NA	240	80	170	41	50	NA	NA	NA	NA	NA	NA	NA	NA
Watermelon	16	20	10	NA	40	67	20	NA	72	NA	NA	NA	NA	70	NA	NA

Cooked															
Broccoli	135	NA	NA	220	NA	410	NA	140	61	NA	NA	NA	NA	86	NA	NA
Cabbage	87	NA	50	140–160	NA	NA	57	30	17	NA	NA	NA	NA	NA	NA	NA
Carrot	690	230	690	400	NA	NA	194	210	47	NA	NA	NA	NA	NA	NA	NA
Cassava	NA	80	20	20	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cauliflower	152	NA	90	310–340	NA	NA	NA	80	47	NA	NA	NA	NA	NA	NA	NA
Okra	69	NA	NA	20	NA	NA	NA	NA	45	NA	NA	NA	NA	NA	NA	NA
Potato	25	40	50	40	NA	NA	1935	10	39	NA	NA	NA	NA	NA	NA	NA
Sweet potato	NA	90–300	30	100–120	NA	NA	550	40	NA	NA	NA	NA	NA	NA	NA	NA
Turnip	249	NA	NA	250	NA	NA	NA	40	20	NA	NA	NA	NA	NA	NA	NA
Processed															
Apple juice	39	NA	20	70	NA	100	36	30	11	NA	NA	NA	NA	8.9	NA	NA
Cassava flour/starch	NA	310	50	NA	NA	NA	NA	10	NA	NA	NA	NA	NA	NA	NA	NA
Fruit cocktail/ mixed fruit, canned	35	NA	NA	20	40	NA	NA	NA	69	NA	NA	NA	NA	NA	NA	NA
Grape juice	53	NA	1	130	NA	30	9	10	17	NA	NA	NA	NA	NA	NA	NA
Grapefruit juice	30	NA	0	50	NA	20	140	NA	29	NA	NA	NA	NA	3	NA	NA
Orange juice	23	NA	NA	30	NA	150	38	NA	22	NA	NA	NA	NA	6	NA	NA
Peach, canned	28	NA	NA	30	NA	10	15	NA	66	NA	NA	NA	NA	167	NA	NA
Pear, canned	13	NA	NA	30	NA	50	NA	NA	36	NA	NA	NA	NA	89.9	NA	NA
Pineapples, canned	3	NA	NA	60	NA	10	21	10	88	NA	NA	NA	NA	147	NA	NA
Raisins	222	NA	NA	NA	150	90	55	120	81	NA	NA	NA	NA	178	NA	NA
Tomato, sauce/ relish/canned	4988	NA	900	1260	NA	1430	2525	2700	102	NA	NA	NA	NA	19.5	NA	NA

(Continued)

Table 21.2 (Continued)

Food	P (mg/kg)								Se (μg/kg)							
	USA	West Africa	Tanzania	Australia	Malaysia	Denmark	Finland	Japan	USA	West Africa	Tanzania	Australia	Malaysia	Denmark	Finland	Japan
Raw																
Apple	109	NA	110	70–90	80–90	174	78	100	0	NA	NA	NA	NA	2.51–2.58	<1.0	NA
Apricot	NA	NA	NA	210	NA	219	110	150	NA	NA	NA	NA	NA	3	10	NA
Asparagus	NA	NA	NA	490	500	520	720	600	NA	NA	NA	NA	NA	10	2	NA
Avocado	467	460	520	480	500	465	310	550	1	NA	NA	NA	NA	8	4	NA
Banana	257	230	220	260	280	273	260	270	3	10	NA	NA	NA	10.4	10	NA
Broccoli	NA	NA	NA	810	660	869	900	890	NA	NA	NA	NA	NA	6	4	NA
Cabbage	NA	340	260	280–400	90	301–341	400	270	NA	10	NA	NA	NA	7.67–10	15	NA
Carrot	279	420	350	360	270	290–331	400	250	2	10	NA	NA	NA	1.0–3	2	NA
Cassava	NA	460	270	720	NA	270	NA	NA	NA	10	NA	NA	NA	NA	NA	NA
Cauliflower	NA	NA	440	500	520	481	500	680	NA	NA	NA	NA	NA	4–6.83	4	NA
Cucumber	228	NA	210	180	210	253	350	360	0	NA	NA	NA	NA	3	2	NA
Eggplant	NA	240	250	280	200	303	220	NA	NA	40	NA	NA	NA	2	4	NA
Grape	221	NA	70	160–190	210	218	200	150	1	NA	NA	NA	NA	3.26	1	NA
Grapefruit	191	NA	80	180	210	195	200	NA	0	NA	NA	NA	NA	2.2	1	NA
Lettuce	225–326	NA	200	260	250	225–300	400	220–490	0–2	NA	NA	NA	NA	2	2	NA
Mango	NA	210	110	140	150	155	NA	120	NA	10	NA	NA	NA	6	NA	NA
Mushrooms	885	NA	860	1100	880	851	896	NA	131	NA	NA	NA	NA	64.7	171	NA
Okra	NA	300	630	NA	320	630	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Onion	280	340	290	360–390	510	401	400	340	13	20	NA	NA	NA	5	5	NA
Orange	221	190	140	200	190	221	166	NA	0	10	NA	NA	NA	0.5	<1.0	NA
Papaya	NA	150	50	150	70	103	NA	110	NA	10	NA	NA	NA	NA	NA	NA
Peach	210	NA	200	210	250	219	200	180	0	NA	NA	NA	NA	1.56	4	NA
Pear	114	NA	110	110	120	188	114	80–130	0	NA	NA	NA	NA	2.12	9	NA
Pineapple	NA	120	70	50	60	139	80	90	NA	trace	NA	NA	NA	6	6	NA
Potato	NA	500	380	560–570	740	553	450	400	NA	20	NA	NA	NA	2.68	6	NA
Pumpkin	NA	NA	NA	390–500	390	330	190	420	NA	NA	NA	NA	NA	NA	0	NA
Sweet potato	NA	380–500	400–550	330	320	470	470	460	NA	10.0–20	NA	NA	NA	NA	6	NA
Tomato	250	410	240	250	280	291	300	260	0	10	NA	NA	NA	3	2	NA
Turnip	NA	NA	NA	270	210	500	500	250	NA	NA	NA	NA	NA	0	3	NA
Watermelon	172	90	110	NA	110	198	100	NA	2	trace	NA	NA	NA	0	1	NA

Cooked															
Broccoli	485	NA	NA	720	NA	670	NA	660	2	NA	NA	NA	NA	16	NA	NA
Cabbage	184	NA	90	260–380	NA	NA	460	200	7	NA	NA	NA	NA	NA	17	NA
Carrot	288	400	NA	350	NA	NA	408	250	3	10	NA	NA	NA	NA	2	NA
Cassava	NA	430	700	700	NA	NA	NA	NA	NA	10	NA	NA	NA	NA	NA	NA
Cauliflower	346	NA	110	470	NA	NA	NA	370	4	NA	NA	NA	NA	NA	NA	NA
Okra	329	NA	NA	600	NA	NA	NA	NA	2	NA	NA	NA	NA	NA	NA	NA
Potato	452	470	500	550–840	NA	NA	451	250	0	20	NA	NA	NA	NA	6	NA
Sweet potato	NA	360	390	360–470	NA	NA	470	420	NA	10.0–20	NA	NA	NA	NA	6	NA
Turnip	317	NA	NA	260	NA	NA	NA	260	0	NA	NA	NA	NA	NA	NA	NA
Processed																
Apple juice	65	NA	70	60	NA	60	60	60	0	NA	NA	NA	NA	0.31	<1.0	NA
Cassava flour/ starch	NA	930	1680	NA	NA	NA	NA	60	NA	NA	NA	NA	NA	NA	NA	NA
Fruit cocktail/ mixed fruit, canned	115	NA	NA	150	60	NA	NA	NA	0	NA	NA	NA	NA	NA	NA	NA
Grape juice	89	NA	26	110	NA	110	200	70	0	NA	NA	NA	NA	NA	1	NA
Grapefruit juice	175	NA	80	130	NA	120	160	NA	0	NA	NA	NA	NA	0	1	NA
Orange juice	263	NA	140	140	NA	216	160	NA	0	NA	NA	NA	NA	0.42	<1.0	NA
Peach, canned	127	NA	NA	150	NA	169	100	NA	0	NA	NA	NA	NA	1.56	5	NA
Pear, canned	70	NA	NA	150	NA	134	NA	NA	0	NA	NA	NA	NA	2.12	NA	NA
Pineapples, canned	66	NA	NA	30	20	100	170	70	0	NA	NA	NA	NA	6	0	NA
Raisins	1079	NA	NA	NA	800	1070	900	900	0	NA	NA	NA	NA	4	4	NA
Tomato, sauce/ relish/canned	296	NA	320	180	NA	190	364	260	3	NA	NA	NA	NA	3	3	NA

(Continued)

Table 21.2 (Continued)

Food	Zn (mg/kg)							
	USA	**West Africa**	**Tanzania**	**Australia**	**Malaysia**	**Denmark**	**Finland**	**Japan**
Raw								
Apple	0	NA	0	0.6–0.8	NA	0.32	<1.0	trace
Apricot	NA	NA	NA	1.5	NA	2	3	1
Asparagus	NA	NA	NA	2	8	4.4	7	5
Avocado	5.3	6	6	5.7	NA	6.4	0	7
Banana	1.6	11	1	1.6	NA	1.76	2	2
Broccoli	NA	NA	NA	6	5	3.9	1	7
Cabbage	NA	2	2	2.6–3	NA	1.45–2.7	2	2
Carrot	1.4	8	2	2	4	1.6–2.7	4	2
Cassava	NA	2	3	23–23.5	NA	3.4	NA	NA
Cauliflower	NA	NA	3	2.5	NA	2.61	5	6
Cucumber	1.1	NA	2	4	NA	1.29	2	2
Eggplant	NA	16	1	0.9	5	1.6	0	NA
Grape	0.3	NA	0	1.2–2.2	NA	0.59	1	1
Grapefruit	0.5	NA	0.7	1.1	NA	0.7	1	NA
Lettuce	1.2–2.2	NA	2	2.3	NA	1.6–2.3	5	2.0–5
Mango	NA	6	0	1.8	NA	0.79	NA	1
Mushrooms	4.1	NA	5	5.6	5	4.77	8	NA
Okra	NA	30	6	NA	13	6	NA	NA
Onion	1.2	2	2	1.8–2.4	3	1.6	4	2
Orange	0.6	1	1	1.5–2	NA	0.68	<1.0	NA
Papaya	NA	14	1	3	NA	0.73	NA	1
Peach	0.9	NA	2	1.2	NA	1.4	1	1
Pear	0.9	NA	1	1	NA	1.24	<1.0	trace-1
Pineapple	NA	22	1	1	NA	0.82	<1.0	1
Potato	NA	3	4	3.1–3.5	NA	3	3	2
Pumpkin	NA	NA	NA	1.4–3	NA	2	<1.0	3
Sweet potato	NA	3.0–4	2.0–3	4.0–5	NA	2	3	2
Tomato	1	11	1	3	2	0.84	2	1
Turnip	NA	NA	NA	2	NA	2.3	2	1
Watermelon	0.9	1	1	NA	0	1	1	NA

Cooked								
Broccoli	2.2	NA	NA	6	NA	4.5	NA	3
Cabbage	0.9	NA	1	2.5–3	NA	NA	2	1
Carrot	1.8	6	NA	2–2.1	NA	NA	4	2
Cassava	NA	2	3	23.5	NA	NA	NA	NA
Cauliflower	1.5	NA	1	2.5–2.6	NA	NA	NA	4
Okra	3.5	NA	NA	6	NA	NA	NA	NA
Potato	2.3	3	3	3.1–4.4	NA	NA	3	2
Sweet potato	NA	3	5	4.1–6.8	NA	NA	3	2
Turnip	1.6	NA	NA	2	NA	NA	NA	1
Processed								
Apple juice	0.1	NA	2	0.4	NA	0.39	<1.0	trace
Cassava flour/starch	NA	7	7	NA	NA	NA	NA	trace
Fruit cocktail/ mixed fruit, canned	0.7	NA	NA	2.3	NA	NA	NA	NA
Grape juice	0.3	NA	0	0.6	NA	0.5	1	1
Grapefruit juice	0.4	NA	0.7	0.6	NA	0.61	<1.0	NA
Orange juice	0.3	NA	1	0.2	NA	0.61	5	NA
Peach, canned	0.7	NA	NA	2	NA	3.1	3	NA
Pear, canned	0.6	NA	NA	2.6	NA	1.3	NA	NA
Pineapples, canned	0.7	NA	NA	0.5	NA	0.82	1	1
Raisins	1.7	NA	NA	NA	NA	3	3	3
Tomato, sauce/ relish/canned	1.8	NA	1	1.6	NA	3	3	1

Sources: data are from databases listed below:
Australia: AUSNUT 2007: Australian Food, Supplement and Nutrient Database 2007 for estimation of population nutrient intakes.
Denmark: DTU Food National Food Institute. Danish Food Composition Databank.
Finland: Fineli® Finnish Food Composition Database. National Institute for Health and Welfare, Finland.
Japan: Sugiyama University. Standard Tables of Food Composition in Japan, 5th Revised Edition, 2000.
Malaysia: NutriWeb Malaysia. Malaysian Foods Composition Database.
Tanzania: Muhimbili University College of Allied and Health Sciences/Tanzania Food and Nutrition Centre/Harvard School of Public Health, 1st edn.
USA: US Food and Drug Administration. Total Diet Study Statistics on Element Results 2006–2008.
West Africa: Food and Agriculture Organization of the United Nations. Composition of Selected Foods from West Africa.
All databases accessed August 2013.

Table 21.3 Toxic elements in some foods from the US FDA database.

Food	As (mg/kg)	Cd (mg/kg)	Hg (mg/kg)	Pb (mg/kg)
Raw				
Apple (red) raw with peel	0	0.002	NA	0
Avocado, raw	0.001	0.015	0	0.002
Banana, raw	0	0.001	NA	0
Cantaloupe raw/frozen	0.008	0.008	NA	0.001
Carrot, baby, raw	0	0.013	NA	0.001
Celery, raw	0	0.04	NA	0
Cucumber, peeled, raw	0.001	0.003	NA	0
Grapefruit, raw	0	0	NA	0.001
Grapes (red/green) raw	0.003	0.001	NA	0.002
Lettuce, iceberg, raw	0	0.051	NA	0
Lettuce, leaf, raw	0.002	0.064	NA	0.005
Mushrooms, raw	0.073	0.005	0.001	0.001
Onion, mature, raw	0	0.014	NA	0.002
Orange (navel/Valencia) raw	0	0.001	NA	0.001
Peach, raw/frozen	0	0.002	NA	0.001
Pear, raw with peel	0.001	0.001	NA	0
Pepper, sweet green, raw	0	0.016	NA	0
Strawberries, raw/frozen	0.001	0.017	NA	0
Tomato, raw	0.001	0.008	0	0
Watermelon, raw/frozen	0	0.002	NA	0
Cooked				
Asparagus, fresh/frozen boiled	0	0.019	NA	0.001
Broccoli, fresh/frozen, boiled	0	0.007	NA	0
Brussels sprouts, fresh/frozen boiled	0	0.006	NA	0
Cabbage, fresh, boiled	0	0.005	NA	0
Carrot, fresh, peeled, boiled	0	0.019	NA	0.001
Cauliflower, fresh/frozen, boiled	0	0.01	0	0
Collards, fresh/frozen, boiled	0.003	0.035	0.001	0.003
Corn, fresh/frozen boiled	0.001	0.003	NA	0
Eggplant, fresh peeled boiled	0.001	0.012	NA	0
Green beans, fresh/frozen, boiled	0	0.001	NA	0
Mixed vegetables, frozen, boiled	0	0.007	NA	0
Okra, fresh, frozen boiled	0.001	0.014	NA	0.001
Potato, baked with peel	0.002	0.026	NA	0.001
Potato, boiled without peel	0	0.019	NA	0
Spinach, fresh/frozen, boiled	0.001	0.124	0	0.003
Summer squash, fresh/frozen, boiled	0	0.002	NA	0
Turnip, fresh/frozen boiled	0	0.019	NA	0.001
Winter squash, fresh/frozen, boiled	0	0.004	NA	0.002
Processed				
Apple juice, bottled	0.005	0.001	0	0.001
Applesauce, bottled	0	0	NA	0.001
Beets, canned	0	0.018	NA	0
Corn, canned	0	0.004	NA	0
Fruit cocktail, canned light syrup	0.002	0.002	NA	0.011
Grape juice, frozen conc. reconst.	0.007	0.001	NA	0.006
Grapefruit juice, frozen conc. reconst.	0	0.0003	NA	0.001
Green beans, canned	0	0.002	NA	0
Lemonade, frozen conc. reconst.	0	0.0001	NA	0

Table 21.3 (Continued)

Food	As (mg/kg)	Cd (mg/kg)	Hg (mg/kg)	Pb (mg/kg)
Orange juice, frozen conc. reconst.	0	0.0003	0	0
Peach, canned light/medium syrup	0	0.0004	NA	0.011
Pear, canned light syrup	0	0.0003	NA	0.007
Pinapples, canned in juice	0	0.004	NA	0.013
Pineapple juice, frozen conc. reconst.	0	0.004	NA	0
Prune juice, bottled	0.004	0.0002	NA	0.002
Raisins	0.014	0.0003	0.0001	0.009
Sweet potatoes, canned	0.001	0.004	NA	0.012
Tomato sauce, plain, bottled	0.001	0.022	NA	0

Source: data from US FDA database.

21.4.3 Nuclear analytical techniques

Having been discovered in 1936 by Hevesy and Levi, instrumental neutron activation analysis (INAA) is now a mature technique which among other applications is suitable for trace element and mineral nutrition research.

In most nuclear analytical laboratories the source of neutrons is typically a small research reactor. After a set period of decay the induced radioactivity of the sample is measured using gamma spectroscopy systems consisting of solid-state hyperpure germanium (HPGe) photon detectors and associated electronics. At the International Centre for Environmental and Nuclear Sciences (ICENS) this source of neutrons is the Safe LOW POwer C(K)ritical Experiment (SLOWPOKE) research reactor. Low-power reactors such as the SLOWPOKE produce irradiated samples that are not highly radioactive so that fewer handling restrictions are required, and administrative procedures may be less stringent. The main disadvantage of low-power reactors is the reduced activation of long-lived isotopes.

The simplest and most common method for analysis is the comparative method. The samples to be analysed are irradiated along with a reference material (RM), followed by measurement of the induced activities. Once the peak areas for the particular elements of interest have been accurately measured for both the sample and RM, it is then possible to perform a simple calculation (equation 21.1) to determine the elemental concentration of a particular element in the sample:

$$\frac{C_{x1}\,M_1}{D_1} = \frac{C_{x2}\,M_2}{D_2} \qquad (21.1)$$

where C_{x1} and C_{x2} are the concentrations of the element of interest in the sample and the RM respectively; M_1 and M_2 are the masses of the sample and the RM, respectively; and D_1 and D_2 are the area count for the gamma lines of interest. The above expression holds for all irradiation facilities mentioned in this chapter. However, when considering core tube irradiation, corrections have to be made for the variation in the flux between the RM and the samples (as they are not in identical positions).

In addition to the flux (ϕ) correction, we must also take into account the fact that the decay times (t_d) of the standard and the sample may not be identical. With these factors taken into account, the concentration of the element of interest is given by equation 21.2:

$$C_{x1} = \frac{C_{x2}\,D_{x1}\,M_2\,\phi_2\,e^{(-\lambda\,td2)}}{D_{x2}\,M_1\,\phi_1\,e^{(-\lambda\,td1)}} \qquad (21.2)$$

This method offers, relatively, the lowest errors; this is due to the fact that the use of unreliable nuclear data and detector efficiencies are eliminated, so the only sources of errors are the standard deviation on the area counts for the gamma line of interest and the errors on the certified values of the concentrations in the RM being used.

Unlike most reactors, quantification by INAA with SLOWPOKE using the in-core irradiation sites does not require co-irradiation of flux monitors or standards. The uniformity, stability and reproducibility of the neutron spectrum within the core and with time facilitates the use of activation constants (k) according to equation 21.3, in which m is the mass of a particular element in

Table 21.4 Essential and toxic elements in Jamaican foods.

Elements	Ca (mg/kg)	Cd (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	K (%)	Na (mg/kg)	Mg (mg/kg)	Mn (mg/kg)	P (%)	Pb (mg/kg)	Zn (mg/kg)
Fruit (*n* = 27)	360	0.029	0.04	0.8	6.7	0.46	9.8	248	1.7	0.032	0.011	2.6
Vegetables: leafy (*n* = 14)	2580	0.4	0.08	0.6	15.5	0.38	90.1	359	8.3	0.042	0.005*	4.2
Vegetables: root (*n* = 16)	390	0.4	0.09	0.7	12.9	0.41	727	157	4.3	0.027	0.01	3.4
Other root crops (*n* = 46)	150	0.2	0.11	1.6	9.6	0.47	48.7	161	1.6	0.027	0.022	5.8

*Value represents LOD × 0.5: Helsel D. Less than obvious: statistical treatment of data below the detection limit. *Environ Sci Technol* 1990;**24**:1766–1774.
Source: data extracted from Howe *et al*. [25].

Table 21.5 Examples of local foods in online databases.

Country	Local foods in database
USA	Collard greens, baby foods (fruit/vegetable varieties), deli coleslaw/potato salad, frozen mixed vegetables
West Africa	Breadfruit, cashew apple, cocoyam, fig, tamarind, taro, yam
Tanzania	Breadfruit, roast banana, guava, jackfruit, soursop, taro, yam
Australia	Beetroot, blueberry, blackberry, boysenberry, mixed fruit drinks/juices, kale, kiwifruit, rhubarb
Malaysia	Bamboo shoot, carambola/starfruit, custard apple, durian, guava, jackfruit, chinese radish (fresh and pickled), soursop, taro
Denmark	Bilberry, black pudding, brussel sprouts, chard, currant, endive, fennel, lingonberry, radish, quince, watercress
Finland	Apple chips, beetroot, cranberry, gooseberry, lingonberry, pickled onion, pickled pumpkin, radish, raspberry
Japan	Coconut powder, coconut milk, cucumber pickles (salted, sour, sweet), horseradish, sweet potato starch

grams, R is the peak area, t_i, t_d and t_c are the times of irradiation, decay and counting respectively, and λ is the decay constant:

$$m = \frac{R}{k \cdot e^{-\lambda t_d} \cdot \left(1 - e^{-\lambda t_i}\right) \cdot \left(1 - e^{-\lambda t_c}\right)} \qquad (21.3)$$

where $k = \varepsilon \cdot \theta \cdot N_A \cdot \sigma \cdot \varphi / A_w$

and ε denotes detector efficiency, θ abundance of the activated nuclide, N_A Avogadro's number = 6.023×10^{23}, σ effective isotopic activation cross-section, φ neutron flux in n/(cm² s) and A_w atomic weight of the irradiated element.

These constants are obtained by the irradiation and counting of analytical-grade single-element standards with identical geometry to that used for samples. These sensitivities can be used over a period of several years [15]. This very convenient standardization method has been called the improved relative (IR) method [16]. The activation constants once accurately measured on a primary spectroscopy system can be readily transferred to other spectroscopy systems using empirical measured efficiency functions thus reducing calibration time [17].

For the determination of short-lived radioisotopes using SLOWPOKE-II in Jamaica, approximately 0.7 g of sample is weighed in precleaned double polyethylene bags and heat sealed in precleaned 7-mL polyethylene. Each sample is irradiated for 3 min at a neutron flux of 5×10^{11} n/(cm² s) and allowed decay periods of approximately 5 and 60 min before counting. For intermediate and long-lived radioisotopes, approximately 1.0 g of sample is weighed in precleaned polyethylene capsules that are then heat sealed in 7-mL polyethylene vials and irradiated for 4 hours at a neutron flux of 1×10^{12} n/(cm² s) and allowed a decay period of 4 days and 14 days respectively before counting [18].

Although it is often stated that NAA is matrix independent, orders of magnitude differences in detection limits for particular elements are obtained depending on the matrix. Many medium- and short-lived elements for instance are severely affected by elements that have either high cross-sections and or high concentrations that produce gamma-rays of energies very close to or identical with the gamma-rays of the elements we wish to quantify. An example of this is ^{203}Hg which occurs at an energy of 279.2 keV and has a spectral interference with ^{76}Se at an energy of 279.5 keV. This is also seen with ^{115}Cd at 336.3 keVand interfering nuclides ^{238}U and ^{58}Fe at 334.4 and 334.7 keV, respectively.

Fast neutron interactions also have an effect on NAA. An example is the case of ^{27}Al due to ^{31}P(n,α)^{27}Al reaction; for biological samples which typically have low mg/kg levels of Al and up to percentage levels of P, correction can be very significant. The correction factors applied to samples are calculated by irradiating small known quantities of pure Al and P and determining the γ peak areas of Al and Mg per microgram of Al, as $_{27}$Mg is produced from both the $_{26}$Mg (n, α) reaction and from the $_{27}$Al (n, p), and the peak area of Al per microgram of P.

The sensitivity of NAA for a particular nuclide is a function of the nuclear reaction cross-section and half-life of the produced nuclide, both of which are related to the fine structure of the nucleus. The LOD is similarly dependent on these nuclear constants but is also dependent on reactor and detector properties (i.e. the neutron spectrum and fluence, detector efficiency). The detection limits are also a function of matrix as this largely determines the analytical peak to background

ratios. The detection limits were calculated using the standard Currie definition [19] for LOD modified for NAA.

X-ray fluorescence is heavily matrix dependent, and sample homogeneity is of utmost important, so the technique creates challenges for analysis of varying types of foods. However, total reflection X-ray fluorscence (TXRF) may be useful for the analysis of low levels of some trace elements, as well as for phosphorus. Samples for analysis by TXRF must also be subjected to mineral decomposition as for spectrophotometric techniques.

21.5 Element data

21.5.1 Toxic elements

Arsenic

The Joint FAO/WHO Expert Committee on Food Additives [20] has suggested a provisional tolerable weekly intake (PTWI) of 0.015 mg/kg of body weight for inorganic arsenic. Inorganic arsenic species, As(III) and As(V) are toxic, whereas organoarsenicals are considered less toxic and arsenobetaine and arsenocholine are considered non-toxic. Plants accumulate arsenic through uptake by roots or atmospheric deposition of airborne arsenic [21]. In the Jamaican database fruits included banana, cucumber, orange, ortanique, plantain, tomato, pumpkin, squash, sweet pepper and zucchini. Total arsenic was assessed for these fruits as well as leafy vegetables (cabbage, callaloo, lettuce, pak choi), root vegetables (cassava, carrot, dasheen, ginger, Irish potato, yam) and other vegetables. The highest arsenic concentration was found in ginger at 0.064 mg/kg. The results were largely below the LOD for the techniques used for analysis. The UK Total Diet Study [14] reported arsenic in various food groups. The categories of green vegetables, other vegetables, canned vegetables, potatoes, fresh fruits and fruit products all had results for inorganic arsenic of less than 0.01 mg/kg. Green vegetables and other vegetables had total arsenic of 0.004 mg/kg and 0.005 mg/kg, respectively. The categories of potatoes, canned vegetables, fresh fruits and fruit products all had results that were below the limit of quantification (LOQ) of 0.005, 0.001, 0.001 and 0.003 mg/kg, respectively. The US FDA databases hold total arsenic concentrations for several fruits and vegetables. Raisins had the highest value of 0.014 mg/kg for total arsenic in the US FDA tables. Bundschuh *et al*. [22], in their 2012 review paper compiled a comprehensive dataset of arsenic concentrations for many foodstuffs including several fruits and vegetables across Latin America. For example, in Bolivia total arsenic in carrots ranged from 0.91 to 3.84 mg/kg dry weight. This compares with reported total arsenic in carrots in Bangladesh of 0.08–8.41 mg/kg dry weight [23]. In mining areas of Mexico both fruits and vegetables had total arsenic concentrations ranging from below the LOD of 0.02 mg/kg up to 11 mg/kg dry weight. In this area oranges had 8.4 mg/kg, avocados 5.7 mg/kg, bananas 2.5 mg/kg and lemons 0.9 mg/kg of total arsenic. Lettuce and cabbage in this area had less than 0.02 mg/kg total arsenic [22]. Raw avocado had a total arsenic concentration of 0.001 mg/kg in the FDA database; raw lettuce leaves had total arsenic results of 0.002 mg/kg. Carrots grown in the vicinity of the metallurgical complex of Vinto-Oruro in Bolivia showed ranges of total arsenic between 66.84 and 94.5 mg/kg dry weight [22]. This review paper also suggested that preparation of foods could affect the concentration of both total and inorganic arsenic. Vegetables grown in uncontaminated and contaminated soils had total arsenic results of less than 12 μg/kg fresh weight and unpeeled carrots grown in soils with arsenic content of 27 mg/kg had a total arsenic concentration of 18 μg/kg fresh weight [21]. In many of the databases only total arsenic was assessed. This makes it somewhat difficult to assess the true toxicity of the fruit or vegetable being investigated. The level of contamination in the soils, as well as additional contamination by atmospheric deposition, and plant type has a significant influence on the concentration of arsenic in the particular fruit or vegetable.

Cadmium

The symptoms of itai itai disease in Japan, caused by the consumption of cadmium-polluted rice, illustrated the potential for dietary exposure to cadmium to have deleterious effects [24]. The World Health Organization (WHO) has suggested a PTWI of 0.007 mg/kg of body weight for cadmium.

Jamaican fruits averaged 0.029 mg/kg, root vegetables averaged 0.4 mg/kg, leafy vegetables averaged 0.4 mg/kg and other root crops averaged 0.2 mg/kg for cadmium, all fresh weight. The maximum value for fruits was 0.16 mg/kg, for leafy vegetables 1.7 mg/kg, and for root vegetables 1.8 mg/kg [25]. Jamaican foods average

10–20 times higher cadmium concentrations than those found in the literature values. These high concentrations are not surprising as many of these crops are grown in bauxitic soils that have naturally elevated levels of several trace elements. Indeed, the cadmium content of some Jamaican foods varies widely, across three orders of magnitude in some cases and may exceed European Union permissible limits [26]. In comparison, fresh fruits in the UK Total Diet Study [14] averaged less than 0.001 mg/kg and fruit products averaged 0.004 mg/kg. Green vegetables in the UK Total Diet Study [14] averaged 0.006 mg/kg, other vegetables averaged 0.007 mg/kg and canned vegetables averaged 0.006 mg/kg. Potatoes were the vegetable category that averaged the highest, with a cadmium content of 0.028 mg/kg. Fresh fruits in the FDA tables ranged from 0.001 mg/kg where detected, to 0.064 mg/kg in lettuce leaves. Iceberg lettuce was second with 0.054 mg/kg. By contrast cooked food ranged from 0.001 mg/kg in green beans to 0.124 mg/kg in fresh boiled spinach. The processed foods in the FDA databases ranged from 0.001 mg/kg in frozen lemonade concentrate to 0.022 mg/kg in tomato sauce. Canned beets had cadmium concentrations of 0.018 mg/kg; second to this was pineapple juice with 0.004 mg/kg and other foods were generally quite low.

Literature from research conducted around the world has given a wide range of concentrations in a variety of fruits and vegetables. Intensively produced tomatoes sampled in Morocco had a cadmium concentration of 0.72–50.4 μg/kg wet weight with a mean of 7.58 μg/kg [27]. This compared with the means or range of means of fresh tomatoes in Kuwait (13.8–31.1 μg/kg), Pakistan (330 μg/kg), Brazil (2.50 μg/kg), Spain (14.2–19.9 μg/kg), Bangladesh (2390 μg/kg), Egypt (0.01 μg/kg) and Greece (1.0–8.0 μg/kg). In Poland a variety of fruits and fruit juices were analysed for cadmium. Strawberries averaged 0.021 mg/kg, cherries averaged 0.007 mg/kg, apples averaged 0.009 mg/kg, pears averaged 0.006 mg/kg and the mean for blackcurrants was 0.007 mg/kg, all on a fresh weight basis. In this study, orange juice had a mean of 0.015 mg/L, grapefruit juice averaged 0.010 mg/L, apple juice averaged 0.017 mg/L and blackcurrant juice averaged 0.021 mg/L for cadmium. The study concluded that the cadmium content of fresh fruits and juices was relatively low [28]. In Shillong, India a study looked at several vegetables; the cadmium ranged from undetected to 0.85 mg/kg dry weight found in beet stem. Leafy vegetables, coriander and lettuce as well as ginger were below LOD. Spinach averaged 0.28 mg/kg dry weight [29].

Lead

Jamaican fruits analysed for lead had a mean concentration of 0.011 mg/kg; leafy vegetables had a mean concentration of below 0.005 mg/kg, root vegetables had a mean of 0.010 mg/kg and other root crops had a mean of 0.022 mg/kg, all fresh weight [25]. The background concentration of lead in Jamaica soils is 45 mg/kg, which is significantly higher than the crustal average of about 13 mg/kg and is also higher than world averages [30]. When compared with fruits from Nigeria, Denmark and the USA, Jamaican fruits have on average higher lead content [25]. In the UK Total Diet Study [14], lead content of green vegetables averaged 0.004 mg/kg, other vegetables averaged 0.013 mg/kg, potatoes were below the LOQ of 0.003 mg/kg, and canned vegetables averaged 0.006 mg/kg. Fruit products had on average 0.007 mg/kg of lead while fresh fruits were below the LOQ of 0.002 mg/kg. The FDA had fresh fruits and vegetables ranging from 0.001 to 0.005 mg/kg with lettuce being the highest. Raw oranges, grapefruit, baby carrots and cantaloupes all had concentrations of 0.001 mg/kg. Lead content of cooked vegetables ranged from 0.001 to 0.003 mg/kg, with collard greens and spinach having the highest content. Processed food had generally higher lead content, ranging from 0.001 mg/kg in apple juice, apple sauce and frozen grapefruit juice to 0.013 mg/kg in canned pineapple juice. Canned sweet potatoes had a lead content of 0.012 mg/kg and raisins were relatively high at 0.009 mg/kg in the FDA database. Da Silva *et al*. [31] list carrots, lettuce, beetroots and raisins among possible food sources for lead.

In fruits analysed in Karachi, Pakistan [32], apples averaged 1.82 mg/kg, apricots averaged 1.6 mg/kg, bananas averaged 3.2 mg/kg, lemons and mangoes averaged 1.9 mg/kg and tomatoes 0.53 mg/kg all on a dry weight basis. In this work, analysis of similar vegetables available at the Yeshwantpur market in India found in some cases strikingly higher results. Apples averaged 23.48 mg/kg, bananas averaged 7.46 mg/kg, lemons averaged 58.7 mg/kg and mangoes averaged 67.20 mg/kg also on a dry weight basis. Elbagermi *et al*. [33] published results for lead content in fruits and vegetables produced and available at a market in Misurata, Libya. Apples averaged 0.2 mg/kg, bananas averaged 0.10 mg/kg,

mangoes averaged 1.8 mg/kg and tomatoes averaged 0.5 mg/kg all on a dry weight basis. Vegetables were also examined in this study; green peppers were highest at 0.47 mg/kg, spinach averaged 0.32 mg/kg with carrots at 0.21 mg/kg. Onions had a lead content of 0.14 mg/kg, cucumbers had a lead content of 0.10 mg/kg and potatoes averaged 0.02 mg/kg.

Many studies have investigated the growing of fruits and vegetables in areas contaminated by proximity to industrial activities or irrigation by wastewater. These activities appear to account for the wide variability that may be found in the lead content of various fruits and vegetables across databases and locations.

Mercury

Mercury tends to bioaccumulate in food chains, especially in contaminated aquatic systems, in its organic form; inorganic mercury does not appreciably accumulate in food chains [34]. Mushrooms may accumulate high concentrations of mercury if grown in soils with high concentrations [34]. Several studies have highlighted the ability of mushrooms to accumulate mercury in high concentrations, even in unpolluted soils [35, 36]. Some species accumulate up to 20 mg/kg dry weight of mercury in unpolluted soils but little information is available on the methylmercury content of mushrooms [36]. Mushrooms do accumulate methylmercury from the soil but also have the ability to methylate inorganic species of mercury [37]. Mushrooms had a value of 0.001 mg/kg fresh weight of total mercury. This was the only unprocessed food that had detectable mercury in the FDA database. Boiled collards (Brassica oleracea) also had 0.001 mg/kg total mercury while raisins had 0.0001 mg/kg mercury, both fresh weights. In the UK 2006 Total Diet Study [14], fresh fruits had mercury concentrations below 0.0005 mg/kg, fruit products has less than 0.0005 mg/kg, green vegetables less than 0.0005 mg/kg, canned vegetables less than 0.0006 mg/kg, potatoes less than 0.001 mg/kg and other vegetables were below the LOQ of 0.007 mg/kg. In the European Food Safety Authority's Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food [38], it was reported that in foods other than fish the concentrations of mercury were generally in the range 1–50 μg/kg and 80% of the samples tested were below the LOQ. The highest results were found in fungi and this again points to mushrooms as a potentially significant non-fish food source of mercury. Based on the research published it appears that the vast majority of commonly consumed fruits and vegetables are not significant sources of mercury.

The literature values suggest that fruits and vegetables are low in toxic elements. Where high concentrations of toxic elements are found in these foods these are likely as a result of high levels of these elements in the soil, whether naturally occurring or as a result of contamination, or possible enrichment due to irrigation with wastewater or fertilizers and pesticides. In the case of mercury, because fish and seafood are the predominant means of dietary exposure, there is relatively little research devoted to analysis of this element in fruits and vegetables compared with arsenic, cadmium and lead.

21.5.2 Macronutrients

Calcium

Calcium concentrations in fruits and vegetables do not vary much for a particular food from country to country, and generally levels are of the order of 10^1 to 10^2 mg/kg. The exception is cassava flour from West Africa, which contains 1170 mg/kg. In many cases calcium was not reported in the databases, and may be seen as more important for nutritional labelling of processed or packaged foods.

Comparison of data from Jamaica, Denmark, the USA, the UK and Nigeria showed significantly lower levels of Ca in Nigerian fruits, 7.65 mg/kg, compared with 360 mg/kg in Jamaican fruits and 113–139 mg/kg in fruits from the other countries [25]. Nigerian vegetables also showed significantly lower levels of Ca than those from the other countries in all vegetable categories, with leafy vegetables especially low (2.50 mg/kg). There was no significant variation in the concentrations in leafy vegetables from the USA, the UK and Denmark (379–484 mg/kg) but Jamaican leafy vegetables were much higher at 2581 mg/kg; root vegetables (208–390 mg/kg) and other root crops (109–403 mg/kg) showed some variation but were within the same order of magnitude.

Magnesium

Fruits and vegetables have relatively low concentrations of magnesium at 10^1 to 10^2 mg/kg. It is also not reported in many instances, and most foods do not show significant variation in concentration between countries. A comparison of fruits and vegetables from Jamaica,

Denmark, the USA, the UK and Nigeria did not show much variation in the Mg content of fruits, but again vegetables from Nigeria exhibited significantly lower concentrations than the other countries [25]: 40 mg/kg in leafy vegetables compared with 168–359 mg/kg from the other countries, 35 mg/kg compared with 126–159 mg/kg for root vegetables, and 14 mg/kg compared with 161–360 mg/kg for other root crops.

Phosphorus

Phosphorus concentrations in fresh fruits and vegetables are mostly less than 500 mg/kg, and generally less than 1000 mg/kg in cooked vegetables. Most foods do not show significant variation in concentration. The notable exception is the higher levels found in raisins, which contain 800–1079 mg/kg of phosphorus, while the other processed foods contain much lower levels. Comparison of data from Jamaica, Denmark, the USA, the UK and Nigeria did not show significant variation in phosphorus levels between countries or across food groupings [25].

Potassium

Most fruits and vegetables have potassium concentrations of the order of 10^3 mg/kg, with some having slightly lower values. Concentrations in fruits and vegetables from other countries are no higher, with the exception of raisins from all countries reporting values of 7320–7850 mg/kg (see Table 21.2). Jamaican fruits and vegetables do not show much variation between food groupings, with concentrations of 3800–4700 mg/kg across all food groups (see Table 21.4). However, data from Nigeria show significantly lower levels of potassium than reported elsewhere, differing by one to two orders of magnitude [25].

Sodium

The concentrations of sodium in fresh fruits and vegetables are very low, with most values under 100 mg/kg. Some values for cooked vegetables are higher, but it may not necessarily be specified if salt was added during the cooking process. Cooked potato from Finland is notable, with a sodium concentration of 1935 mg/kg. Root vegetables from Jamaica, averaging 727 mg/kg, also show higher levels of sodium (Table 21.5) and vegetables from Nigeria also contain somewhat higher levels of sodium [25]. The highest concentrations of sodium in the foods considered are found in processed tomato sauce/relish/canned tomatoes, containing 900–4988 mg/kg. Salt is obviously added during processing, and it is noteworthy that the highest concentration, from the USA, is almost twice the second highest, 2700 mg/kg, from Japan.

21.5.3 Trace elements

Chromium

From online publicly accessible databases only food from Denmark exhibited concentrations of chromium. Of this, pineapple and its juice both had chromium content of 110 mg/kg fresh weight. Raisins (88 mg/kg) had the second highest chromium content and apple juice had 26 mg/kg. Excluding pineapples, fresh fruits and vegetables ranged from undetected to 21 mg/kg, found in mushrooms. Pumpkins had 20 mg/kg, pears 18 mg/kg and both cucumber and cabbages 17 mg/kg.

Jamaican fruits averaged 0.04 mg/kg, leafy vegetables averaged 0.08 mg/kg, root vegetables averaged 0.09 mg/kg and other root crops 0.11 mg/kg on a fresh weight basis [25]. In comparison the UK Total Diet Study [14] found that green vegetables averaged less than the LOQ of 0.008 mg/kg, potatoes averaged 0.031 mg/kg, other vegetables averaged 0.024 mg/kg and canned vegetables had a mean of 0.039 mg/kg. Fresh fruits averaged below the LOQ of 0.007 mg/kg and fruit products had a mean of 0.017 mg/kg. Farid and Enani [39] published, among other measurands, data on chromium in several fruit juices available on the Saudi Arabian market. The mean concentration in apple juice was 6.39 μg/kg, in orange juice 5.93 μg/kg and in mango juice 7.64 μg/kg.

Chromium content of fruits in Karachi City, Pakistan ranged from 2.86 mg/kg in tomatoes to 4.34 mg/kg in bananas on a dry weight basis. Mangoes averaged 4.1 mg/kg, apricots 3.97 mg/kg, lemons averaged 3.97 mg/kg, apples averaged 3.93 mg/kg and dates had a mean of 4.18 mg/kg [32]. In general there was little variability in this database. In Shillong City, India vegetables ranged from 0.33 to 2.81 mg/kg (found in turmeric) with a mean of 1.03 mg/kg, all dry weight [29]. Some of the more common vegetables such as lettuce had a mean chromium content of 0.34 mg/kg, ginger averaged 1.34 mg/kg, spinach averaged 0.43 mg/kg, and coriander averaged 0.64 mg/kg. In Bangalore City, chromium content ranged from undetected in guavas and apples in the K.R. market to 227.50 mg/kg in lemons in the Yeshwantpur markets. Between the two markets in

Bangalore, the minimum detectable chromium was 8.75 mg/kg found in apples. Other notable averages for the fruits sampled include pomegranates (68 mg/kg in K.R market and 140 mg/kg in Yeshwantpur), bananas (37.87 mg/kg in K.R. market and 88.32 mg/kg in Yeshwantpur), mangoes (85.71 mg/kg in K.R market and 37.36 mg/kg in Yeshwantpur), oranges (32.82 mg/kg in K.R. market and 61.25 mg/kg in Yeshwantpur) and pears (47.61 mg/kg in K.R. market and 88.32 mg/kg in Yeshwantpur) [40]. There is obviously great variability in chromium content within this dataset, which is on a dry weight basis but these means appear to be significantly higher than those found in other locations.

Copper

Copper is an essential micronutrient for human nutrition. Jamaican research on elements including copper showed fruits averaging 0.8 mg/kg, leafy vegetables averaging 0.6 mg/kg, root vegetables averaging 0.7 mg/kg and other root crops averaging 1.6 mg/kg, fresh weight [25]. Green vegetables in the UK Total Diet Study [14] had a mean copper content of 0.580 mg/kg, potatoes averaged 1.12 mg/kg, other vegetables averaged 0.808 mg/kg and canned vegetables averaged 1.29 mg/kg. Fresh fruits averaged 0.786 mg/kg and fruit products had a mean of 0.544 mg/kg copper in this database. Australian and Finnish databases do not report copper, but other national food composition tables do report this measurand. The FDA tables report a range of undetected to a maximum of 3.3 mg/kg in raisins; the West African database reports a minimum of 0.2 mg/kg in cabbage and papaya and a maximum of 3.3 mg/kg in pineapples; Tanzanian tables range from undetected, to 4.0 mg/kg in potato; Malaysian foods range from undetected to 1 mg/kg in onions; Danish databases range from 0.06 mg/kg in apple juice to 4.2 mg/kg in mushrooms and Japanese food tables range from 0.2 mg/kg in several raw, cooked and processed fruits and vegetables including cabbage, grape juice and cooked turnips to 3.9 mg/kg in raisins. In similar food categories there was no significant variability in the means of copper between Jamaican and other countries. In comparison with the 0.8 mg/kg for Jamaican fruits, Denmark fruits averaged 0.93 mg/kg, US fruits averaged 0.75 mg/kg and UK fruits averaged 0.79 mg/kg. For root vegetables Denmark averaged 1.6 mg/kg, the US averaged 1.3 mg/kg and the UK averaged 1.0 mg/kg compared with Jamaica's 1.6 mg/kg. For root vegetables Denmark averaged 0.64 mg/kg, the UK averaged 0.85 mg/kg and the US averaged 0.7 mg/kg compared with 0.7 mg/kg for Jamaica. There was somewhat more variability in leafy vegetables with Denmark having a mean of 0.36 mg/kg, the UK averaging 0.76 mg/kg and the US averaging 0.32 mg/kg compared with Jamaica's 0.6 mg/kg [25].

Copper values ranged from 0.02 to 0.56 mg/kg with a mean of 0.17 mg/kg fresh weight in tomatoes sampled from Morocco [27]. In Bangladesh, Jolly *et al*. [41] analysed several vegetables for a number of elements including copper. Among the more common vegetables, spinach averaged 5.59 mg/kg, tomatoes averaged 3.62 mg/kg, radishes averaged 4.45 mg/kg, cauliflower had a mean of 4.59 mg/kg and carrots had a mean of 5.35 mg/kg, all dry weight. In fruits and vegetables sampled in Hyderabad, Pakistan, spinach averaged 5.0 mg/kg and mint averaged 19 mg/kg dry weight for leafy vegetables. For stem and root vegetables, potatoes averaged 5.0 mg/kg, gingers averaged 8.0 mg/kg, while onion and carrots both averaged 1.4 mg/kg, dry weight. Fruits such as tomatoes averaged 1.3 mg/kg while bananas averaged 1.1 mg/kg and okras and cucumbers both averaged 1.0 mg/kg [42]. Spinach sampled in Libya showed similar concentrations of copper to the Bangladesh and Pakistan research with a mean of 5.32 mg/kg dry weight. Carrots were also similar to the Bangladesh study at 5.0 mg/kg but varied from the Pakistan value.

Iodine

While only four of the online databases referenced included data for iodine, the data were included due to the element's essentiality for thyroid function. The levels of iodine reported in fruits and vegetables are generally quite low, with most being less than 20 μg/kg. However, some processed products contain higher concentrations of iodine, 40 μg/kg or more in fruit cocktail and canned pineapples from the USA, and in apple juice and grape juice from Australia. Considerably higher levels were also found in raw and cooked carrots from West Africa (190 and 200 μg/kg, respectively) and in raw mushrooms (235 μg/kg) and cooked sweet potato (119 μg/kg) from Finland. A survey of fruits and vegetables on the Swiss market found concentrations of 0.3–13 μg/kg (mean 3 μg/kg) in fruits and 1–22 μg/kg (mean 5 μg/kg) in vegetables [43]. While iodine data are not readily available for many fruits and vegetables, it appears that most are not significant sources of the

element, and the most significant dietary source would likely be iodized table salt.

Iron

Iron is a trace element that is essential for human nutrition. In excess it is also toxic. Several fruits and vegetables are good sources of non-haem iron [44]. Jamaican fruits average 6.7 mg/kg, leafy vegetables average 15.5 mg/kg, root vegetables average 12.9 mg/kg and other root crops average 9.6 mg/kg, all fresh weight [25]. The Japanese database ranges from trace to 23 mg/kg in raisins, although lettuce varies widely from 3.0 to 24 mg/kg. The FDA tables range from undetected to 19.8 mg/kg in raisins. Lettuce ranges from 2.8 to 15.2 mg/kg. The West African database ranges from 1 mg/kg in oranges to 46 mg/kg in cassava starch/flour. In the Tanzanian tables, iron content ranges from 0.6 mg/kg in grapefruits to 50 mg/kg in lettuce. The Australian food tables have a minimum of 0.1 mg/kg in orange juice to 10 mg/kg in okras. The Malaysian database ranges from 2 mg/kg in watermelons and apples to 46 mg/kg in onions. Denmark fruits and vegetables have a minimum of 1 mg/kg in pears to 24 mg/kg in raisins. Finnish fruits and vegetables range from undetected to 33 mg/kg in mushrooms. Somewhat surprisingly the UK Total Diet Study [14] did not include iron.

Iron in tomatoes sampled from Morocco ranged from 0.28 mg/kg to 1.89 mg/kg with a mean of 0.73 mg/kg, all fresh weight [27]. In vegetables sampled from Shillong City, India, iron content ranged from 73.2 to 251.3 mg/kg with a mean of 196.19 mg/kg dry weight [29]. The lowest value for iron in this study was in spinach which is surprising since in general spinach is considered a good source of non-haem iron [44]. The highest value was found in turmeric. Fruits sampled in Bangalore City, also in India, ranged from a low of 43.99 mg/kg in lemons in the Yeshwantpur market to a maximum of 344.01 mg/kg in pears in the K.R. market, all dry weight [40]. Zahir *et al*. [32] examined fruits sampled in Karachi City, Pakistan and these ranged from 7.9 mg/kg in tomatoes to 24.7 mg/kg in apples. In comparison, fruits and vegetables sampled for iron content in Hyderabad, Pakistan ranged from 14 mg/kg in potatoes to 290 mg/kg in mint, on a dry weight basis. Tomatoes were also relatively low in this study at 15 mg/kg while spinach was second with 220 mg/kg [42], further reiterating the status of green leafy vegetables as significant sources of iron. Jolly *et al*. [41] found iron to range from 32.90 to 210.30 mg/kg dry weight in vegetables in Bangladesh. The highest iron content was in spinach, with *Amaranthus*, a relative of spinach, averaging 195.00 mg/kg dry weight.

Manganese

The Australia, Finland, Malaysia and West Africa databases do not have manganese in their list of elements. The other databases from Denmark, Japan, Tanzania and the FDA have values for a number of fruits and vegetables. The maximum value for manganese in most of these databases is from either fresh pineapple fruit or pineapple juices. The range of maximums across these databases is 11.5–17 mg/kg on a fresh weight basis. The exception is the Tanzanian database where sweet potato ranges from 5.6 to 40 mg/kg. In the FDA database all foods apart from pineapple juice are under 3 mg/kg. In the Japan database roughly 83% of foods with quoted concentrations for manganese are under 3 mg/kg.

Analysis of Jamaican fruits and vegetables showed some variations, with fruits averaging 1.7 mg/kg, leafy vegetables averaging 8.3 mg/kg, root vegetables averaging 4.3 mg/kg and other root crops averaging 1.6 mg/kg on a fresh weight basis. The maximum value found across all elements was cowpea (*Vinga unguiculata*) with a manganese content of 27 mg/kg fresh weight [25]. The UK Total Diet Study [14] had fresh fruits averaging 1.56 mg/kg, and fruit products averaging 4.56 mg/kg. The mean for green vegetables was 2.06 mg/kg, potatoes 1.58 mg/kg, other vegetables 1.54 mg/kg and canned vegetables 1.71 mg/kg.

Although manganese is an essential element in human nutrition, it is regarded as a heavy metal. For this reason it is a measurand in several research publications on fruits and vegetables. The range of manganese in tomatoes sampled from Morocco was 0.06–1.11 mg/kg fresh weight. This was compared with tomatoes in Finland (mean 18 mg/kg), Mexico (mean 5 mg/kg) and Saudi Arabia (mean 7.4 mg/kg) and Australia (mean 20 mg/kg), with all results on a fresh weight basis. Tomatoes from Spain ranged from 0.32 to 6.3 mg/kg [27]. In fruits sampled in Karachi City, including apples, apricots, bananas, lemons, mangoes and tomatoes among others, manganese contents averaged between 0.013 and 0.612 mg/kg dry weight [32]. In data from Bangalore markets, India [40] manganese content of fruits analysed ranged from 8.75 to 417.39 mg/kg dry weight. Bananas from the Yeshwantpur Market recorded the

maximum concentration; at the K.R. market also in Bangalore the maximum of 272.13 mg/kg was found in pears. In Shillong City, India [29] vegetables including beet leaves, beet stem, coriander, ginger, lettuce and spinach ranged from 14.17 to 127.97 mg/kg with a mean value of 61.22 mg/kg dry weight. The lowest levels were found in lettuce and the highest in beet leaves.

Nickel

Nickel has never been shown to have an essential role in human nutrition [3]. The primary means of exposure to nickel for the general population is via diet, although only about 1% is absorbed from foods. Nickel is most commonly seen as an allergen and anywhere from 10 to 20% of the population is allergic to this metal [45]. Many databases do not report nickel, likely because it is not an essential element and because it is usually found at low levels in the environment and so very powerful and sensitive techniques are required to detect this measurand in foods [45].

In the UK Total Diet Study [14], nickel content in green vegetables averaged 0.086 mg/kg, potatoes averaged 0.07 mg/kg, other vegetables averaged 0.079 mg/kg and canned vegetables averaged 0.338 mg/kg. Fresh fruits averaged 0.036 mg/kg and fruit products averaged 0.066 mg/kg. Only the FDA and Denmark databases report nickel concentrations. In the FDA tables the nickel content of various foods ranges from undetected to 301 μg/kg in avocados on a fresh weight basis. Apart from lettuce, which ranges from 65 to 135 μg/kg, and tomato sauce, which averages 102 μg/kg, no other foods have means over 100 μg/kg, although peaches are relatively close at 93 μg/kg. The minimum detected nickel is found in mushrooms at 2 μg/kg with apples also low at 5 μg/kg. In the Danish table nickel ranges from undetected to a maximum of 597 μg/kg in avocadoes; fresh pineapples are also relatively high at 500 μg/kg. The minimum detected nickel is found in grapefruit juice and mushrooms at 3 μg/kg. Several processed fruit and vegetable products are relatively high in nickel as well. These include raisins at 178 μg/kg, canned peaches at 167 μg/kg and canned pineapples at 147 μg/kg. In comparison, the FDA reports 81, 36 and 88 μg/kg for these foods, respectively. Peaches in the Danish table average 151 μg/kg. Watermelon is similar in both tables at 72 μg/kg in the FDA tables and 70 μg/kg in the Denmark table.

Jolly *et al*. [41] only detected nickel in radishes, averaging 0.87 mg/kg and cauliflower with a mean of 0.94 mg/kg dry weight. All other vegetables were below the LOD of 0.65 mg/kg. Indian vegetable nickel content ranged from 1.27 to 9.60 mg/kg with a mean of 4.75 mg/kg dry weight. In fruits sampled in Pakistan, nickel content ranged from 0.144 mg/kg in tomatoes to 5.033 mg/kg in mangoes on a dry weight basis. Dates had the second highest nickel content at 1.818 mg/kg and bananas third with 1.316 mg/kg [32]. In eastern Serbia, apples, pears, apricots and peaches were analysed for several measurands including nickel. These fruits were sampled from areas in close proximity to industrial activity. Apples averaged 4.39 mg/kg, pears averaged 4.14 mg/kg, peaches averaged 1.26 mg/kg and apricots averaged 9.37 mg/kg all on a dry weight basis [46]. In comparison, fruits sampled in Bangalore City showed even higher ranges for nickel content. A maximum of 97.72 mg/kg was found in apples in the K.R. market while apples in the Yeshwantpur market averaged 47.43 mg/kg. Grapes sampled from the K.R. market had 44.42 mg/kg nickel while from the Yeshwantpur market these fruits averaged 37.15 mg/kg, all dry weight [40]. By comparison, where mangoes had undetectable nickel in the Yeshwantpur market and 14.07 mg/kg in the K.R. market, the 5.033 mg/kg in mangoes in Karachi, Pakistan represented the maximum value. The influence of soil geochemistry and irrigation practices among other factors may be responsible for the variability in this non-essential element.

Selenium

Selenium is not widely reported for fruits and vegetables in the online databases surveyed and the levels reported in most are less than 20 μg/kg, except for raw mushrooms with reported concentrations of 131 μg/kg (USA), 64.7 μg/kg (Denmark) and 171 μg/kg (Finland).

Concentrations for some foods may also be found in peer-reviewed publications. Fresh apricots grown in several regions of Turkey were found to contain selenium concentrations of 84–124 μg/kg (mean 108 μg/kg), while dried apricots contained 260–360 μg/kg (mean 312 μg/kg) [47]. Apricots are high in selenium compared with other fruits, but concentrations may vary significantly depending on variety and growing region [47].

Fruits and vegetables from Thailand were found to contain on average 6 and 12 μg/kg of selenium, respectively [48]. The concentrations in fruits and vegetables grown in eastern Croatia ranged from 6.3 μg/kg (cucumber)

to 66.1 μg/kg (cabbage), while other purchased fruits and vegetables ranged from 7.6 μg/kg (orange) to 151.6 μg/kg (mushroom) [49]. Fruits and vegetables grown or purchased in south-eastern Spain were mostly found to contain low levels of selenium (most <5 μg/kg), with the highest concentrations of 28.11 μg/kg and 29.65 μg/kg being found in two varieties of cultivated mushrooms; olives were found to contain 12.96 μg/kg [50]. Analysis of fruits and vegetables in the Slovak Republic also yielded mostly low levels of selenium, with the maximum of 239.8 μg/kg found in a variety of mushroom [51]. Fruits and vegetables from Slovenia had concentrations of 0.3–77 μg/kg, the highest value being found in cabbage [52]. Fruits and vegetables purchased in Greece contained 3.4 and 6.5 μg/kg of selenium, respectively [53]. Selenium in fresh fruits and vegetables, canned vegetables, fresh fruits and fruit products from the UK contain from less than 5 to 18 μg/kg [14]. Most fruits and vegetables do not contain significant amounts of selenium, with mushrooms and apricots being the clear exceptions.

Zinc

Zinc is an essential element and as such is found in many national food databases. Jamaican fruits average 2.6 mg/kg, leafy vegetables average 4.2 mg/kg, root vegetables average 3.4 mg/kg and other root crops average 5.8 mg/kg, fresh weight [25]. In the UK Total Diet Study [14], the mean for green vegetables is 3.26 mg/kg, potatoes average 3.66 mg/kg, other vegetables a mean of 2.62 mg/kg and canned vegetables a mean of 3.33 mg/kg. Fresh fruits average 0.89 mg/kg and fruit products average 0.61 mg/kg. The FDA databases range from undetected to a maximum of 5.3 mg/kg in avocados. The Japanese database ranges from trace in several foods to 7 mg/kg in broccoli and avocados. The Malaysian database does not have many foods with zinc concentrations but these range from undetected in watermelon to 13 mg/kg in okra. The Danish food database records a minimum concentration of zinc of 0.32 mg/kg in apples and a maximum of 6.4 mg/kg in avocados; okra has 6 mg/kg. The Tanzanian database ranges from undetected in apples, grapes, mangoes and grape juice to 7 mg/kg in cassava flour. It is noteworthy that both okra and avocados have a zinc content of 6 mg/kg. The West African database ranges from 1 mg/kg in orange and watermelon to 30 mg/kg in okra; pineapples have a zinc content of 22 mg/kg. The Australian database ranges from 0.2 mg/kg in orange juice to 23.5 mg/kg in both raw and cooked cassava samples. The Finnish food database ranges from undetected to 8 mg/kg in mushrooms. As reported in Howe *et al*. [25] in a comparison between the means of Jamaican fruits and vegetable groups and others including Denmark, the UK, USA and Nigeria, except for legumes the zinc content is fairly similar between these countries and Jamaica.

Zinc is also a heavy metal and because of this is a measurand of interest in many research publications. The zinc content of tomato samples from Morocco ranged from 0.08 to 1.16 mg/kg with a mean of 0.44 mg/kg, fresh weight [27]. This was compared to the zinc content of tomatoes around the world. Mexican tomatoes averaged 7 mg/kg, Saudi Arabian tomatoes averaged 14.4 mg/kg, Pakistani tomatoes averaged 2.5 mg/kg, Egyptian tomatoes averaged 7.7 mg/kg, Finnish tomatoes averaged 12 mg/kg and Bangladeshi tomatoes averaged 29 mg/kg, all fresh weight. Zinc content of tomatoes from Spain ranged from 6.9 to 36 mg/kg, all fresh weight [27]. The analysis of zinc content in fruit juices available on the Saudi Arabian market found apple juice averaging 0.52 mg/kg, a mean of 0.89 mg/kg for orange juice, and mango juice averaging 0.49 mg/kg [39]. Elbagermi *et al*. [33] analysed for zinc in several fruits and vegetables sampled in Libya. Converting the dry weight into wet weight because of the given moisture content of fruits and vegetables, the authors found that zinc ranged from a minimum of 0.004 mg/kg in green peppers to a maximum of 1.76 mg/kg in potatoes. Spinach sampled averaged 1.70 mg/kg and onions averaged 1.55 mg/kg, all fresh weight. Tomatoes averaged 0.51 mg/kg, carrots had a mean of 0.52 mg/kg and cucumbers averaged 0.55 mg/kg. This makes the green pepper extremely low in comparison with the other vegetables. The vegetables averaged 0.94 mg/kg fresh weight while the fruits averaged 0.44 mg/kg, with the lowest being mangoes at 0.11 mg/kg and the maximum bananas averaging 1.59 mg/kg. Other fruits of note were melons (0.62 mg/kg), peaches (0.57 mg/kg) and apples (0.35 mg/kg). Fresh fruits were sampled from two markets in Bangalore city and concentrations found based on sample dry weight. The zinc content of these fruits ranged from 17.40 mg/kg in grapes from the K.R. market to 45.64 mg/kg in bananas in the Yeshwantpur market [40]. Comparing these with the dry weight concentrations for the foods sampled in Libya shows variability. For instance the dry weight concentration of the bananas sampled in Libya was 6.84 mg/kg while the bananas in the K.R.

market was 32.04 mg/kg. Mangoes averaged 0.634 mg/kg dry weight in the Libyan study, while in the Bangalore study the dry weight concentrations were 38.53 and 28.97 mg/kg for the K.R. market and Yeshwantpur market, respectively. Finally, apples in Libya averaged 2.34 mg/kg dry weight while they averaged 31.97 mg/kg between the two markets in Bangalore city.

Abbreviations

AAS: Atomic absorption spectrophotometry
FAAS: Flame atomic absorption spectrophotometry
FAO: Food and Agriculture Organization of the United Nations
FDA: Food and Drug Administration
FFQ: Food frequency questionnaire
GFAAS: Graphite furnace atomic absorption spectrophotometry
HPGe: Hyperpure germanium
ICP-MS: Inductively coupled plasma mass spectroscopy
ICP-OES: Inductively coupled plasma optical emission spectroscopy
INFOODS: International Network of Food Data Systems
INAA: Instrumental neutron activation analysis
ICENS: International Centre for Environmental and Nuclear Sciences
JECFA: Joint FAO/WHO Expert Committee on Food Additives
LOD: Limit of detection
LOQ: Limit of quantification
NAA: Neutron activation analysis
PTWI: Provisional tolerable weekly intake
RM: Reference material
SLOWPOKE: Safe LOW POwer C(K)ritical Experiment
TDS: Total Diet Study
TXRF: Total reflection X-ray fluorescence
WHO: World Health Organization

References

1 Food and Agriculture Organization of the United Nations/World Health Organization. *Fruits and Vegetables for Health. Report of a Joint FAO/WHO Workshop*, 1–3 September 2004, Kobe, Japan. Available at http://www.fao.org/ag/magazine/fao-who-fv.pdf.

2 US Food and Drug Administration. *Total Diet Study Statistics on Element Results*, 2006–2008. College Park, MD, 2010.

3 McDowell LR. *Minerals in Animal and Human Nutrition*. Academic Press, San Diego, CA, 1992.

4 Ridge I, Bell M, Murphy P, Parker P. *Biology: Plant Physiology: Form and Function*, 2nd edn. Hodder Education, London, 1991.

5 Food and Agriculture Organization of the United Nations. *The State of Food and Agriculture*. FAO, Rome, 2013.

6 Wolmarans P, Danster NA. Characteristics of the South African Food Composition Database, an essential tool for the nutrition fraternity in the country: Part I. *S Afr J Clin Nutr* 2008;**21**:308–313.

7 Food and Agriculture Organization of the United Nations. *Composition of Selected Foods from West Africa*. FAO, Rome, 2010.

8 Lukmanji Z, Hertzmark E. *Tanzania Food Composition Tables*. Muhimbili University College of Allied and Health Sciences, Tanzania Food and Nutrition Centre and Harvard School of Public Health, Dar es Salaam, Tanzania, 2008.

9 Food Standards Australia New Zealand (FSANZ). *AUSNUT 2007: Australian Food, Supplement and Nutrient Database 2007 for estimation of population nutrient intakes*. FSANZ, Canberra, 2008.

10 NutriWeb Malaysia. Malaysian Foods Composition Database. Available at http://www.nutriweb.org.my/searchfood.php.

11 DTU Food National Food Institute. Danish Food Composition Databank. Available at http://www.foodcomp.dk/v7/fcdb_default.asp

12 National Institute for Health and Welfare. *Fineli® Finnish Food Composition Database*. National Institute for Health and Welfare, Finland. Available at http://www.fineli.fi/index.php?lang=en

13 Sugiyama University. Standard Tables of Food Composition in Japan, 5th revised edition, 2000. Available at http://database.food.sugiyama-u.ac.jp/index_asia.php

14 Food Standards Agency. *Survey on measurement of the concentrations of metals and other elements from the 2006 UK Total Diet Study*. Food Standards Agency, London, 2009. Available at http://tna.europarchive.org/20140306205048/http://www.food.gov.uk/science/research/surveillance/fsisbranch2009/survey0109

15 Kennedy G, St-Pierre J, Wang K *et al*. Activation constants for Slowpoke and MNS reactors calculated from the neutron spectrum and k0 and Q0 values. *J Radioanal Nucl Chem* 2000;**245**:167–172.

16 Kennedy G, St-Pierre J. NAA with the relative improved method and the interactive computer program EPAA. *J Radioanal Nucl Chem* 1993;**169**:471–481.

17 Vutchkov M, Grant CN, Lalor GC, Preston J. Standardization of the Slowpoke-2 reactor in Jamaica for routine NAA. *J Radioanal Nucl Chem* 2000;**224**:355–359.

18 Lalor GC, Vutchkov MK, Grant C, Preston J, Favaro DIT, Figueiredo AMG. INAA of trace elements in biological materials using the SLOWPOKE-2 reactor in Jamaica. *J Radioanal Nucl Chem* 2000;**244**:263–266.

19 Currie LA. Limits for qualitative detection and quantitative determination: application to radiochemistry. *Anal Chem* 1968;**40**:586–593.
20 Joint FAO/WHO Expert Committee on Food Additives. *Evaluation of Certain Contaminants in Food: Seventy-second Report of the Joint FAO/WHO Expert Committee on Food Additives*. WHO Technical Report Series 959. WHO, Geneva, 2010.
21 Agency for Toxic Substances and Disease Registry (ATSDR).Toxicological profile for arsenic, August 2007. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=22&tid=3.
22 Bundschuh J, Litter MI, Parvez P *et al*. One century of arsenic exposure in Latin America: a review of history and occurrence from 14 countries. *Sci Total Environ* 2012; **429**:2–35.
23 Laizu J. Speciation of arsenic in vegetables and their correlation with inorganic phosphate levels. *Bangladesh J Pharmacol* 2007;**2**:88–94.
24 Inaba T, Kobayashi E, Suwazono Y *et al*. Estimation of cumulative cadmium intake causing Itai-itai disease. *Toxicol Lett* 2005;**159**:192–201.
25 Howe A, Hoo Fung L, Lalor G, Rattray R, Vutchkov M. Elemental composition of Jamaican foods 1: a survey of five food crop categories. *Environ Geochem Health* 2005;**27**:19–30.
26 Lalor GC. Review of cadmium transfers from soil to humans and its health effects in the Jamaican environment. *Sci Total Environ* 2008;**400**:162–172.
27 Luis G, Hernandez C, Rubio C *et al*. Trace elements and toxic metals in intensively produced tomatoes (Lycopersicon esculentum). *Nutr Hosp* 2012;**27**:1605–1609.
28 Krejpcio Z, Sionkowski S, Bartela J. Safety of fresh fruits and juices available on the Polish markets as determined by heavy metal residues. *Polish J Environ Stud* 2005;**14**:877–881.
29 Marbaniang DB, Baruah P, Decruse R, Dkhar ER, Diengdoh DF, Niongpur CL. Study of the trace metal concentration in some local vegetables available in Shillong City, Meghlaya, India. *Int J Environ Prot* 2012;**2**:24–48.
30 Lalor G. *A Geochemical Atlas of Jamaica*. Canoe Press, Kingston, Jamaica, 1996.
31 da Silva ALO, Barrocas PRG, do Couto Jacob S, Moreira JC. Dietary intake and health effects of selected toxic elements. *Braz J Plant Physiol* 2005;**17**:79–93.
32 Zahir E, Naqvi II, Udin SM. Market basket survey of selected metals in fruits from Karachi City (Pakistan). *J Basic Appl Sci* 2009;**5**:47–52.
33 Elbagermi MA, Edwards HGM, Alajtal AI. Monitoring of heavy metal content in fruits and vegetables collected from production and market sites in the Misurata area, Libya. *ISRN Anal Chem* 2012; Article ID 827645. doi:10.5402/2012/827645.
34 Agency for Toxic Substances and Disease Registry (ATSDR).Toxicological profile for mercury, March 1999. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=115&tid=24.
35 Melgar MJ, Alonso J, García MA. Mercury in edible mushrooms and underlying soil: bioconcentration factors and toxicological risk. *Sci Total Environ* 2009;**407**: 5328–5334.
36 Kalač P, Svoboda L, Havlíčková B. Contents of cadmium and mercury in edible mushrooms. *J Appl Biomed* 2004;**2**: 15–20.
37 Fischer RG, Rapsomanikis S, Andreae MO, Baldi F. Bioaccumulation of methylmercury and transformation of inorganic mercury by macrofungi. *Environ Sci Technol* 1995;**29**:993–999.
38 European Food Safety Authority. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA J* 2012;**10**:2985.
39 Farid SM, Enani MA. Levels of trace elements in commercial fruit juices in Jeddah, Saudi Arabia. *Med J Islamic World Academy of Sciences* 2010;**18**:31–38.
40 Mahdavian SE, Somashekar RK. Heavy metals and safety of fresh fruits in Bangalore City: a case study. *Kathmandu University J Sci Eng Tech* 2008;**1**:17–27.
41 Jolly YN, Islam A, Akbar S. Transfer of metals from soil to vegetables and possible health risk assessment. *SpringerPlus* 2013;**2**:385–392.
42 Ismail F, Anjum MR, Mamon AR, Kazi TG. Trace elements of vegetables and fruits of Hyderabad retail market. *Pakistan J Nutr* 2011;**10**:365–372.
43 Haldimann M, Alt A, Blanc A, Blondeau K. Iodine content of food groups. *J Food Compos Anal* 2005;**18**:461–471.
44 National Institutes of Health. Iron in diet. Available at http://www.nlm.nih.gov/medlineplus/ency/article/002422.htm.
45 Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for nickel, August 2005. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=245&tid=44
46 Kalinovic JV, Serbula SN, Kalinovic TS, Ilic AA. Content of heavy metals and sulphur in fruits sampled in vicinity of mining–metallurgical complex. In: *16th International Research/Expert Conference 'Trends in the development of machinery and associated technology'*, 10–12 September 2012, Dubai, UAE.
47 Munzuroglu O, Karatas F, Geckil H. The vitamin and selenium contents of apricot fruit of different varieties cultivated in different geographical regions. *Food Chem* 2003;**83**:205–212.
48 Sirichakwal P, Puwastien P, Polngam J, Kongkachuichai R. Selenium content of Thai foods. *J Food Compos Anal* 2005;**18**:47–59.
49 Klapec T, Mandić ML, Grgić J, Primorac LJ, Perl A, Krstanovic C. Selenium in selected foods grown or purchased in eastern Croatia. *Food Chem* 2004;**85**: 445–452.
50 Diaz-Alarcon JP, Navarro-Alarcon M, Lopez-Garcia de la Serrana H, Lopez-Martinez MC. Determination of selenium

levels in vegetables and fruits by hydride generation atomic absorption spectrometry. *J Agric Food Chem* 1994;**42**: 2848–2851.

51 Kadrabova J, Madaric A, Ginter E. The selenium content of selected food from the Slovak Republic. *Food Chem* 1997;**58**:29–32.

52 Smrkolj P, Pograjc L, Hlastan-Ribic C, Stibilj V. Selenium content in selected Slovenian foodstuffs and estimated daily intakes of selenium. *Food Chem* 2005;**90**:691–697.

53 Pappa EC, Pappas AC, Surai PF. Selenium content in selected foods from the Greek market and estimation of the daily intake. *Sci Total Environ* 2006;**372**:100–108.

CHAPTER 22
Cereals and pulses

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Abstract: Available and commonly used methodologies for determining the mineral composition of cereals and pulses have been reviewed in recently published papers from 2000 to 2013. Data on the presence of major, minor, trace and ultratrace elements in barley, maize, millet, oat, rice, rye, wheat and other cereal products, together with beans, chickpeas, lentils, peas, peanuts and soybean, from different origins have been compiled to provide a picture of the state of the art in the field and the potential contribution of cereals and pulses to the daily intake of minerals.

Keywords: cereals, pulses, trace elements, arsenic speciation, selenium speciation

22.1 The importance of cereals and pulses in the daily intake of minerals

Cereals and pulses are staple foodstuffs of all the regional diets around the world; they have a key socioeconomic role in countries such as China, India, the USA and Brazil. The most important cereals include maize, rice and wheat; these three top the worldwide production rankings, just below sugar cane, with production in 2012 of 872.1, 719.7 and 670.9 million tonnes, corresponding to a trade value of US$53.6, 18.6 and 79.3 billion, respectively. With regard to pulses, soybean production in 2012 was 241.8 million tonnes, corresponding to a value of US$60.7 billion [1].

Cereals and pulses have an important nutritional value because of their high content of protein, carbohydrates, dietary fibres and minerals such as Cr, Cu, Fe, Mn, Mo, Ni, Se and Zn [2–4] and help to prevent and manage diabetes [5], cardiovascular and renal disease [6–8] and some types of cancer [9]. The most popular cereals consumed by humans are barley (*Hordeum vulgare*), maize (*Zea mays*), millet (*Pennisetum typhoides*), oat (*Avena sativa*), rice (*Oryza sativa*), rye (*Secale cereale*) and wheat (*Triticum aestivum*, *Triticum durum*); derived cereal products, such as pasta, breakfast cereals and bread, are commonly consumed around the world. Some of the most frequently consumed pulses include beans such as pinto beans, kidney beans and green beans (*Phaseoulus vulgaris*), mungbeans (*Phaseoulus aereus*) cowpea beans (*Vigna unguiculata*), azuki beans (*Vigna angularis*), black beans (*Vigna mungo*) and broad beans (*Vicia faba*), followed by chickpeas (*Cicer arietinum*), lentils (*Lens culinaris*), peas (*Pisum sativum*), peanuts (*Arachis hypogaea)* and soybean (*Glycine max*), and these play an important role in different cultures.

Rice is considered to be much more nutritious and a much better source of minerals than other cereals [10], especially in the developing countries due to its daily consumption [11, 12]. For example, for Chinese people, it serves as the major source of Fe, Ca and Zn [13]; it is also, together with wheat, the main source of Mn for humans [14]. However, rice is also a major source of inorganic As forms in the human diet [15]. Although most edible vegetables do not accumulate As at a high rate, rice, carrots and certain others are the exceptions [16]. Some authors have found that rice-producing areas such as Bangladesh, West Bengal, China and Taiwan typically manifest very high As contents in the soil and water, contributing to the high content of As in rice [16]. Conversely, other authors have found higher levels of As in the rice produced in the USA and Europe and have established a global 'normal' range of 0.08–0.2 μg/g in rice [17].

Regarding the mineral content of wheat, many studies have been conducted because of its numerous uses

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in derived wheat products [18]. For example, bread is a source of energy and also supplies irreplaceable nutrients to humans in many countries worldwide [13, 19–24]. Pasta obtained from wheat is a widely consumed foodstuff in Italy and is also a food item of growing popularity in many countries worldwide [22, 23, 25, 26]. Several publications have focused on how levels of minerals are affected by processing, such as the milling of wheat and derived product manufacture [27, 28]. Especially important is wheat Se input; studies have shown that the Se content of cereal crops in different countries and even different regions can vary as much as 1000 times depending on the soil [29, 30]. However, fertilization of soils and effective root uptake of Se in wheat has enabled the development of supplementation programmes to introduce this element into diets deficient in selenium [31].

The scientific literature also highlights the general importance of cereals as one of the main sources of Cr, whose required dietary intake is not usually achieved in the industrialized nations (it has even been named the 'geriatric' nutrient) [32]; a similar situation exists with Mn, Fe, Ni, Mo and Zn. Pulses are important sources of Cu, Fe, Mn, Mo, Ni and Zn, all essential elements required for good health [33, 34]. On the other hand, harmful elements such as As, Pb, Hg, Br and Cd are also present in cereals and pulses due to plant root uptake from contaminated soils and water, or by direct foliate absorption via the use of fumigants such as methyl bromide [33, 35]. Therefore it is absolutely necessary to assess the content of microelements that are considerate essential (e.g. Ca, K, P, Fe, Ni, Cu Zn, Cr, Mn, Co, Ni and Mo) and those which could be potentially toxic at high levels (e.g. Al, Ti, Ba, Br, Pb, Hg, Cd and As) [34] in both cereals and pulses due to their high consumption by humans. Data need to be collected if not available, and updated for all regions of the world. This information will be useful in setting baseline levels that may help in the preparation of balanced and therapeutic diets containing the correct amounts of trace elements [36, 37] and provides an opportunity to develop supplementation programmes for elements like Se [38, 39] and Fe [40, 41] that are deficient in the diets of many populations.

Additionally, studies on the speciation of minerals in these basic foods and the evaluation of bioavailability [13, 33, 40, 42–44] of the considered elements are of great importance in the correct evaluation of human diets in different countries.

22.2 Methods to determine the mineral composition of cereals and pulses

This chapter reviews the most relevant papers from the recent scientific literature (2000–2013) that provide data about the mineral composition of cereals and pulses. Information on sample, sample origin, analytical methodology employed and analysed elements has been summarized in five tables. Table 22.1 (rice) and Table 22.2 (wheat) have been compiled separately due to the large number of papers on these topics. In Table 22.3 we have summarized the studies on the other consumed cereals, such as barley, maize, millet, oat and rye. Table 22.4 summarizes the papers covering products derived from cereals and Table 22.5 compiles information about the mineral profile of pulses.

22.2.1 Analytical procedures

In our review of the literature, the techniques commonly used to determine the presence of mineral elements in cereals and pulses include atomic absorption spectrometry (AAS), comprising 30% of the papers reviewed, inductively coupled plasma mass spectrometry (ICP-MS) [20, 76, 140], also comprising 30% of the papers, and inductively coupled plasma optical emission spectroscopy (ICP-OES), also comprising 30%. The other techniques comprise a much smaller proportion and include methods based on atomic fluorescence spectrometry (AFS) [55, 97], voltammetry [18, 89], neutron activation analysis (NAA) [23, 29] and molecular spectrometry [117].

With regard to AAS techniques, flame AAS (FAAS) remains a popular technique for trace element determination and is usually used in combination with a preconcentration step that allows determination of measurable analyte concentrations. For example, solid-phase extraction [82, 122, 130, 158, 163], generally involving chelating resins or metal complexation followed by retention on an adsorbent support or the use of nanoparticles [50, 147] or knotted reactors [78], together with cloud point extraction [132, 134, 157] have been employed in recent years to increase the

Table 22.1 Published papers on total mineral content of rice with reference to sample treatment and analytical techniques.

Sample	Origin	Elements	Treatment	Technique	Reference
Rice	Spain (market)	As	MAD with HNO_3–H_2O_2	ICP-MS	[25]
Rice	—	As	MAD	ICP-MS	[45]
Rice	China	Cd	Solid sampling	SS-ETV-AFS	[35]
Rice and rice flour	Greece, India, Thailand	As	MAD with HNO_3	ETAAS	[46]
Rice	Iran	Fe and Mn	Hot plate with HNO_3–H_2O_2	UV/Vis	[47]
Rice	Iran	Mn and Pb	Dry ashing, HNO_3	SPE-FAAS	[48]
Rice	Iran	Pb	Dry ashing, HCl	CPE-ETAAS	[49]
Rice	Iran	Pb	Heating with HNO_3	MSPE (magnetic)-FAAS	[50]
Rice	Turkey	Sb	MAD with HNO_3–H_2O_2	GFAAS	[51]
Rice	China	Se	Hot plate with HNO_3–$HClO_4$	CPE-GFAAS	[52]
Rice	China	Se	Digestive stove with HNO_3–H_2O_2	HFS-AAS	[53]
Rice	South Asia, Malaysia, Vietnam, Thailand	As	MAD with HNO_3–H_2O	ICP-MS	[54]
Rice	China	Cd	MAD with HNO_3–H_2O_2, heating with H_2O_2	Electrolytic HG-AFS	[55]
Rice	India, Pakistan, Thailand, USA, Guyana, Italy (Jamaican market)	Ag, Al, As, B, Ba, Br, Ca, Cd, Ce, Co, Cr, Cs, Cu, Eu, Fe, Hf, Hg, K, La, Mg, Mn, Mo, Na, P, Rb, S, Sb, Sc, Se, Sm, Sr, Th, Ti, U, V and Zn	Heating block with HCl–HNO_3	ICP-OES, FAAS, INAA, TXRF	[56]
Rice	China	As	Microwave with HNO_3	DRC-ICP-MS	[57]
Rice	Japan	As, Ca, Fe, K, Mg, Mn, Na, P and Zn	Microwave with HNO_3–H_2O_2	ICP-OES, ICP-MS	[58]
Rice	Taiwan, USA, India, Italy, Thailand, Canada, Sri Lanka, France, Spain, Egypt, Switzerland, Uruguay, Himalaya, Laos, Cambodia, Pakistan, China, Turkey, Madagascar, Japan	As	High-pressure asher with HNO_3	ICP-MS	[59]
Rice	Brazil	Cd	Digester block and cold finger with HNO_3–H_2O_2	ETAAS	[60]
Rice	Japan, Australia, USA, China, Thailand	Sr and Pb	Digestion with HNO_3–H_2O_2	HR-ICP-MS	[61]
Rice	Taiwan (market)	As and Se	MAD with HNO_3–H_2O_2	DRC-ICP-MS	[62]
Rice	Spain, Japan, Brazil, India	Al, As, Ba, Bi, Ca, Cd, Ce, Cr, Co, Cu, Er, Eu, Fe, Ho, K, La, Li, Mg, Mn, Mo, Na, Nd, Ni, Pb, Pr, Se, Sm, Sr, Ti, Tl, Yb and Zn	Digestion with H_2SO_4–H_2O_2	ICP-OES	[63]

(Continued)

Table 22.1 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Rice and rice semolina	Brazil (market)	As	Microwave with HNO_3–H_2O_2	DRC-ICP-MS	[64]
Rice semolina	Spain (market)	Sb, Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[65]
Rice	Italy	As	High-pressure asher with HNO_3	ICP-MS	[66]
Rice	Spain (market)	As	MAD with HNO_3–H_2O_2	ICP-MS	[67]
Rice (Se-enriched)	China	Se	Digestion block with HNO_3	ICP-MS	[68]
Rice	Brazil, Japan, Spain, Thailand	Hg	MAD with HNO_3–H_2O_2	HG-AFS	[14]
Rice and rice semolina	Spain (market)	As, Bi, Sb, Se and Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[69]
Rice	—	As	Heating block with HNO_3	ICP-MS	[70]
Rice	China, Japan	Al, As, B, Ba, Ca, Cd, Co, Cr, Cs, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Se, Sr, Tl, V and Zn	MAD or dry ashing	ICP-OES, ICP-MS	[71]
Rice	Italy, Spain, China, Japan, USA, Thailand, Bangladesh, Pakistan (market, internet)	As	Microwave with HNO_3–H_2O_2	ICP-MS	[72]
Rice	Turkey	As	Microwave with HNO_3–H_2O_2	SPE-HG-AAS	[73]
Rice	Japan	As	Microwave with HNO_3–$HClO_4$–HF	GFAAS	[74]
Rice	India	Se	Microwave with HNO_3–H_2O_2	DRC-ICP-MS	[75]
Rice	—	Al, Ba, Ca, Cd, Co, Cu, Fe, K, Na, Mg, Mn, Mo, Ni, P, Rb, S, Sr, V and Zn	MAD with HNO_3–H_2O_2	ICP-MS	[76]
Rice	—	Ca, Cu, K, Mg, Mn, P and Zn	—	ICP-OES	[77]
Rice	China	Cd	MAD	TS-FF-AAS	[78]
Rice	India	As	Digestion with HNO_3–H_2O_2	AHG-ICP-MS, FI-UV-HG-ICP-MS	[79]
Rice	India, Italy, UK market	As	MAD with HNO_3–H_2O_2	ICP-MS	[80]
Rice	Italy (market)	Se	MAD with HNO_3–H_2O_2–NaCl	HG-ICP-MS, DPASV	[81]
Rice	Turkey	Cu and Fe	Hot plate with HNO_3–$HClO_4$	SPE-FAAS	[82]
Rice	Turkey	As	Microwave with HNO_3–H_2O_2	HG-AAS	[83]
Rice	USA	As	Microwave with HNO_3	ICP-MS	[84]
Rice	China, Egypt, France, Ghana, India, Italy, Japan, Philippines, Spain, Thailand, USA	Se	Microwave with HNO_3	ICP-MS	[85]
Rice	—	Dy, Hf, Rb, Sc, Se	Solid sampling	PCINAA	[86]

Sample	Origin	Elements	Treatment	Technique	Reference
Rice flour	Brazil	Mn	Heating with HNO_3–H_2O	FI-CPE-FAAS	[87]
Rice	China	Mn	Dry ashing	Fluorimetry	[88]
Rice	India	Cd, Cr, Cu, Fe, K, Mn, Pb and Zn	UV photolysis with HNO_3–H_2O_2	ICP-OES	[12]
Rice flour	Iran	Mn	Ultrasounds with HNO_3	AAS, ASV	[89]
Rice flour	Japan	As	MAD with HNO_3–$HClO_4$–HF	DRC-ICP-MS	[90]
Rice	Japan	Ca, Cu, Fe, K, Mg, Mn, P and Zn	MAD and heating with HNO_3–H_2O_2–HF	ICP-OES, micro-PIXE	[91]
Rice	India	As	Dry ashing	HG-AAS	[92]
Rice	West Bengal, India (market)	As	Dry ashing with ashing aid	HG-AAS	[93]
Rice	Taiwan (market)	Hg	Microwave with HNO_3	ICP-MS	[94]
Rice	USA	As	Digestion with HNO_3–H_2O_2	ICP-OES	[95]
Rice	—	As	Extraction in mild acid conditions and moderate heat	Bioluminiscence	[96]
Rice	China	Pb	MAD with HNO_3–H_2O_2	FI-HG-AFS	[97]
Rice	China	Fe and Zn	MAD with HNO_3– H_2O_2–HF	ICP-MS	[13]
Rice	Spain	As	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[98]
Rice	Spain, China (Libyan market)	Co, Cr, Fe, Sc and Zn	Solid sampling	INAA	[26]
Rice	Turkey	Se	MAD with HNO_3–H_2O_2	SPE-GFAAS	[99]
Rice	Turkey	Co, Cu, Fe and Pb	MAD with HNO_3–H_2O_2	SPE-FAAS	[100]
Rice	—	As, Cd, Cr, Hg, Pb, Sb and Sn	MAD with HNO_3 and microwave-assisted evaporation	ICP-MS	[101]
Rice	Brazil	Al, Ca, Co, Cr, Cu, Fe, K, Mn, Mo, Ni, P, Pb, Ti and Zn	—	SR-TXRF	[34]
Rice flour	Brazil	Cd, Co, Cu and Ni	Digestion bomb with HNO_3–H_2O	SPE-FAAS	[102]
Rice flour	Brazil	Fe, Mn and Zn	Focalized microwave with HNO_3–H_2O_2	FAAS	[103]
Rice flour	Brazil	Cd. Co, Ni	Digestion bomb with HNO_3–H_2O	SPE-FAAS	[104]
Rice flour	Brazil	Cu	Heating with HNO_3–H_2O	SPE-FAAS	[105]
Rice	China	Mg	Sand bath with $HClO_4$	Polarography	[106]
Rice	China (Libyan market)	Hg and Se	Solid sampling	INAA, RNAA	[29]
Rice	India	Br, Ca, Cl, Co, Cr, Fe, K, Hg, Mn, Na, P, Rb, Sc, Se, Th and Zn	Solid sampling	INAA	[107]
Rice	Korea	As, Cd, Hg and Pb	Dry ashing	ICP-OES, TDA-AAS	[108]
Rice	Uruguay	As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni and Zn	MAD with HNO_3 and dry ashing with ashing aid $Mg(NO_3)_2$	ETAAS, FAAS, CV-AAS	[109]

(Continued)

Table 22.1 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Rice and rice flour	—	Ag, Al, Ba, Bi, Cd, Cr, Cu, Fe, Ga, In, Mg, Mn, Pb and Zn	Hot plate with HNO_3–H_2SO_4 or HNO_3–H_2O_2–H_2SO_4; or dry ashing	ICP-OES	[110]
Rice	Brazil	Ca, Cu, Fe, K, Mg, Mn, Na, P, Se and Zn	MAD with HNO_3–H_2O_2	ICP-MS	[111]
Rice	Burkina Faso, France, China, Senegal, Madagascar	Fe and Zn	Dry ashing and hot plate with HNO_3	FAAS	[43]
Rice, flour	China	Al, Cu, Fe, Ni and V	Dry ashing	RP-HPLC-UV-Vis	[112]
Rice	Spain	As	Dry ashing	FI-HG-AAS	[113]
Rice	Spain	Fe, Mn and Zn	MAD with HNO_3–H_2O_2	FAAS	[114]
Rice	Spain, India (Spanish market)	As	Ultrasound probe with HNO_3–H_2O_2. MAD with HNO_3–H_2O_2	ICP-MS	[115]
Rice and rice bean	Thailand	Se	Heating block with HNO_3–$HClO_4$–HCl	Fluorimetry	[116]
Rice	Thailand (market)	Ca, Fe, Mg, P and Zn	MAD with HNO_3	CFD-ICP-OES	[42]
Rice	Brazil	Cu	HNO_3–$HClO_4$	FAAS	[21]
Rice	China	Pb	Dry ashing	UV/Vis	[117]
Rice flour	China	Cd	Hot plate with HNO_3–$HClO_4$–HCl	CV-AAS	[118]
Rice	—	As	MAD with HNO_3–H_2O_2–H_2O	Q-ICP-MS	[119]
Rice	—	Cu, Cr, Fe, La, V and Zn	Slurry PTFE	ETV-ICP-OES	[120]
Rice and rice flour	China	Cd	MAD with HNO_3–H_2O_2	UV/Vis	[121]
Rice	Turkey	Cd, Co, Cu, Fe, Ni, Pb and Zn	MAD with HNO_3–H_2O_2	SPE-FAAS	[122]
Rice	Chile	Cu, Fe and Zn	Digestion with HNO_3–H_2SO_4–$HClO_4$	AAS	[22]
Rice	—	As	MAD with HNO_3–H_2O_2	ICP-MS	[123]
Rice	—	As	Extraction with MeOH/H_2O 1 : 1	HG-AFS	[16]
Rice	Pakistan	As, Br, Hg, Sb and Se	Solid sampling	INAA	[36]
Rice	Taiwan (market)	Cd, Cr, Cu, Hg and Pb	Ultrasound with Triton X-100 and HNO_3 and ascorbic acid	USS-ETV-DRC-ICP-MS	[124]
Rice	China	Se	Sand bath with HNO_3–$HClO_4$	HG-AFS	[125]
Rice	China	Se	Sand bath with HNO_3–$HClO_4$	HG-AFS	[38]
Rice	Italy	As, Cd, Co, Cr, Cu, Fe, Mn, Pb, V and Zn	MAD with HNO_3– H_2O_2	ICP-OES, Q-ICP-MS, HR-ICP-MS	[10]
Rice	—	As	MAD with HNO_3	ICP-MS	[126]
Rice	—	Al, Br, Ca, Cl, Co, Fe, K, La, Mg, Mn, Na, Rb, Sm and Zn	Solid sampling	INAA	[33]
Rice	Japan	Al, Ba, Ca, Cd, Co, Cr, Cs, Cu, Fe, K, Mg, Mn, Mo, Ni, P, Pb, Rb, Sr and Zn	MAD with HNO_3–$HClO_4$	ICP-OES, HR-ICP-MS	[127]

Table 22.1 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Rice	Canada	B, Ca, Cl, Co, Cu, I, K, Mg, Mn, Na, Rb, S, Ti and V	Solid sampling	NAA	[23]
Rice	Vietnam	Al, As,Ca, Cd, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P and Zn	MAD with HNO_3–H_2O_2	ICP-OES, ICP-MS, FAAS	[11]
Rice	—	Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sm, Tb, Tm, Y and Yb	MW with HNO_3–H_2O_2	ICP-MS	[128]

Table 22.2 Published papers on total mineral content of wheat with reference to sample treatment and analytical techniques.

Sample	Origin	Elements	Treatment	Technique	Reference
Wheat flour	Spain (market)	As	MAD with HNO_3–H_2O_2	ICP-MS	[25]
Wheat bran	Iran	Fe	Dry ashing	Optical membrane sensor	[40]
Wheat seeds	Iran	Br, K and Na	Solid sampling	NAA	[129]
Wheat	Iran	Cd, Cu, Pb	Heating with HNO_3	SPE-FAAS	[130]
Wheat flour	Taiwan (market)	As and Se	MAD with HNO_3–H_2O_2	DRC-ICP-MS	[62]
Wheat bran, flour and semolina	Spain (market)	Sb, Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[65]
Wheat	Japan, Australia, USA, Canada	Sr and Pb	Digestion with HNO_3–H_2O_2	HR-ICP-MS	[61]
Wheat	Italy (market)	As	Digestion block with HNO_3–H_2O_2	DCR-ICP-MS	[19]
Wheat seeds	Iran	Fe	Ultrasounds with HNO_3, hydrazine	MCPE-DSPV	[131]
Wheat flour, semolina and bran	Spain (market)	As, Bi, Sb, Se and Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[69]
Wheat	Turkey	As	Microwave with HNO_3–H_2O_2	SPE-HG-AAS	[73]
Wheat flour	Brazil	Mn	Digestion bomb with HNO_3–H_2O	CPE-FAAS	[132]
Wheat	China	Ca, Cu, Fe, K, Mg, Mn, P and Zn	MAD with HNO_3	ICP-OES	[133]
Wheat	—	Dy, Hf, Rb, Sc, Se	Solid sampling	PCINAA	[86]
Wheat	Taiwan (market)	Hg	Microwave with HNO_3	ICP-MS	[94]
Wheat flour	Iran	Mn	Ultrasounds with HNO_3	ASV	[89]
Wheat flour	Brazil	Cu and Zn	Digestion bomb with HNO_3–H_2O_2	FS-FAAS	[134]

(Continued)

Table 22.2 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Wheat semolina	Spain	As	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[98]
Wheat flour	UK	Se	MAD with HNO_3–H_2O_2	ICP-MS	[20]
Wheat	Turkey	Se	MAD with HNO_3–H_2O_2	SPE-GFAAS	[99]
Wheat	Turkey	Co, Cu, Fe and Pb	MAD with HNO_3–H_2O_2	SPE-FAAS	[100]
Wheat	Russia	As. S, Se and Te	Heating with TMAH and HCl	Spectrophotometry UV/Vis	[135]
Wheat meal	Italy (market)	Cr, Cu, Fe, Mn, Mo, Pb, Sb, Sn and Zn	Platinum crucible with with HCl–HNO_3–H_2SO_4	SWASV, SWV, AAS	[136]
Wheat meal and wholemeal	Italy	Cr, Cu, Pb, Sb, Sn, Tl and Zn	Platinum crucible with HCl–HNO_3–H_2SO_4	ASV	[18]
Wheat flour	Libya, Italy, Tunisia (Libyan market)	Hg and Se	Solid sampling	INAA, RNAA	[29]
Wheat	India	Br, Ca, Cl, Co, Cr, Fe, K, Hg, Mn, Na, P, Rb, Sc, Se, Th and Zn	Solid sampling	INAA	[107]
Wheat flour	Brazil	Cd and Fe	Solid sampling	SS-HR-CS-ETAAS	[41]
Wheat flour	Brazil	Fe, Mn and Zn	MAD with HNO_3–H_2O_2	FAAS	[103]
Wheat	Serbia	As, Cd, Cu, Fe, Hg, Mn, Pb and Zn	Heating under reflux with HNO_3, $HClO_4$	FAAS, GFAAS, CV-AAS	[137]
Wheat and wheat flour	Romania	Se	Digestion bomb with HNO_3–H_2O_2	GFAAS	[30]
Wheat meal and whole meal	Italy	Cr, Cu, Pb, Sb, Sn and Tl	Platinum crucible with HCl–HNO_3–H_2SO_4	SWASV and SWV	[138]
Wheat	Hungary	Se	MAD with HNO_3–H_2O_2, HCl	FI-HG-GFAAS	[139]
Wheat meal	Brazil	Cu	HNO_3–$HClO_4$	FAAS	[21]
Wheat and wheat flour	—	Ag, Al, Ba, Bi, Cd, Cr, Cu, Fe, Ga, In, Mg, Mn, Pb and Zn	Hot plate with HNO_3–H_2SO_4 or HNO_3–H_2O_2–H_2SO_4; or dry ashing	ICP-OES	[110]
Wheat and wheat semolina	—	Cd, Cr, Fe, Ni and Pb	MAD with HNO_3–H_2O_2	ICP-MS	[140]
Wheat	—	Cd, Cr, Fe, Ni and Pb	MAD	ICP-MS	[27]
Wheat meal and whole meal	Italy	Cr, Cu, Pb, Sb, Sn and Zn	Platinum crucible with HCl–HNO_3–H_2SO_4	SWASV	[141]
Wheat meal and wholemeal	Italy	As, Cd, Cu, Fe, Mn, Pb, Se and Zn	Block digestor with HCl	SWASV, SWCSV, SWV	[142]
Wheat	Pakistan	As, Br, Hg, Sb and Se	Solid sampling	INAA	[36]
Wheat meal and wholemeal	Italy	Cd, Cu, Pb, Sb and Zn	Block digestor with HCl	DPASV	[143]
Wheat bran, semolina flour, brown flour	—	Ag, Al, As, Au, Ba, Br, Ca, Ce, Cd, Cl, Co, Cr, Cs, Cu, Dy, Eu, Fe, Ga, Hf, Hg, I, In, Ir, K, La, Lu, Mg, Mn, Mo, Na, Nd, Ni, Rb, Sb, Sc, Se, Sm, Sn, Sr, Ta, Tb, Te, Th, Ti, U, V, W, Yb, Zn, and Zr	Solid sampling	NAA	[144]
Wheat flour	USA, Canada, Europe	Cd, Pb, Se and Sr	MAD with HNO_3–HCl	HR-ICP-MS, MS	[28]

Table 22.2 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Wheat	—	Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Se, Sn, V and Zn	MAD with HNO_3–H_2O_2, MAD with HNO_3–$HClO_4$	Q-ICP-MS	[145]
Wheat (normal and Se-enriched)	Austria	Se	—	HG-ICP-MS	[146]
Wheat	—	Al, Br, Ca, Cl, Co, Fe, K, La, Mg, Mn, Na, Rb, Sm and Zn	Solid sampling	INAA	[33]
Wheat, wheat flour	Canada	B, Ca, Cl, Co, Cu, I, K, Mg, Mn, Na, Rb, S, Ti and V	Solid sampli ng	NAA	[23]

Table 22.3 Published papers on total mineral content of barley, maize, millet, oat and rye with reference to sample treatment and analytical techniques.

Sample	Origin	Elements	Treatment	Technique	Reference
Barley	Turkey	Pb	Hot plate with HNO_3–H_2O_2	SPE-FAAS	[147]
Barley seeds	Iran	Fe	Ultrasounds with HNO_3, hydrazine	DSPV	[131]
Barley	Japan, Australia, USA, Canada	Sr and Pb	Digestion with HNO_3–H_2O_2	HR-ICP-MS	[61]
Barley	Poland	Fe	Electric plate with HNO_3–H_2O_2	Spectrophotometric	[148]
Barley	—	Co, Cu, Ni and Zn	Hot plate with HNO_3–$HClO_4$	PCRBFNs, PCFFNNs	[149]
Barley	Italy (market)	Se	MAD with HNO_3–H_2O_2–NaCl	HG-ICP-MS, DPASV	[81]
Barley	India	Br, Ca, Cl, Co, Cr, Fe, K, Hg, Mn, Na, P, Rb, Sc, Se, Th and Zn	Solid sampling	INAA	[107]
Barley (normal and Se-enriched)	Austria	Se	—	HG-ICP-MS	[146]
Maize flour	Spain (market)	Sb, Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[65]
Maize flour	Brazil	Mn	Ultrasounds with HNO_3 and Triton X-100	SS-ETAAS	[14]
Maize grits and flakes	Poland	Fe	Electric plate with HNO_3–H_2O_2–H_2O	Spectrophotometric	[148]
Maize flour	Spain (market)	As, Bi, Sb, Se and Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[69]
Maize bran	—	Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn	MAD. Clossed-vessel with HNO_3; open-vessel with HNO_3–H_2O_2–HCl	ICP-OES	[150]
Maize	—	Dy, Hf, Rb, Sc, Se	Solid sampling	PCINAA	[86]

(Continued)

Table 22.3 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Maize	Italy (market)	Se	MAD with HNO_3–H_2O_2–NaCl	HG-ICP-MS, DPASV	[81]
Maize flour	Brazil	Mn	Heating with HNO_3–H_2O	FI-CPE-FAAS	[87]
Maize flour	Brazil	Cu and Zn	Digestion bomb with HNO_3–H_2O_2	FS-FAAS	[134]
Maize meal	Italy	Cr, Cu, Pb, Sb, Sn, Tl and Zn	Platinum crucible with HCl–HNO_3–H_2SO_4	ASV	[18]
Maize meal	Italy (market)	Cr, Cu, Fe, Mn, Mo, Pb, Sb, Sn and Zn	Platinum crucible with HCl–HNO_3–H_2SO_4	SWASV, SWV	[136]
Maize	Ghana	Ba, Br, ca, Cl, Co, Cu, Dy, K, Mg, Mn, Na, Rb, S, Sr, Ti, Th, U, V and Zn	Solid sampling	INAA	[151]
Maize flour	Brazil	Cd and Fe	Solid sampling	SS-HR-CS-ETAAS	[41]
Maize starch and flour	Brazil	Fe, Mn and Zn	MAD with HNO_3–H_2O_2	FAAS	[103]
Maize flour	Brazil	Cu	Heating with HNO_3–H_2O	SPE-FAAS	[105]
Maize flour	Czech Republic (market)	Fe, Co, Cu, Mn, Mo, Ni, P and Zn	MAD with HNO_3	ICP-MS	[152]
Maize	India	Br, Ca, Cl, Co, Cr, Fe, K, Hg, Mn, Na, P, Rb, Sc, Se, Th and Zn	Solid sampling	INAA	[107]
Maize	Libya (market)	Hg and Se	Solid sampling	INAA, RNAA	[29]
Maize and maize flour	—	Ag, Al, Ba, Bi, Cd, Cr, Cu, Fe, Ga, In, Mg, Mn, Pb and Zn	Hot plate with HNO_3–H_2SO_4 or HNO_3–H_2O_2–H_2SO_4; or dry ashing	ICP-OES	[110]
Maize meal	Brazil	Cu	HNO_3–$HClO_4$	FAAS	[21]
Maize	Burkina Faso, France, China, Senegal, Madagascar	Fe and Zn	Dry ashing and hot plate with HNO_3, HCl	FAAS	[43]
Maize flour	China	Cd	hot plate with HNO_3–$HClO_4$–HCl	CV-AAS	[118]
Maize	Hungary	Se	MAD with HNO_3–H_2O_2, HCl	FI-HG-GFAAS	[139]
Maize	Chile	Cu, Fe and Zn	Digestion with HNO_3–H_2SO_4–$HClO_4$	AAS	[22]
Maize meal	Italy	Cr, Cu, Pb, Sb, Sn and Zn	Platinum crucible with HCl–HNO_3–H_2SO_4	SWASV	[141]
Maize meal	Italy	As, Cd, Cu, Fe, Mn, Pb, Se and Zn	Block digestor with HCl	SWASV, SWCSV, SWV	[142]
Maize meal	Brazil	Fe	—	Photoacoustic	[153]
Maize meal	Italy	Cd, Cu, Pb, Sb and Zn	Block digestor with HCl	DPASV	[143]

Table 22.3 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Maize	Nigeria	Fe, Zn	HNO_3–$HClO_4$	ICP-OES	[44]
Maize	Chile	As	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	FI-HG-AAS	[154]
Millet	China	Cd	MAD with HNO_3–H_2O_2, heating with H_2O_2	Electrolytic HG-AFS	[55]
Millet grits	Poland	Fe	Electric plate with HNO_3–H_2O_2	Spectrophotometric	[148]
Millet	China	Mn	Dry ashing	Fluorimetry	[88]
Millet	Ghana	Ba, Br, ca, Cl, Co, Cu, Dy, K, Mg, Mn, Na, Rb, S, Sr, Ti, Th, U, V and Zn	Solid sampling	INAA	[151]
Millet	India	Br, Ca, Cl, Co, Cr, Fe, K, Hg, Mn, Na, P, Rb, Sc, Se, Th and Zn	Solid sampling	INAA	[107]
Millet	Burkina Faso, France, China, Senegal, Madagascar	Fe and Zn	Dry ashing and hot plate with HNO_3, HCl	FAAS	[43]
Millet	—	Al, Br, Ca, Cl, Co, Fe, K, La, Mg, Mn, Na, Rb, Sm and Zn	Solid sampling	INAA	[33]
Oat rolled and bran	Poland	Fe	Electric plate with HNO_3–H_2O_2	Spectrophotometric	[148]
Oat	Brazil	Mn	Digestion bomb with HNO_3–H_2O	CPE-FAAS	[132]
Oat	Brazil	Cu and Zn	Digestion bomb with HNO_3–H_2O_2	FS-FAAS	[134]
Oat	Russia	Se	Heating with TMAH and HCl. Oxidation with antimony (V) complex	Spectrophotometry UV/Vis	[135]
Oat flour	Brazil	Fe, Mn and Zn	MAD with HNO_3–H_2O_2	FAAS	[103]
Rye flaks and flour	Spain (market)	Sb, Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[65]
Rye flakes and flour	Spain (market)	As, Bi, Sb, Se and Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[69]
Rye flour	Czech Republic (market)	Fe, Co, Cu, Mn, Mo, Ni, P and Zn	MAD with HNO_3	ICP-MS	[152]
Rye	Hungary	Se	MAD with HNO_3–H_2O_2, HCl	FI-HG-GFAAS	[139]
Rye (normal and Se-enriched)	Austria	Se	—	HG-ICP-MS	[146]

Table 22.4 Published papers on total mineral content of cereal products with reference to sample treatment and analytical techniques.

Sample	Origin	Elements	Treatment	Technique	Reference
Bread, pasta, biscuit and breakfast cereal	Spain (market)	As	MAD with HNO_3–H_2O_2	ICP-MS	[25]
Rice and rice crackers	Spain (market)	As	MAD with HNO_3–H_2O_2	ICP-MS	[67]
Bread	Italy (market)	As	Digestion block with HNO_3–H_2O_2	DCR-ICP-MS	[19]
Infant cereals	—	Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn	Clossed-vessel microwave with HNO_3. Open-vessel microwave with HNO_3–H_2O_2–HCl	ICP-OES	[150]
Crisped rice and puffed rice, rice crackers and noodles (breakfast cereal)	UK (market, internet)	As	Microwave with HNO_3	ICP-MS	[155]
Cuscus and pasta	Tunisia, Libya (Libyan market)	Co, Cr, Fe, Sc and Zn	Solid sampling	INAA	[26]
Bread wholemeal	—	Se	MAD with HNO_3–H_2O_2	ICP-MS	[20]
Cuscus and pasta	Libya, Italy, Tunisia (Libyan market)	Hg and Se	Solid sampling	INAA, RNAA	[29]
Biscuit and bread	Brazil	Cd and Fe	Solid sampling	SS-HR-CS-ETAAS	[41]
Maize sausage	—	As, Cd, Cr, Hg, Pb, Sb and Sn	MAD with HNO_3 and microwave-assisted evaporation	ICP-MS	[101]
Bread, biscuit and pasta	Brazil	Cu	HNO_3–$HClO_4$	FAAS	[21]
Pasta	—	Cd, Cr, Fe, Ni and Pb	MAD with HNO_3–H_2O_2	ICP-MS	[140]
Bread and pasta	Chile	Cu, Fe and Zn	Digestion with HNO_3–H_2SO_4–$HClO_4$	AAS	[22]
Cereal breakfast	Spain	Cr	HNO_3–H_2SO_4–$HClO_4$	GFAAS	[32]
Bread	Turkey	Se and Zn	Solid sampling	INAA	[24]
Bread	Bulgaria	Ca, Fe, Mg and Zn	MW with HNO_3	ICP-OES	[13]
Pasta	Canada	B, Ca, Cl, Co, Cu, I, K, Mg, Mn, Na, Rb, S, Ti and V	Solid sampling	NAA	[23]

Table 22.5 Published papers on total mineral content of pulses with reference to sample treatment and analytical techniques.

Sample	Origin	Elements	Treatment	Technique	Reference
Alfalfa	—	Se	Enzymatic digestion (protease XIV)	ICP-MS	[156]
Bean (canned)	Turkey	Cd, Cu, Fe and Pb	MAD with HNO_3–H_2O_2	CPE-FAAS	[157]
Bean (string, blank, read)	Spain (market)	As, Bi, Sb, Se and Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[69]
Bean (broad)	Iran	Cd, Co, Cr, Cu, Fe, Pb and Zn	Hot plate and dry ashing with HNO_3–H_2O_2–HCl–$HClO_4$	SPE-FAAS	[158]

Table 22.5 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Bean (cowpea)	Brazil	Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Ni, P, Pb, Sb, Se, Sr, V and Zn,	MAD with HNO_3–H_2O_2	ICP-OES, ICP-MS, DRC-ICP-MS	[159]
Bean (kidney)	India (market)	As	Dry ashing with ashing aid	HG-AAS	[93]
Bean (black, green)	—	K	Solid sampling	NAA	[160]
Bean	Brazil (market)	Al, Ca, Co, Cr, Cu, Fe, K, Mn, Mo, Ni, P, Pb, Ti and Zn	—	SR-TXRF	[34]
Bean	Brazil	Br, Ca, Co, Cs, Fe, K, Rb and Zn	Solid sampling	INAA	[41]
Bean (mung)	China	Mg	Sand bath with $HClO_4$	Polarography	[106]
Bean (kidney)	Turkey	Fe, Mn and Zn	MAD with HNO_3–H_2O_2–HCl	FAAS	[37]
Bean (green, mung)	India	Cr, Mo and W	—	ICP-OES	[161]
Bean (black, red, kidney, etc.)	Brazil	Cu	HNO_3–$HClO_4$	FAAS	[21]
Bean (cowpea, mung)	Burkina Faso, France, China, Senegal, Madagascar	Fe and Zn	Dry ashing and hot plate with HNO_3, HCl	FAAS	[43]
Bean (green, mung)	India	Cr, Mo and W	—	ICP-OES	[161]
Bean (green)	Italy	Mn	Heating with HCl	dASCP	[162]
Bean (broad)	Spain	Cd	Continuous ultrasound with HNO_3–H_2O	FI-SPE-FAAS	[163]
Bean	Spain	Fe, Mn and Zn	MAD with HNO_3–H_2O_2	FAAS	[114]
Bean (mung, cowpea)	Thailand	Se	Heating block with HNO_3–$HClO_4$–HCl	Fluorimetry	[116]
Bean (mung)	Thailand (market)	Ca, Mg, P, Fe and Zn	MAD with HNO_3	CFD-ICP-OES	[42]
Bean	—	Ba, Ca, Mg, Mn, Sr, Fe, Co, Ni and Se	HNO_3	ICP-OES	[164]
Bean	Chile	Cu, Fe and Zn	Digestion with HNO_3–H_2SO_4–$HClO_4$	AAS	[22]
Bean	Chile	As	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	FI-HG-AAS	[154]
Bean (haricot, kidney)	Spain (market)	Al, Cd, Cr, Cu, Fe, Ni and Pb	Digestion block with HNO_3	ETAAS	[165]
Bean	Pakistan	As, Br, Hg, Sb and Se	Solid sampling	INAA	[36]
Bean (red, mung, horse gram)	India	Al, Br, Ca, Cl, Co, Fe, K, La, Mg, Mn, Na, Rb, Se, Sm and Zn	Solid sampling	INAA	[33]
Bean (black, red, mung)	Japan, India	Al, Ba, Br, Ca, Ce, Cl, Co, Cs, Dy, Eu, Fe, K, Lu, Mg, Mn, Na, Rb, Sb, Sc, Sm, Sr and Zn	Solid sampling	NAA, PAA	[166]
Bean (green)	Canada	B, Ca, Cl, Co, Cu, I, K, Mg, Mn, Na, Rb, S, Ti and V	Solid sampling	NAA	[23]

(Continued)

Table 22.5 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Chickpea	Spain (market)	As, Bi, Sb, Se and Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[69]
Chickpea	Turkey	Fe, Mn and Zn	MAD with HNO_3–H_2O_2–HCl	FAAS	[37]
Chickpea	Spain	Cd	Continuous ultrasound with HNO_3–H_2O	FI-SPE-FAAS	[163]
Chickpea	Spain	Fe, Mn and Zn	MAD with HNO_3–H_2O_2	FAAS	[114]
Chickpea	Spain (market)	Al, Cd, Cr, Cu, Fe, Ni and Pb	Digestion block with HNO_3	ETAAS	[165]
Chickpea	India	Al, Br, Ca, Cl, Co, Fe, K, La, Mg, Mn, Na, Rb, Se, Sm and Zn	Solid sampling	INAA	[33]
Chickpea	India	Al, Ba, Br, Ca, Ce, Cl, Co, Cs, Dy, Eu, Fe, K, Lu, Mg, Mn, Na, Rb, Sb, Sc, Sm, Sr and Zn	Solid sampling	NAA, PAA	[166]
Lentil	—	Se	Enzymatic digestion (protease XIV)	ICP-MS	[156]
Lentil	Iran	Fe	Ultrasound with HNO_3, hydrazine	MCPE-DSPV	[131]
Lentil	Spain (market)	As, Bi, Sb, Se and Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[69]
Lentil	—	Se	MAD with HNO_3–H_2O_2	ICP-MS	[167]
Lentil	Turkey	Fe, Mn and Zn	MAD with HNO_3–H_2O_2–HCl	FAAS	[37]
Lentil	Spain	Cd	Continuous ultrasound with HNO_3–H_2O	FI-SPE-FAAS	[163]
Lentil	Spain	Fe, Mn and Zn	MAD with HNO_3–H_2O_2	FAAS	[114]
Lentil	Chile	Cu, Fe and Zn	Digestion with HNO_3–H_2SO_4–$HClO_4$	AAS	[22]
Lentil	Spain (market)	Al, Cd, Cr, Cu, Fe, Ni and Pb	Digestion block with HNO_3	ETAAS	[165]
Lentil	Pakistan	As, Br, Hg, Sb and Se	Solid sampling	INAA	[36]
Lentil	Czech Republic (market)	Co, Cu, Fe, Mn, Mo, Ni, P, Se and Zn	MAD with HNO_3–H_2O_2	ICP-MS, ICP-OES	[168]
Pea (canned)	Turkey	Cd, Cu, Fe and Pb	MAD with HNO_3–H_2O_2	CPE-FAAS	[157]
Pea	Iran	Cd,Cr, Co, Cu, Pb and Zn	Hot plate and dry ashing with HNO_3–H_2O_2, HCl–$HClO_4$	SPE-FAAS	[158]
Pea (canned)	Brazil	Cu	HNO_3–$HClO_4$	FAAS	[21]
Pea	Czech Republic	Co, Cu, Fe, P, Mn, Mo, Ni and Zn	MAD with HNO_3	ICP-MS	[169]
Pea	Chile	Cu, Fe and Zn	Digestion with HNO_3–H_2SO_4–$HClO_4$	AAS	[22]
Pea	Spain (market)	Al, Cd, Cr, Cu, Fe, Ni and Pb	Digestion block with HNO_3	ETAAS	[165]
Pea	Czech Republic (market)	Co, Cu, Fe, Mn, Mo, Ni, P, Se and Zn	MAD with HNO_3–H_2O_2	ICP-MS, ICP-OES	[168]
Pea	Japan	Al, Ba, Br, Ca, Ce, Cl, Co, Cs, Dy, Eu, Fe, K, Lu, Mg, Mn, Na, Rb, Sb, Sc, Sm, Sr and Zn	Solid sampling	NAA, PAA	[166]

Table 22.5 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Peanut seeds	China, Japan	B, Ba, Ca, Cd, Co, Cu, Fe, Ga, K, Mg, Mn, Mo, Na, Ni, P, Rb, Sr, and Zn	MAD with HNO_3–H_2O_2–HF	ICP-MS	[170]
Peanut	Australia	B, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Ni, P, Se and Zn	MAD with HNO_3–H_2O_2–H_2O	ICP-OES, ICP-MS, DRC-ICP-MS	[171]
Peanut	Greece	Al, Ba, Cd, Cr, Cu, Fe, Mg, Mn, Pb and Zn	MAD with HNO_3–H_2SO_4–H_2O_2	ICP-OES	[172]
Peanut	Ghana	Ba, Br, ca, Cl, Co, Cu, Dy, K, Mg, Mn, Na, Rb, S, Sr, Ti, Th, U, V and Zn	Solid sampling	INAA	[151]
Peanut	Spain	Cd	Continuous ultrasound with HNO_3–H_2O	FI-SPE-FAAS	[163]
Peanut	Thailand	Se	Heating block with HNO_3–$HClO_4$–HCl	Fluorimetry	[116]
Peanut	Chile	Cu, Fe and Zn	Digestion with HNO_3–H_2SO_4–$HClO_4$	AAS	[22]
Peanut	Spain (market)	Al, Cd, Cr, Cu, Fe, Ni and Pb	Digestion block with HNO_3	ETAAS	[165]
Peanut	Spain (market)	Cu and Fe	Digestion with H_2SO_4–H_2O_2	FAAS	[173]
Peanut	Italy (market)	Ca, Cu, Fe, Mg and Zn	Dry ashing, HNO_3	FAAS	[174]
Bean	Brazil	Se	MAD with HNO_3–H_2O_2, heating with HCl	CF-HG-ETAAS	[175]
Soybean meal	Iran	Co, Cu, Ni and Zn	Hot plate with HNO_3–$HClO_4$	PCRBFNs, PCFFNNs	[149]
Soybean	Burkina Faso, France, China, Senegal, Madagascar	Fe and Zn	Dry ashing and hot plate with HNO_3, HCl	FAAS	[43]
Soybean	Thailand	Se	Heating block with HNO_3–$HClO_4$–HCl	Fluorimetry	[116]

sensitivity of FAAS for trace element determination of cereals and pulses.

Despite the general trend observed worldwide of the declining use of single-element techniques in favour of multi-element analysis of foods, in the case of cereals the growing concern about As in rice and Se supplementation in wheat has led to numerous studies focused only on one of these elements. In fact, the number of reviewed papers is similar for both multi-element and single-element determinations. Studies aiming to establish the mineral profile of cereals or pulses obviously use multi-element determination techniques such as ICP-OES and ICP-MS, although several studies employed NAA and even voltammetry techniques. For As and Se determinations, the technique of choice should be ICP-MS due to its excellent sensitivity, followed by electrothermal AAS (ET-AAS), hydride generation AAS (HG-AAS) or AFS. Other elements of interest such as Cu, Cd, Fe, Mn and Pb are determined by using the aforementioned AAS techniques and also by ultraviolet/visible (UV/Vis) spectrophotometry.

22.2.2 Sample digestion

Most procedures employed for mineral determination in cereals and pulses require previous sample decomposition, with the exception of NAA methods which are

suitable to be applied directly to the dry sample. In the reviewed literature, wet digestions (85%) are clearly preferred over dry ashing (15%) [37, 110, 114]. For wet sample treatment, the use of microwave-assisted digestion (MAD) is twice as common as the use of convective heating via hot plates, digestion blocks, bomb digestors, high-pressure ashers and sand baths, and the recent use of ultrasound-assisted digestion. MAD is being increasingly used since this method facilitates rapid dissolution of the sample matrix, requires low amounts of acids and oxidizing reagents and causes minimal contamination of the sample prior to element analysis, being generally used in closed reactors under pressure. Usually MAD precedes analysis by ICP-OES and ICP-MS, whereas dry ashing and wet digestion with convective heating precede AAS. With regard to the use of acids in wet digestion of both cereals and pulses, procedures use a mixture of HNO_3 and H_2O_2 extensively (around 40% of methods), followed by the use of only HNO_3 (15%). Other alternatives involve the single or combined use of HCl, H_2SO_4, $HClO_4$ and HF.

22.3 Mineral composition of cereals and pulses

Figure 22.1 shows the concentration ranges of trace elements determined in rice from published studies. As can be seen, K, Mg and P are the elements present in highest concentrations in rice samples at thousands of micrograms per gram, followed by Ca, Fe, Mn, Na, Rb and Zn and finally by Cu, all at microgram per gram levels. Ba, Cr, As, Mo and Ni have concentrations up to 100 ng/g, whereas Cd, Se, Pb, Sc and Co are present in samples with some fluctuations and Hg is under 100 ng/g.

Figure 22.2 shows the concentration ranges of trace elements determined in wheat from recently published studies. As can be seen, K, Mg and Ca can be found at the highest concentrations (μg/g) followed by Fe, Mn, Na, Rb and Zn and finally by Cs and Al. Mo and Ni are clearly in the range 100–1000 ng/g, while Pb and Se exhibit fluctuations at the aforementioned levels. As, Cd, Co, Cr and Hg are present at the lowest concentrations. In comparison with rice, wheat exhibits a significant positive difference in the content of Cs and probably a negative difference in the content of Cu. Wheat has a slightly higher concentration of Ca but lower concentrations of As and Cr compared with rice, these differences being about one order of magnitude.

Figure 22.3 shows average data from studies of different cereals. Sodium in barley, maize and millet is the element with the highest concentration (at percentage level), followed by K, Mg and Ca. Fe, Mn and Zn are in the range 10–100 μg/g, whereas Cu and Cr are below 10 μg/g. As, Se, Pb, Hg, Cd, Ni and Mo are present at the nanogram per gram level but it is difficult to draw general conclusions because of fluctuations in their concentrations as a function of type of sample and sample origin. The high amount of Pb in barley and As in maize should be emphasized. It can also be seen that cereals exhibit higher levels of Na and Cr in comparison with rice and wheat.

Concentrations of elements in pulses are summarized in Figure 22.4. The six types of pulses have similar mineral profiles. K is the element with the highest concentration (at percentage level), while P, Mg and Ca are present in the upper thousands of micrograms per gram. Fe followed by Zn, Mn and Na are in the range 100–1000 μg/g range and Cu, Mo and Ni are also present at low microgram per gram levels. At the nanogram per gram level, Se, Cd, Co, Pb, As, Cr and Hg are reported for many pulses. The high content of Se in beans, Mo in lentils and Mn and Ni in peanuts should be noted. In comparison with cereals, pulses have high levels of K, Ca, Fe, Cu, Mo and Ni and low levels of Na and Cr.

22.4 Speciation of mineral compounds in cereals and pulses

Traditionally, speciation studies have focused on foods of marine origin and meat, being less concerned with fruits, legumes and cereals. However, recent studies have stressed the importance of speciation in foods like rice and wheat that are consumed worldwide and which can extract relatively high amounts of toxic elements such as As, Hg, Sb and Te from contaminated soils and water. Moreover, the development of very sensitive analytical tools for the determination of these elements based on HG-AFS and ICP-MS, with detection in liquid chromatography, has increased the number of studies in this field. As can be seen in Tables 22.6 and 22.7, studies of As, Se Sb, Te and Hg have been published in the last few years.

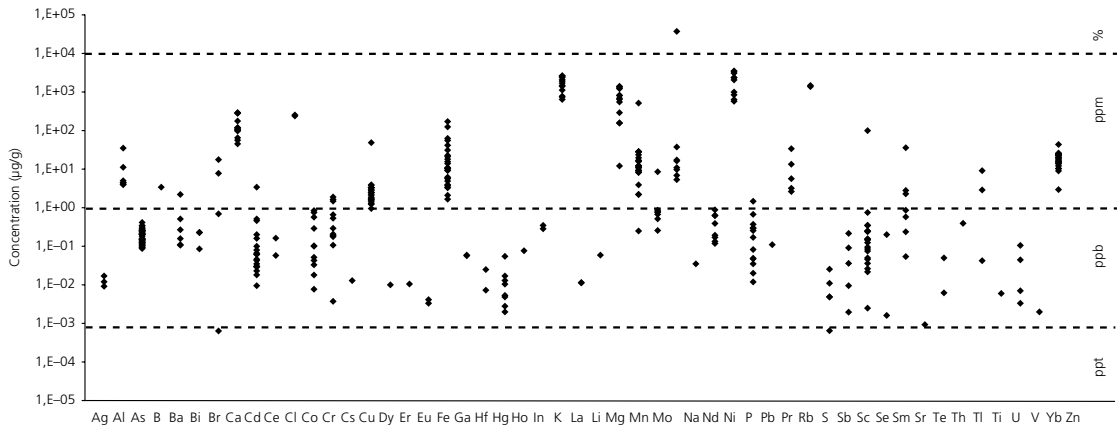


Figure 22.1 Concentration ranges of elements determined in rice from published studies.

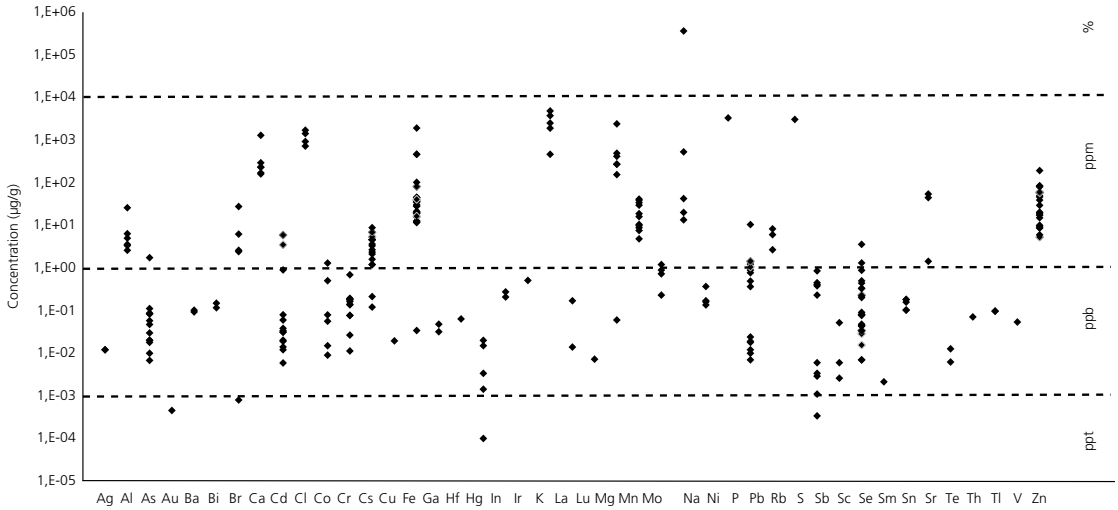


Figure 22.2 Concentration ranges of elements determined in wheat from published studies.

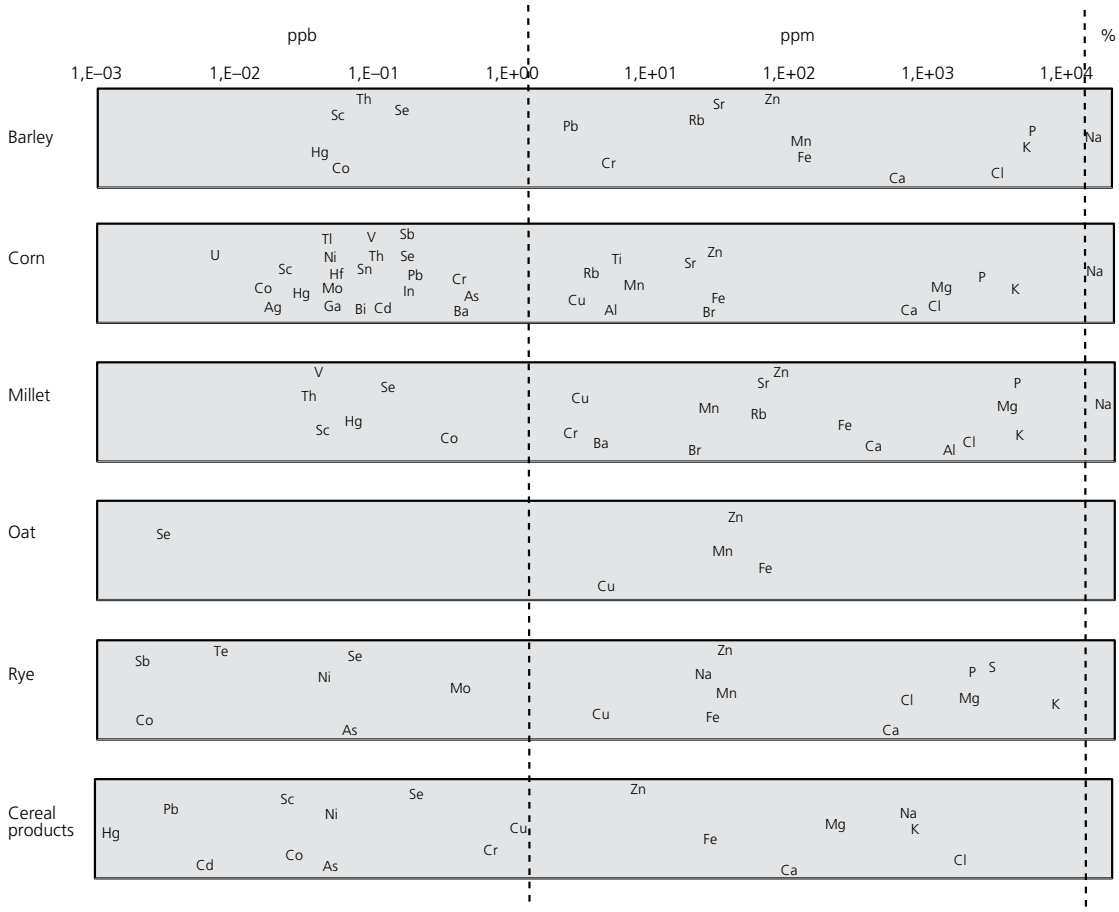


Figure 22.3 Concentration ranges of elements determined in other cereals and cereal products (bread, pasta, breakfast cereals) from published studies.

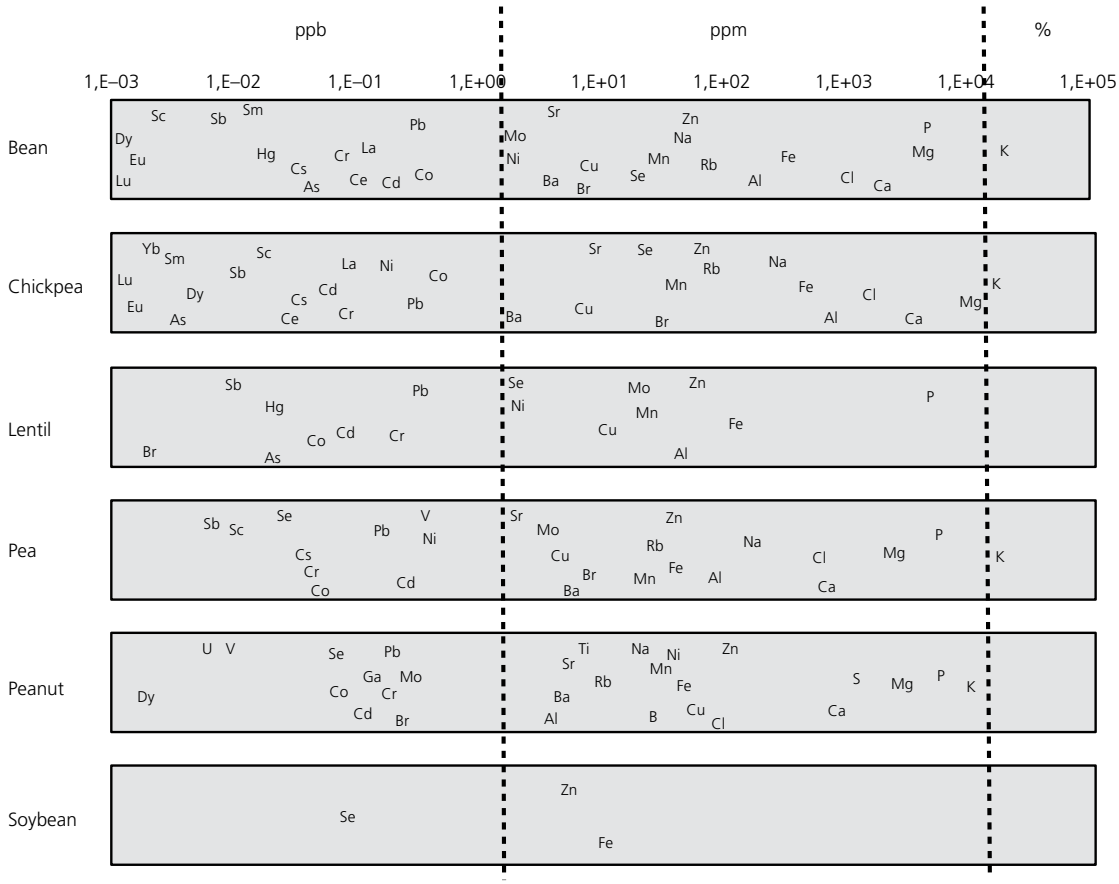


Figure 22.4 Concentration ranges of elements determined in pulses from published studies.

Table 22.6 Published papers on speciation of minerals in cereals with reference to sample treatment and analytical techniques.

Sample	Species	Treatment	Technique	Reference
Barley	Se(VI), SeCys, SeMet	Enzymolysis with protease at 37 °C, 4 h	HPLC-ICP-MS	[146]
Maize flour	Sb(III), Sb(V), Te(IV), Te(VI)	Ultrasound extraction with 1 mol/L H_2SO_4 and centrifuge, extraction with 0.1% (w/v) EDTA and centrifuge. Mix extracts	HG-AFS by proportional equations at different measurement conditions	[65]
Maize flour	P, Mn, Fe, Co, Ni, Cu, Zn, Mo	Extraction with 0.02 mol/L Tris-HCl, 1 h	SEC-ICP-MS	[152]
Maize	i-As	Acid digestion: solvent extraction with HCl digestion overnight, reduction with hydrazine-HBr, extraction with $CHCl_3$ (three times), back-extraction to HCl phase (twice), followed by dry ashing with ashing aid at 450 °C	FI-HG-AAS	[154]
Rice and bulgur	As(III), As(V), DMA, MMA	Acid extraction with 0.28 mol/L HNO_3 at 95 °C water bath, 90 min	HPLC-ICP-MS	[45]
Rice	i-As, DMA, MMA	Microwave-assisted extraction with 1% (v/v) HNO_3 + 1% (v/v) H_2O_2	HPLC-ICP-MS	[25]
Rice and rice cracker	i-As	Extraction with 0.1 mol/L HNO_3 and 3% H_2O_2 at 90 °C, 1 h, and oxidation all i-As to As(V)	SPE-HG-AAS	[177]
Rice	i-Se, o-Se	Ultrasound extraction with water followed by cyclohexane	Selective extraction, CPE-GFAAS	[52]
Rice	As(III), As(V), DMA, MMA	Extraction with water at 90 °C, 4 h, followed by enzymolysis with α-amylase 37 °C, 30 min	HPLC-ICP-MS	[54]
Rice, rice flour	i-As, As(III), As(V) by difference	*Total inorganic As*: ultrasound extraction with 1 mol/L HNO_3, 15 min and centrifuge, extraction with 0.1 % (w/v) EDTA vortex and centrifuge. Supernatants to ETAAS *As(III)*: microwave-assisted extraction 1 mol/L HNO_3, 85 °C, liquid–liquid extraction with EDTA-APDC-MIBK vortex and centrifuge. Organic phase to ETAAS for As(III), and determine As(V) by difference from T-iAs	Selective extraction, ETAAS	[46]
Rice	As(III), As(V), DMA, MMA	Microwave-assisted extraction with 1% v/v HNO_3 at 95 °C, 30 min	HPLC-ICP-MS	[57]
Rice	As(III), As(V), DMA, MMA	Extraction with water at 90 °C, 3 h	HPLC-ICP-MS	[58]
Rice	As(III), As(V), DMA, MMA	Acid extraction with 0.28 mol/L HNO_3 at 95 °C heating block, 90 min	HPLC-ICP-MS	[59]
Rice	As(III), As(V), MMA, DMA $(Se\text{-}Cys)_2$, SeMeSeCys, SeMet, Se(IV), Se(VI)	Enzymolysis with protease XIV and α-amylase microwave-assisted, 70 °C, 30 min	IC-DRC-ICP-MS	[62]
Rice	i-As	Acid digestion: solvent extraction with HCl digestion overnight, reduction with hydrazine-HBr, extraction with $CHCl_3$ (three times), back-extraction to HCl phase (twice) and dilution	Selective extraction, ICP-MS	[67]
Rice	Se(IV), Se(VI), $SeCys_2$, SeMet	Enzymolysis with protease and lipase at 37 °C, 16 h	CE-ICP-MS	[68]

(Continued)

Table 22.6 (Continued)

Sample	Species	Treatment	Technique	Reference
Rice and rice semolina	Sb(III), Sb(V), Te(IV), Te(VI)	Ultrasound extraction with 1 mol/L H_2SO_4 and centrifuge, extraction with 0.1% (w/v) EDTA and centrifuge. Mix extracts	HG-AFS by proportional equations at different measurement conditions	[65]
Rice	As(III), As(V), DMA, MMA	Acid extraction with 2% v/v HNO_3 at 95 °C water bath, 90 min	HPLC-ICP-MS	[64]
Rice crackers	i-As	Acid digestion: solvent extraction with HCl digestion overnight, reduction with hydrazine-HBr, extraction with $CHCl_3$ (three times), back-extraction to HCl phase (twice) and dilution	Selective extraction, ICP-MS	[67]
Rice	As(III), As(V), MMA, DMA	Acid extraction with 0.28 mol/L HNO_3 at 95 °C heating block, 90 min	HPLC-ICP-MS	[70]
Rice	Se(VI), SeMet, MeSeCys	Ultrasound-assisted enzymolysis with α-amylase 60 s and protease 180 s	HPLC-ICP-DRC-MS	[75]
Rice	As(III), As(V), DMA, MMA	Acid extraction with 0.15 mol/L HNO_3 at 100 °C heating block, 2 h	HPLC-ICP-MS	[72]
Rice	As(III), As(V), DMA	Water extraction at 90 °C heating block, 3 h	HPLC-ICP-MS	[74]
Rice	As(III), As(V), MMA, DMA, AsB	Extraction with methanol/water (1 : 1), 10 h at 55 °C	LC-UV-HG-ICP-MS	[79]
Rice	Se(IV), Se(VI), $SeCys_2$, SeOMet, SeMet	Ultrasound -assisted enzymolysis with α-amylase at 37 °C, 30 min and protease XIV at 45 °C, 2 h	RP-HPLC-ICP-MS, SAX-HPLC-ICP-MS	[39]
Rice	i-As = As(III + V), o-As = MMA + DMA	Microwave-assisted extraction with 1% (v/v) HNO_3 + 1% (v/v) H_2O_2	HPLC-ICP-MS	[80]
Rice	As(III), As(V), DMA, MMA Se(IV), Se(VI), SeMet	Microwave-assisted enzymolysis with protease XIV and α-amylase 37 °C, 30 min	IC-ICP-MS	[178]
Rice	i-As, DMA, MMA	Microwave-assisted extraction with 0.5 mol/L trifluoroacetic acid at 80 °C, 10 min	HPLC-ICP-MS	[84]
Rice	Se(VI), SeMet, SeCys, MeSeCys		Micro-XANES	[85]
Rice (crackers, crisped, noodles, puffed)	i-As, o-As	Microwave-assisted extraction with 1% v/v HNO_3 at 95 °C, 30 min	HPLC-ICP-MS	[155]
Rice flour	As(III), As(V), MMA, DMA	Microwave-assisted extraction with water at 80 °C, 30 min	HPLC-ICP-MS	[90]
Rice	As(III), As(V), DMA, MMA	Extraction with 2 mol/L trifluoroacetic acid at 100 °C, 6 h	HPLC-HG-AFS	[92]
Rice	As(III), As(V), DMA	Ultrasound-assisted extraction with 2 mol/L trifluoroacetic acid at 75 °C, 6 h	HPLC-ICP-DRC-MS	[95]
Rice	Hg(II), MeHg, EtHg	Microwave-assisted extraction with 0.5% (v/v) 2-mercaptoethanol in 5% methanol at 60 °C, 3 min	LC-VG-ICP-MS	[94]
Rice	As(III), As(V), DMA, MMA	Extraction with 2 mol/L trifluoroacetic acid at 100 °C, 6 h	HPLC-HG-AFS	[93]

Sample	Species	Treatment	Technique	Reference
Rice	As(III), As(V), MMA, DMA	Ultrasound extraction with 1 mol/L H_3PO_4 + 0.1% (w/v) Triton XT-114 and centrifuge, extraction with 0.1% (w/v) EDTA and centrifuge. Mix extracts	HG-AFS by proportional equations at different measurement conditions	[98]
Rice and rice cracker	As(III)	(1) Grinding rice to powder, (2) mixing with an aqueous solution containing pancreatic enzymes, (3) mechanical shearing, (4) extraction in mild acid conditions and moderate heat, and (5) centrifugation and pH neutralization	Bioluminescence	[96]
Rice	i-As	Acid digestion: solvent extraction with HCl digestion overnight, reduction with hydrazine-HBr, extraction with $CHCl_3$ (three times), back-extraction to HCl phase (twice), followed by dry ashing with ashing aid at 450 °C	FI-HG-AAS	[113]
Rice	As(III), As(V), MMA, DMA	Ultrasound-assisted enzymolysis with protease XIV and α-amylase, 3 min	LC-ICP-MS	[115]
Rice and rice cereals	As(III), As(V), MMA, DMA, AsB	Ultrasound extraction (three times) with 25, 25 and 20 mL methanol/water (1 : 1) for 1 h, 30 min, 30 min and centrifuge. Reduction volume to 5 mL	HPLC-ICP-MS	[179]
Rice	As(III), As(V), MMA, DMA	Ultrasound extraction with methanol/water (1 : 1) at 50 °C, 2 h, centrifuge. Repeat extraction four times. Concentrate the supernatants to 0.5 mL at 50 °C, 6 h, and dilute to 15 mL	HPLC-Q-ICP-MS	[119]
Rice	As(III), As(V), MMA, DMA, AsB	Ultrasound extraction with methanol/water (1 : 1) at 55 °C, 10 h, centrifuge. Repeat extraction twice. Combined extracts evaporated to dryness and diluted	HPLC-ICP-MS	[16]
Rice and rice cereals	As(III), As(V), MMA, DMA	Ultrasound extraction with methanol/water (1 : 1), 30 min, centrifuge. Repeat extraction twice. Combined extracts evaporated and diluted	HPLC-ICP-MS	[180]
Rice	As(III), As(V), MMA, DMA	Enzymolysis with α-amylase 60 °C, 24 h	IC-ICP-MS	[123]
Rice	As(III) + As(V), MMA, DMA	Extraction with 2 mol/L trifluoroacetic acid at 100 °C, 6 h	IC-ICP-MS	[126]
Rye flakes and flour	Sb(III), Sb(V), Te(IV), Te(VI)	Ultrasound extraction with 1 mol/L H_2SO_4 and centrifuge, extraction with 0.1% (w/v) EDTA and centrifuge. Mix extracts	HG-AFS by proportional equations at different measurement conditions	[65]
Rye flour	Fe, Co, Cu, Mn, Mo, Ni, P, Zn	Extraction with 0.02 mol/L Tris-HCl, 1 h	SEC-ICP-MS	[152]
Rye	Se(VI), SeCys, SeMet	Enzymolysis with protease at 37 °C, 4 h	HPLC-ICP-MS	[146]
Wheat flour	i-As, DMA, MMA	Microwave-assisted extraction with 1% (v/v) HNO_3 + 1% (v/v) H_2O_2	HPLC-ICP-MS	[25]
Wheat	Se(VI), SeMet	Enzymolysis with Tris-HCl buffer with protease and lipase, incubation at 37 °C, 18 h and centrifuge. Repeat the procedure with fresh buffered enzyme solution	HPLC-ICP-MS	[20]

(Continued)

Table 22.6 (Continued)

Sample	Species	Treatment	Technique	Reference
Wheat bran, flour, semolina	Sb(III), Sb(V), Te(IV), Te(VI)	Ultrasound extraction with 1 mol/L H_2SO_4 and centrifuge, extraction with 0.1% (w/v) EDTA and centrifuge. Mix extracts	HG-AFS by proportional equations at different measurement conditions	[65]
Wheat flour	As(III), As(V), MMA, DMA $(SeCys)_2$, SeMeSeCys, SeMet, Se(IV), Se(VI)	Enzymolysis with protease XIV and α-amylase microwave-assisted 70 °C, 30 min	IC-DRC-ICP-MS	[62]
Wheat	Hg(II), MeHg, EtHg	Microwave-assisted extraction with 0.5% v/v 2-mercaptoethanol in 5% methanol at 60 °C, 3 min	LC-VG-ICP-MS	[94]
Wheat	As(III), As(V), DMA, MMA	Microwave-assisted extraction with 0.16 mol/L HNO_3 at 95 °C, 30 min	HPLC-ICP-MS	[19]
Wheat semolina	As(III), As(V), MMA, DMA	Ultrasound extraction with 1 mol/L H_3PO_4 + 0.1% (w/v) Triton XT-114 and centrifuge, extraction with 0.1% (w/v) EDTA and centrifuge. Mix extracts	HG-AFS by proportional equations at different measurement conditions	[98]
Wheat	Se(VI), SeCys, SeMet	Enzymolysis with protease at 37 °C, 4 h	HPLC-ICP-MS	[146]
Biscuit, bread, breakfast cereal, pasta, maize snack	i-As, DMA, MMA	Microwave-assisted extraction with 1% (v/v) HNO_3 + 1% (v/v) H_2O_2	HPLC-ICP-MS	[25]
Bread and pasta	As(III), As(V), DMA, MMA	Microwave-assisted extraction with 0.16 mol/L HNO_3 at 95 °C, 30 min	HPLC-ICP-MS	[19]

Table 22.7 Published papers on speciation of minerals in pulses with reference to sample treatment and analytical techniques.

Sample	Species	Treatment	Technique	Reference
Beans (kidney)	As(III), As(V), DMA, MMA	Extraction with 2 mol/L trifluoroacetic acid at 100 °C, 6 h	HPLC-HG-AFS	[93]
Bean (green, mung)	Cr, Mo and W	Liquid–liquid extraction/separation with membrane	ICP-OES	[147]
Bean (white seeds)	Cd, Co, Cu, Fe, Mn, Mo, Ni, P, S, Se, Zn	Extraction with 0.02 mol/L Tris-HCl, 1 h	SEC-ICP-MS	[168]
Beans	i-As	Acid digestion: solvent extraction with HCl digestion overnight, reduction with hydrazine-HBr, extraction with $CHCl_3$ (three times), back-extraction to HCl phase (twice), followed by dry ashing with ashing aid at 450 °C	FI-HG-AAS	[154]
Pea seeds	Co, Cu, Fe, Mn, Mo, Ni, P, Zn	Extraction with 0.02 mol/L Tris-HCl, 1 h	SEC-ICP-MS	[169]
Peanut	Cu, Mn, Ni Zn	Extraction with 0.1 mol/L NaOH vortex, 30 min	SEC-UV-ICP-MS	[181]
Soybean flour	Cd, Co, Cu, Fe, Mn, Mo, Ni, P, S, Se, Zn	Extraction with 0.02 mol/L Tris-HCl, 1 h	SEC-ICP-MS	[168]

Chromatograph-based speciation has been mainly employed, followed by selective extraction and other non-chromatographic methods based on the use of proportional equations to differentiate inorganic and organic species and to check different oxidation states for which the capability of hydride generation of the target elements can be controlled. In order to provide a picture of the present state of speciation of minerals in cereals and pulses, the total content of As, Se and other elements are summarized in Figures 22.5, 22.6 and 22.7, together with a relation description of the different species identified and quantified in rice, wheat and other cereals and beans.

Arsenic is by far the most studied element and speciation of As(III), As(V), dimethylarsenic acid (DMA) and monomethylarsenic acid in rice produced in different areas around the world and also in wheat and wheat-based products, like bread, pasta or semolina, has been examined. It should be noted that arsenobetaine has been determined in only two studies on rice [16, 146], As(III) or DMA being the species with the highest concentration in rice, followed by As(V).

Several non-chromatography studies have focused on the determination of total inorganic arsenic and, to a lesser extent, total organic arsenic. With regard to sample origin and its influence on the presence of As species in rice, it seems that rice from the USA is richer in methylated arsenic than rice produced in Europe and Asia [15]. Based on the studies by Zavala *et al*. [95], this may be explained by the fact that US rice has a higher total As content than that found in other countries. Additional research has identified that there are two different types of rice, inorganic arsenic type and DMA type, with grains of the former able to transport inorganic As present in water and soil and grains of the latter able to transform these species into DMA [17]. However, Zhao *et al*. [15] discussed the ability of the plants to transform As, showing that methylated As species in rice are derived from the soil. In fact, it seems well demonstrated that rice is a significant source of inorganic As for humans and that the speciation of As, Ge and Si in rice grains could be the key to elucidating the mechanism of arsenate transportation, and thus there is special interest in the use of high-sensitivity and high-resolution instruments [176].

Selenium, selenomethionine (SeMet), Se(VI), selenocystine and, to a lesser extent Se(IV), have been demonstrated (Figure 22.6), the differentiation between total inorganic Se and total organic Se also being reported [52]. This shows the predeominance of total organic Se, especially as SeMet, the most bioavailable species in human intake [31]. The concentration of Se in these samples ranged from 9 ng/g [146] to 99,100 ng/g [75], with high values around hundreds of nanograms per gram in many cases.

Total inorganic Hg and total organic Hg have been determined in rice and wheat flour [94], with concentrations being of the same order for both types of compounds. On the other hand, studies with Sb showed that total concentration was between 0.3 and 2.9 ng/g, with concentrations of Sb(III) and Sb(V) of the same order of magnitude. With regard to Te, total concentration was between 0.9 and 20 ng/g, concentrations of Te(VI) being slighly higher than those of Te(IV).

In short, it can be concluded that additionall speciation studies are required in order to clearly identify the mechanism of absorption of minerals from soil and water, and the detoxification pathways. In addition, it would be useful to extend these studies to other elements like Cr and Fe and chemical species such as arsenosugars to complete our knowledge on the presence of essential and toxic elements in important foods.

22.5 Challenges in the mineral composition of cereals and pulses

In-depth study of the mineral profile of cereals and pulses is mandatory based on both their importance in the human diet and the continuous increase in production of rice and wheat worldwide, related to the constant growth of the population. Thus in the near future there is likely to be an increase in studies related to the mechanisms of absorption of minerals from soil and water by the plants and the bioavailability of species for humans, especially with regard to correct evaluation of the appropriate areas for cultivation and to clearly identify the risks of using previously unemployed soils.

Another important issue related to cereals concerns the possibility of the enrichment of these basic foods by minerals traditionally deficient in the diets of some areas, in order to prevent illness and to improve the health of the population, especially those where diets are poor in fish, meat and fresh vegetables. However, in order to achieve this, multi-element and speciation studies are required to provide a complete picture of the benefits of

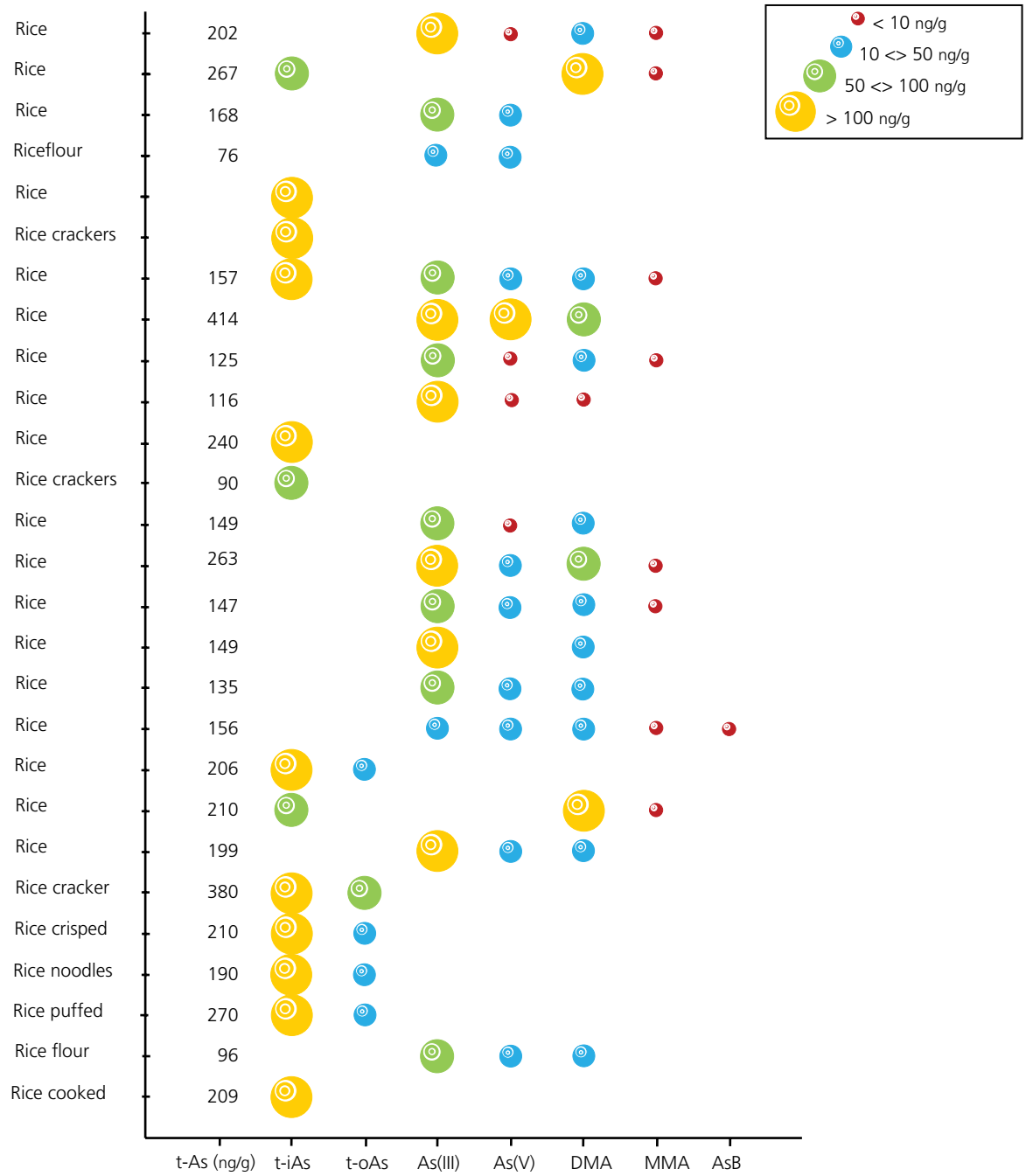


Figure 22.5 Speciation of As in cereals and pulses. (See insert for colour representation of the figure.)

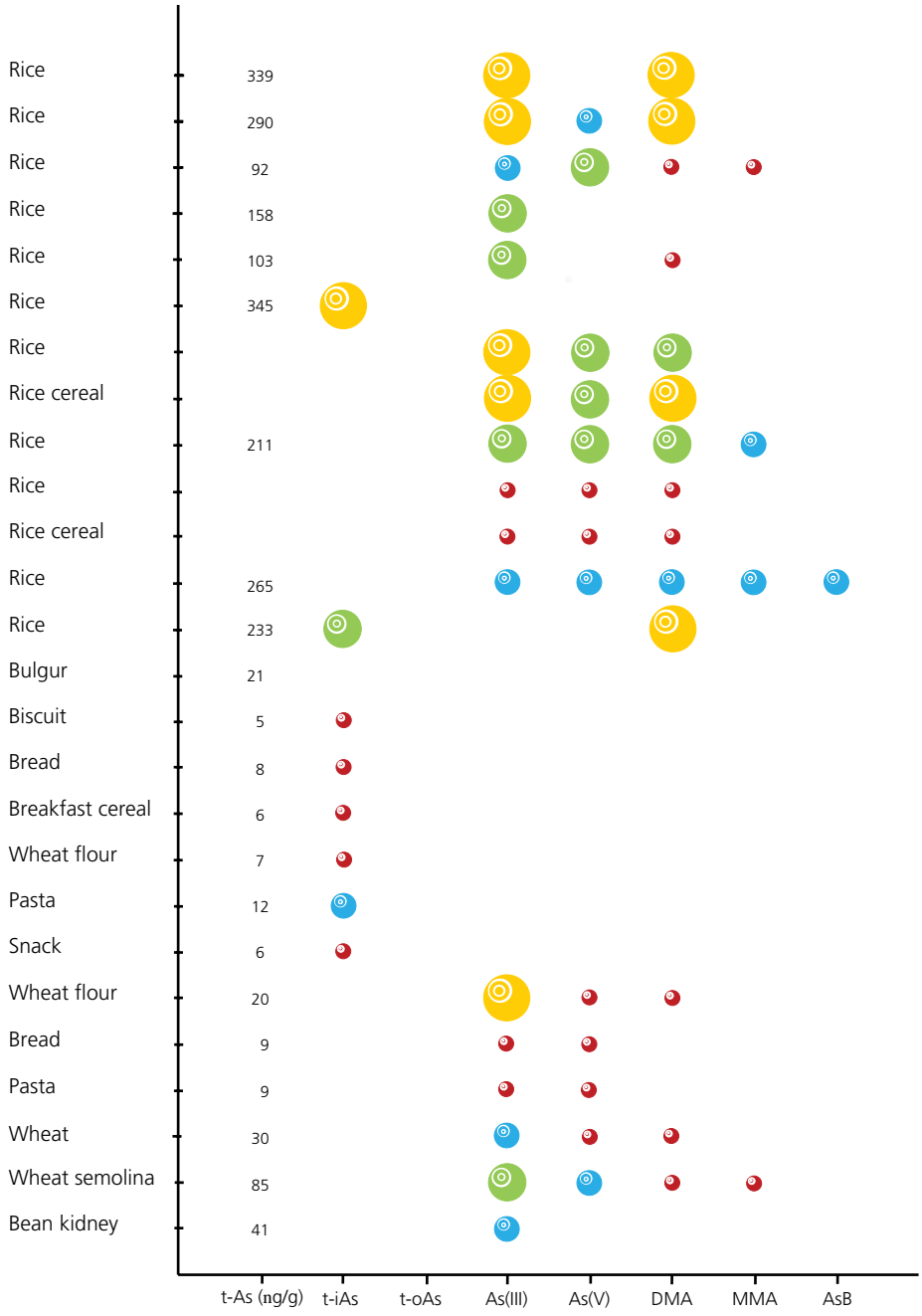


Figure 22.5 (Continued)

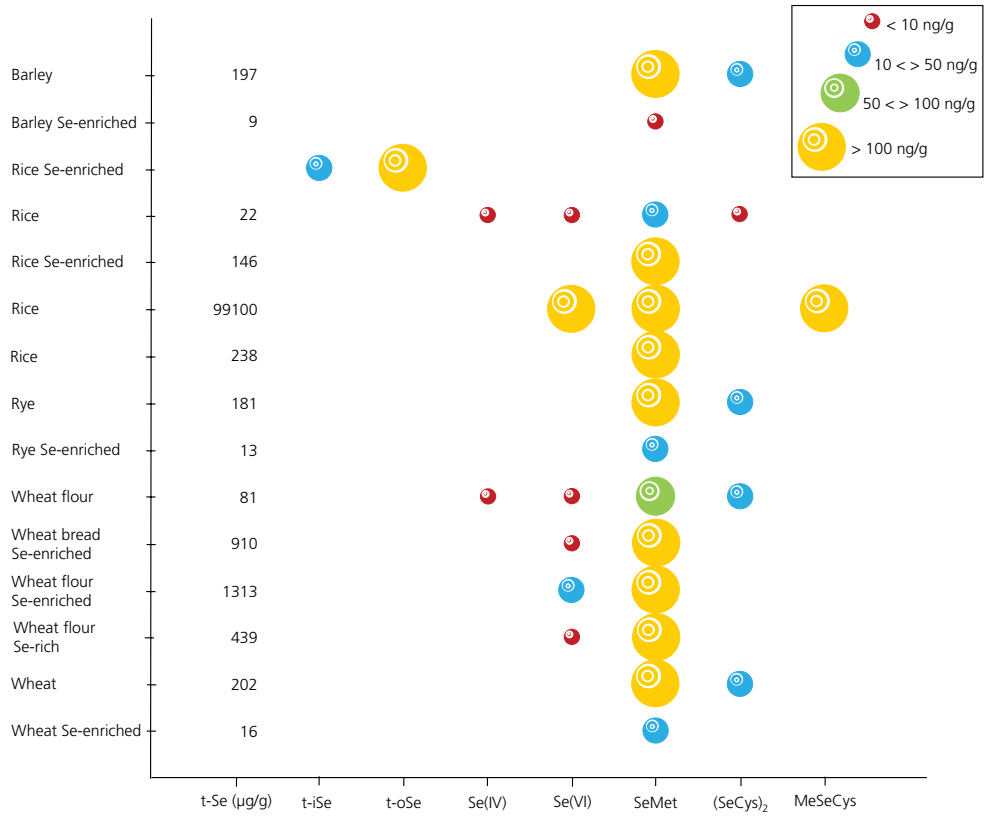


Figure 22.6 Speciation of Se in cereals and pulses. (See insert for colour representation of the figure.)

adding essential elements to wheat and rice, in particular an evaluation of their practical absorption in the human body because metabolic pathways depend on their chemical forms and the presence of other elements.

To guarantee the safety of cereals and pulses and to correctly adjust the addition of minerals to these basic foods, it is essential that there are new developments in methodologies that increase their sensitivity and selectivity, a serious improvement in demonstrating speciation at low concentrations, and an increase in application studies providing data on cereals and pulses produced from areas with different soils and water.

Abbreviations

AAS: Atomic absorption spectrometry
AHG-ICP-MS: Automated hydride generation inductively coupled plasma mass spectrometry
AsB: Arsenobetaine
ASV: Anodic stripping voltammetry
CFD-ICP-OES: Continuous-flow dialysis inductively coupled plasma optical emission spectrometric
CPE-ETAAS: Cloud point extraction electrothermal atomic absorption spectrometry
CPE-FAAS: Cloud point extraction flame atomic absorption spectrometry
CPE-GFAAS: Cloud point extraction graphite furnace atomic absorption spectrometry
CV-AAS: Cold vapour atomic absorption spectrometry
DASCP: Derivative anodic stripping chronopotentiometry
DMA: Dimethylarsenic acid
DPASV: Differential pulse anodic stripping voltammetry
DRC-ICP-MS: Dynamic reaction cell inductively coupled plasma mass spectrometry
DSPV: Differential surface photovoltage
ET-AAS: Electrothermal atomic absorption spectrometry

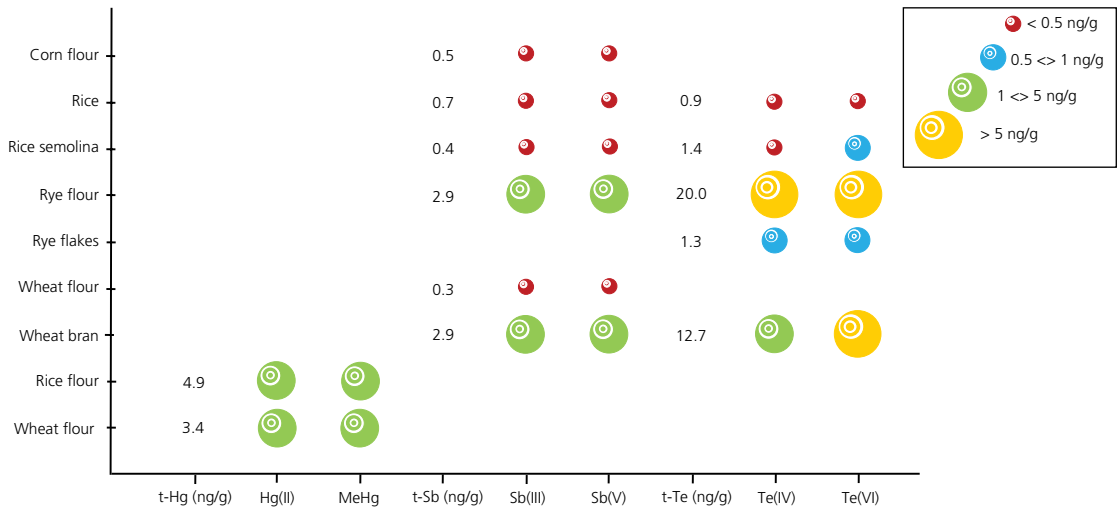


Figure 22.7 Speciation of Hg, Sb and Te in cereals and pulses. (See insert for colour representation of the figure.)

ETV-ICP-OES: Electrothermal vaporization inductively coupled plasma optical emission spectrometry
FAAS: Flame atomic absorption spectrometry
Fe-CAS-ODTA TX100: Complexes of Fe(III) with Chromazurol S (CAS) in the presence of octadecyltrimethylammonium chloride (ODTA) and Triton X-100 (TX100)
FI-CPE-FAAS: Flow injection cloud point extraction flame atomic absorption spectrometry
FI-HG-AAS: Flow injection hydride generation atomic absorption spectrometry
FI-HG-AFS: Flow injection hydride generation atomic fluorescence spectrometry
FI-UV-HG-ICP-MS: Flow injection ultraviolet visible photolysis hydride generation inductively coupled mass spectrometry
FS-FAAS: Fast sequential flame atomic absorption spectrometry
GF-AAS: Graphite furnace atomic absorption spectrometry
HFS-AAS: Hydride generation atomic absorption spectrometry
HG-AAS: Hydride generation atomic absorption spectrometry
HG-AFS: Hydride generation atomic fluorescence spectrometry
HG-ICP-MS: Hydride generation inductively coupled plasma mass spectrometry
HR-CS-GFAAS: High-resolution continuum source graphite furnace atomic absorption spectrometry
HR-ICP-MS: High-resolution inductively coupled plasma mass spectrometry
IC-DRC-ICP-MS: Ion chromatography dynamic reaction cell inductively coupled plasma mass spectrometry.
ICP-MS: Inductively coupled plasma mass spectrometry
ICP-oa-TOF-MS: Inductively coupled plasma orthogonal acceleration time-of-flight mass spectrometry
ICP-OES: Inductively coupled plasma optical emission spectrometry
ICP-ORS-MS: Inductively coupled plasma octopole reaction systems mass spectrometry
INAA: Instrumental neutron activation analysis
IR-MS: Isotope-ratio mass spectrometry
MAD: Microwave-assisted digestion
MCPE: Modified carbon paste electrode
MeHg: Methylmercury
MeSeCys: Se-methylselenocysteine
MMA: Monomethylarsenic acid
MSPE-FAAS: Magnetic solid-phase extraction flame atomic absorption spectrometry
NAA: Neutron activation analysis
PCFFNNs: Principal component-feed forward neural networks
PCINAA: Pseudo-cyclic instrumentation neutron activation analysis
PCRBFNs: Principal component-radial basis function networks
PIXE: Particle-induced X-ray emission
Q-ICP-MS: Inductively coupled plasma quadrupole mass spectrometry
RNAA: Radiochemical neutron activation analysis
RP-HPLC-UV-vis: Reversed-phase high performance liquid chromatography ultraviolet visible spectrophotometry
$(SeCys)_2$: Selenocystine
SeMet: Selenomethionine
SPE-FAAS: Solid-phase extraction flame atomic absorption spectrometry
SPE-GFAAS: Solid-phase extraction graphite furnace atomic absorption spectrometry
SPE-HG-AAS: Solid-phase extraction hydride generation atomic absorption spectrometry
SR-TXRF: Synchrotron radiation total reflection X-ray fluorescence analysis
SS-ETAAS: Solid sampling electrothermal atomic absorption spectrometry
SS-ETV-AFS: Solid sampling electrothermal vaporization atomic fluorescence spectrometry
SS-HR-CS-ETAAS: Solid sampling high-resolution continuum source electrothermal atomic absorption spectrometry
SWASV: Square-wave anodic stripping voltammetry
SWC-SV: Square-wave cathodic stripping voltammetry
SWV: Square-wave voltammetry
t-As: Total arsenic
TDA-AAS: Thermal decomposition, amalgamation and atomic absorption spectrometry
t-Hg: Total mercury
t-iAs: Total inorganic arsenic
t-iSe: Total inorganic selenium
t-oAs: Total organic arsenic
t-oSe: Total organic selenium
t-Sb: Total antimony
t-Se: Total selenium
TS-FF-AAS: Thermospray flame furnace atomic absorption spectrometry

t-Te: Total tellurium

USS-ETV-DRC-ICP-MS: Ultrasonic slurry sampling electrothermal vaporization dynamic reaction cell inductively coupled plasma mass spectrometry

UV/Vis: Ultraviolet/visible spectrophotometry

References

1 FAOSTAT Food and Agriculture Organization of the United Nations. http://faostat.fao.org/ (accessed February 2014).

2 Aisbitt B, Caswell H, Lunn J. Cereals: current and emerging nutritional issues. *Nutr Bull* 2008;**33**:169–185.

3 Bouchenak M, Lamri-Senhadji M. Nutritional quality of legumes, and their role in cardiometabolic risk prevention: a review. *J Med Food* 2013;**16**:185–198.

4 Gupta UC, Gupta SC. Sources and deficiency diseases of mineral nutrients in human health and nutrition: a review. *Pedosphere* 2014;**24**:13–38.

5 Belobrajdic DP, Bird AR. The potential role of phytochemicals in wholegrain cereals for the prevention of type-2 diabetes. *Nutr J* 2013;**12**:62.

6 Bernstein AM, Titgemeier B, Kirkpatrick K, Golubic M, Roizen MF. Major cereal grain fibers and psyllium in relation to cardiovascular health. *Nutrients* 2013;**5**:1471–1487.

7 Singhal P, Kaushik G, Mathur P. Antidiabetic potential of commonly consumed legumes: a review. *Crit Rev Food Sci Nutr* 2014;**54**:655–672.

8 Rebello CJ, Greenway FL, Finley JW. A review of the nutritional value of legumes and their effects on obesity and its related co-morbidities. *Obes Rev* 2014;**15**:392–407.

9 Wang Y, Wang Z, Fu L, Chen Y, Fang J. Legume consumption and colorectal adenoma risk: a meta-analysis of observational studies. *PLoS ONE* 2013;**8**(6):e67335. doi:10.1371/journal.pone.0067335.

10 d'Ilio S, Alessandrelli M, Cresti R, Forte G, Caroli S. Arsenic content of various types of rice as determined by plasma-based techniques. *Microchem J* 2002;**73**:195–201.

11 Phuong TD, Chuong PV, Khiem DT, Kokot S. Elemental content of Vietnamese rice. Part 1. Sampling, analysis and comparison with previous studies. *Analyst* 1999;**124**:553–560.

12 Manjusha R, Dash K, Karunasagar D, Arunachalam J. Determination of trace elements in Indian rice by ETAAS and ICP-AES. *Atom Spectrom* 2008;**29**:70–80.

13 Liang JF, Han BZ, Han LZ, Nout MJR, Hamer RJ. Iron, zinc and phytic acid content of selected rice varieties from China. *J Sci Food Agric* 2007;**87**:504–510.

14 da Silva DG, Matos GD, dos Santos AMP, da Paz Fiuza G, de Jesus RM. A procedure using slurry sampling for the determination of manganese in corn flour by ET AAS. *Anal Method* 2011;**3**:2625–2629.

15 Zhao FJ, Zhu YG, Meharg AA. Methylated arsenic species in rice: geographical variation, origin, and uptake mechanisms. *Environ Sci Technol* 2013;**47**:3957–3966.

16 Pizarro I, Gomez M, Palacios MA, Camara C. Evaluation of stability of arsenic species in rice. *Anal Bioanal Chem* 2003;**376**:102–109.

17 Booth B. Arsenic speciation varies with type of rice. *Environ Sci Technol* 2008;**42**:3484–3485.

18 Locatelli C. Voltammetric methods for the simultaneous determination of trace metals in foods, plant tissues and soil. *J Sci Food Agric* 2007;**87**:305–312.

19 d'Amato M, Aureli F, Ciardullo S, Raggi A, Cubadda F. Arsenic speciation in wheat and wheat products using ultrasound- and microwave-assisted extraction and anion exchange chromatography-inductively coupled plasma mass spectrometry. *J Anal Atom Spectrom* 2011;**26**:207–213.

20 Warburton E, Goenaga-Infante H. Methane mixed plasma-improved sensitivity of inductively coupled plasma mass spectrometry detection for selenium speciation analysis of wheat-based food. *J Anal Atom Spectrom* 2007;**22**:370–376.

21 Ferreira KS, Gomes JC, Chaves JBP. Copper content of commonly consumed food in Brazil. *Food Chem* 2005;**92**:29–32.

22 Olivares M, Pizarro F, de Pablo S, Araya M, Uauy R. Iron, zinc, and copper: contents in common Chilean foods and daily intakes in Santiago, Chile. *Nutrition* 2004;**20**:205–212.

23 Soliman K, Zikovsky L. Determination of bromine, calcium, chlorine, cobalt, copper, iodine, potassium, magnesium, manganese, sodium, rubidium, sulfur, titanium, vanadium in cereals, oils, sweeteners and vegetables sold in Canada by neutron activation analysis. *J Food Compos Anal* 1999;**12**:85–89.

24 Aras NK, Nazli A, Zhang W, Chatt A. Dietary intake of zinc and selenium in Turkey. *J Radioanal Nucl Chem* 2001;**249**:33–37.

25 Llorente-Mirandes T, Calderón J, Centrich F, Rubio R, López-Sánchez JF. A need for determination of arsenic species at low levels in cereal-based food and infant cereals. Validation of a method by IC-ICPMS. *Food Chem* 2014;**147**:377–385.

26 Alamin MB, Mhapes AA, Bejey AM *et al*. Determination of essential and toxic elements in Libyan foodstuff using instrumental neutron activation analysis (INAA). *J Radioanal Nucl Chem* 2007;**271**:247–250.

27 Cubadda F, Raggi A, Marconi E. Effects of processing on five selected metals in the durum wheat food chain. *Microchem J* 2005;**79**:97–102.

28 Branch S, Burke S, Evans P, Fairman B, Wolff Briche CSJ. A preliminary study in determining the geographical origin of wheat using isotope ratio inductively coupled plasma mass spectrometry with carbon-13, nitrogen-15 mass spectrometry. *J Anal Atom Spectrom* 2003;**18**:17–22.

29 Alamin MB, Bejey AM, Kucera J, Mizera J. Determination of mercury and selenium in consumed food items in Libya using instrumental and radiochemical NAA. *J Radioanal Nucl Chem* 2006;**270**:143–146.

30 Alexiu V, Vladescu L. Optimization of a chemical modifier in the determination of selenium by graphite furnace atomic absorption spectrometry and its application to wheat and wheat flour analysis. *Anal Sci* 2005;**21**:137–141.

31 López-Bellido Garrido FJ, López Bellido L. Selenium and health: reference values and current status of Spanish population. *Nutr Hosp* 2013;**28**:1396–1406.

32 Mateos CJ, Aguilar MV, Martinez-Para M. Determination of the chromium content in commercial breakfast cereals in Spain. *J Agric Food Chem* 2003;**51**:401–405.

33 Balaji T, Acharya RN, Nair AGC *et al*. Multielement analysis in cereals and pulses by k0 instrumental neutron activation analysis. *Sci Total Environ* 2000;**253**:75–79.

34 Vives AES, Moreira S, Brienza SMB, Zucchi OLA, Nascimento VFF. Synchrotron radiation total reflection X-ray fluorescence (SR-TXRF) for evaluation of food contamination. *J Radioanal Nucl Chem* 2006;**270**:147–153.

35 Mao XF, Liu JX, Huang YT *et al*. Assessment of homogeneity and minimum sample mass for cadmium analysis in powdered certified reference materials and real rice samples by solid sampling electrothermal vaporization atomic fluorescence spectrometry. *J Agric Food Chem* 2013;**61**: 848–853.

36 Waheed S, Zaidi JH, Ahmad S. Instrumental neutron activation analysis of 23 individual food articles from a high altitude region. *J Radioanal Nucl Chem* 2003;**258**: 73–81.

37 Erdogan S, Erdemoglu SB, Kaya S. Optimisation of microwave digestion for determination of Fe, Zn, Mn and Cu in various legumes by flame atomic absorption spectrometry. *J Sci Food Agric* 2006;**86**:226–232.

38 Hu QH, Chen LC, Xu J, Zhang YL, Pan GX. Determination of selenium concentration in rice and the effect of foliar application of Se-enriched fertiliser or sodium selenite on the selenium content of rice. *J Sci Food Agric* 2002;**82**: 869–872.

39 Fang Y, Zhang Y, Catron B, Chan Q, Hu Q, Caruso JA. Identification of selenium compounds using HPLC-ICPMS and nano-ESI-MS in selenium-enriched rice via foliar application. *J Anal Atom Spectrom* 2009;**24**:1657–1664.

40 Bagher-Gholivand M, Babakhanian A, Mohammadi M, Moradi P, Kiaie SH. Novel optical bulk membrane sensor and its application for determination of iron in plant and cereal samples. *J Food Compos Anal* 2013;**29**: 144–150.

41 Santos LGC, Fernandes EAN, Bacchi MA, Tagliaferro FS, Tsai SM. Evaluation of conventional and organic beans by instrumental neutron activation analysis. *J Radioanal Nucl Chem* 2006;**270**:249–252.

42 Judprasong K, Ornthai M, Siripinyanond A, Shiowatana J. A continuous-flow dialysis system with inductively coupled plasma optical emission spectrometry for in vitro estimation of bioavailability. *J Anal Atom Spectrom* 2005;**20**: 1191–1196.

43 Lestienne I, Icard-Verniere C, Mouquet C, Picq C, Treche S. Effects of soaking whole cereal and legume seeds on iron, zinc and phytate contents. *Food Chem* 2005;**89**:421–425.

44 Oikeh SO, Menkir A, Maziya-Dixon B, Welch R, Glahn RP. Assessment of concentrations of iron and zinc and bioavailable iron in grains of early-maturing tropical maize varieties. *J Agric Food Chem* 2003;**51**:3688–3694.

45 Sofuoglu SC, Güzelkaya H, Akgül T, Kavcar P, Kurucaovali F, Sofuoglu A. Speciated arsenic concentrations, exposure, and associated health risks for rice and bulgur. *Food Chem Toxicol* 2014;**64**:184–191.

46 Pasias IN, Thomaidis NS, Piperaki EA. Determination of total arsenic, total inorganic arsenic and inorganic arsenic species in rice and rice flour by electrothermal atomic absorption spectrometry. *Microchem J* 2013;**108**:1–6.

47 Abbasi-Tarighat M, Shahbazi E, Niknam K. Simultaneous determination of Mn^{2+} and Fe^{3+} as 4,4′((4-cholorophenyl) methylene)bis(3-methyl-1-phenyl-1H-pyrazol-5-ol) complexes in some foods, vegetable and water samples by artificial neural networks. *Food Chem* 2013;**138**:991–997.

48 Tarigh GD, Shemirani F. Magnetic multi-wall carbon nanotube nanocomposite as an adsorbent for preconcentration and determination of lead(II) and manganese(II) in various matrices. *Talanta* 2013;**115**:744–750.

49 Shakerian F, Dadfarnia S, Shabani AMH, Esfahani GS. Preconcentration and determination of lead(II) by microextraction based on suspended cadion covered zirconia nanoparticles in a surfactant media. *Microchim Acta* 2013;**180**:1225–1232.

50 Ensafi AA, Rabiei S, Rezaei B, Allafchian AR. Magnetic solid-phase extraction to preconcentrate ultra trace amounts of lead(II) using modified-carbon nanotubes decorated with NiFe2O4 magnetic nanoparticles. *Anal Method* 2013;**5**:3903–3908.

51 Mendil D, Bardak H, Tuzen M, Soylak M. Selective speciation of inorganic antimony on tetraethylenepentamine bonded silica gel column and its determination by graphite furnace atomic absorption spectrometry. *Talanta* 2013;**107**: 162–166.

52 Sun M, Liu G, Wu Q. Speciation of organic and inorganic selenium in selenium-enriched rice by graphite furnace atomic absorption spectrometry after cloud point extraction. *Food Chem* 2013;**141**:66–71.

53 Wang YD, Wang X, Wong YS. Generation of selenium-enriched rice with enhanced grain yield, selenium content and bioavailability through fertilisation with selenite. *Food Chem* 2013;**141**:2385–2393.

54 Nookabkaew S, Rangkadilok N, Mahidol C, Promsuk G, Satayavivad J. Determination of arsenic species in rice from Thailand and other Asian countries using simple extraction and HPLC-ICP-MS analysis. *J Agric Food Chem* 2013;**61**:6991–6998.

55 Zhang WB, Yang XA, Xue JJ, Wang SB. Green method for the determination of Cd in rice and water samples based on electrolytic hydride generation and atomic fluorescence spectrometry. *J Anal Atom Spectrom* 2012;**27**:928–936.

56 Antoine JMR, Fung LAH, Grant CN, Dennis HT, Lalor GC. Dietary intake of minerals and trace elements in rice on the Jamaican market. *J Food Compos Anal* 2012;**26**: 111–121.

57 Sun GX, Van de Wiele T, Alava P, Tack F, Du Laing G. Arsenic in cooked rice: effect of chemical, enzymatic and microbial processes on bioaccessibility and speciation in the human gastrointestinal tract. *Environ Pollut* 2012;**162**: 241–246.

58 Narukawa T, Hioki A, Chiba K. Speciation and monitoring test for inorganic arsenic in white rice flour. *J Agric Food Chem* 2012;**60**:1122–1127.

59 Huang JH, Fecher P, Ilgen G, Hu KN, Yang J. Speciation of arsenite and arsenate in rice grain: verification of nitric acid based extraction method and mass sample survey. *Food Chem* 2012;**130**:453–459.

60 da Silva DG, Junior MMS, Silva LOB, Portugal LA, Matos GD, Ferreira SLC. Determination of cadmium in rice by electrothermal atomic absorption spectrometry using aluminum as permanent modifier. *Anal Method* 2011;**3**:2495–2500.

61 Ariyama K, Shinozaki M, Kawasaki A, Ishida Y. Strontium and lead isotope analyses for determining the geographic origins of grains. *Anal Sci* 2011;**27**:709–714.

62 Tsai CY, Jiang SJ. Microwave-assisted extraction and ion chromatography dynamic reaction cell inductively coupled plasma mass spectrometry for the speciation analysis of arsenic and selenium in cereals. *Anal Sci* 2011;**27**: 271–276.

63 Gonzálvez A, Armenta S, de la Guardia M. Geographical traceability of 'arros de Valencia' rice grain based on mineral element composition. *Food Chem* 2011;**126**: 1254–1260.

64 Batista BL, Souza JMO, De Souza SS, Barbosa F Jr. Speciation of arsenic in rice and estimation of daily intake of different arsenic species by Brazilians through rice consumption. *J Hazard Mater* 2011;**191**:342–348.

65 Reyes MNM, Cervera ML, de la Guardia M. Determination of inorganic species of Sb and Te in cereals by hydride generation atomic fluorescence spectrometry. *J Braz Chem Soc* 2011;**22**:197–203.

66 Dufailly V, Nicolas M, Richoz-Payot J, Poitevin E. Validation of a method for arsenic speciation in food by ion chromatography-inductively coupled plasma/mass spectrometry after ultrasonic-assisted enzymatic extraction. *J AOAC Int* 2011;**94**:947–958.

67 Fontcuberta M, Calderon J, Villalbí JR *et al*. Total and inorganic arsenic in marketed food and associated health risks for the Catalan (Spain) population. *J Agric Food Chem* 2011;**59**:10013–10022.

68 Zhao Y, Zheng J, Yang M, Yang G, Wu Y, Fu F. Speciation analysis of selenium in rice samples by using capillary electrophoresis-inductively coupled plasma mass spectrometry. *Talanta* 2011;**84**:983–988.

69 Matos-Reyes MN, Cervera ML, Campos RC, de la Guardia M. Total content of As, Sb, Se, Te and Bi in Spanish vegetables, cereals and pulses and estimation of the contribution of these foods to the Mediterranean daily intake of trace elements. *Food Chem* 2010;**122**:188–194.

70 Huang JH, Ilgen G, Fecher P. Quantitative chemical extraction for arsenic speciation in rice grains. *J Anal Atom Spectrom* 2010;**25**:800–802.

71 Li B, Wang XP. Analysis of 27 mineral elements in the rice samples collected from China and Japan by using ICP-OES and ICP-MS. *Guangpuxue Yu Guangpu Fenxi* 2010;**30**: 2260–2264.

72 Nishimura T, Hamano-Nagaoka M, Sakakibara N, Abe T, Maekawa Y, Maitani T. Determination method for total arsenic and partial-digestion method with nitric acid for inorganic arsenic speciation in several varieties of rice. *J Food Hyg Soc Japan* 2010;**51**:178–181.

73 Uluozlu OD, Tuzen M, Mendil D, Soylak M. Determination of As(III) and As(V) species in some natural water and food samples by solid-phase extraction on Streptococcus pyogenes immobilized on Sepabeads SP 70 and hydride generation atomic absorption spectrometry. *Food Chem Toxicol* 2010;**48**:1393–1398.

74 Narukawa T, Chiba K. Heat-assisted aqueous extraction of rice flour for arsenic speciation analysis. *J Agric Food Chem* 2010;**58**:8183–8188.

75 Cubadda F, Aureli F, Ciardullo S *et al*. Changes in selenium speciation associated with increasing tissue concentrations of selenium in wheat grain. *J Agric Food Chem* 2010;**58**: 2295–2301.

76 Laursen KH, Hansen TH, Persson DP, Schjoerring JK, Husted S. Multi-elemental fingerprinting of plant tissue by semi-quantitative ICP-MS and chemometrics. *J Anal Atom Spectrom* 2009;**24**:1198–1207.

77 Horita H. Collaboratory study for determination of inorganic elements in milled rice measured by ICP-OES. *Bunseki Kagaku* 2009;**58**:107–112.

78 Wen XD, Wu P, Xu KL, Wang JW, Hou XD. On-line precipitation-dissolution in knotted reactor for thermospray flame furnace AAS for determination of ultratrace cadmium. *Microchem J* 2009;**91**:193–196.

79 Sengupta MK, Dasgupta PK. An automated hydride generation interface to ICPMS for measuring total arsenic in environmental samples. *Anal Chem* 2009;**81**:9737–9743.

80 Raab A, Baskaran C, Feldmann J, Meharg AA. Cooking rice in a high water to rice ratio reduces inorganic arsenic content. *J Environ Monit* 2009;**11**:41–44.

81 Toniolo R, Tubaro F, Bin S *et al*. Single-step microwave digestion of food and biological samples for the quantitative conversion of Se into the +4 oxidation state. *Talanta* 2009;**78**:753–758.

82 Tokalioglu S, Livkebabci A. A new solid-phase extraction method for the determination of Cu(II) and Fe(III) in various samples by flame atomic absorption spectrometry using N-benzoyl-N-phenylhydroxylamine. *Microchim Acta* 2009;**164**:471–477.

83 Tuzen M, Citak D, Mendil D, Soylak M. Arsenic speciation in natural water samples by coprecipitation-hydride generation atomic absorption spectrometry combination. *Talanta* 2009;**78**:52–56.

84 Heitkemper DT, Kubachka KM, Halpin PR, Allen MN, Shockey NV. Survey of total arsenic and arsenic speciation in US-produced rice as a reference point for evaluating change and future trends. *Food Addit Contam B* 2009;**2**: 112–120.

85 Williams PN, Lombi E, Sun GX *et al*. Selenium characterization in the global rice supply chain. *Environ Sci Technol* 2009;**43**:6024–6030.

86 Nyarko BJB, Akaho EHK, Fletcher JJ, Chatt A. Neutron activation analysis for Dy, Hf, Rb, Sc and Se in some Ghanaian cereals and vegetables using short-lived nuclides and Compton suppression spectrometry. *Appl Radiat Isot* 2008;**66**:1067–1072.

87 Lemos VA, Baliza PX, de Carvalho AL, Oliveira R, Teixeira LS, Bezerra MA. Development of a new sequential injection in-line cloud point extraction system for flame atomic absorption spectrometric determination of manganese in food samples. *Talanta* 2008;**77**:388–393.

88 Jiang ZT, Li R. Determination of manganese in food by polymer-phase fluorescence quenching method with eosin in the presence of phenanthroline. *Chem Anal (Warsaw, Pol.)* 2008;**53**:659–671.

89 Rezaei B, Ghiaci M, Sedaghat ME. A selective modified bentonite-porphyrin carbon paste electrode for determination of Mn(II) by using anodic stripping voltammetry. *Sensors Actuators B* 2008;**B131**:439–447.

90 Narukawa T, Inagaki K, Kuroiwa T, Chiba K. The extraction and speciation of arsenic in rice flour by HPLC-ICP-MS. *Talanta* 2008;**77**:427–432.

91 Ogiyama S, Tagami K, Uchida S. The concentration and distribution of essential elements in brown rice associated with the polishing rate: use of ICP-AES and micro-PIXE. *Nucl Instrum Method Phys Res B* 2008;**266**:3625–3632.

92 Signes A, Mitra K, Burló F, Carbonell-Barrachina AA. Contribution of water and cooked rice to an estimation of the dietary intake of inorganic arsenic in a rural village of West Bengal, India. *Food Addit Contam A* 2008;**25**:41–50.

93 Signes-Pastor AJ, Mitra K, Sarkhel S *et al*. Arsenic speciation in food and estimation of the dietary intake of inorganic arsenic in a rural village of West Bengal, India. *J Agric Food Chem* 2008;**56**:9469–9474.

94 Lin LY, Chang LF, Jiang SJ. Speciation analysis of mercury in cereals by liquid chromatography chemical vapor generation inductively coupled plasma-mass spectrometry. *J Agric Food Chem* 2008;**56**:6868–6872.

95 Zavala YJ, Gerads R, Gürleyük H, Duxbury JM. Arsenic in rice: II. Arsenic speciation in USA grain and implications for human health. *Environ Sci Technol* 2008;**42**:3861–3866.

96 Baumann B, van der Meer JR. Analysis of bioavailable arsenic in rice with whole cell living bioreporter bacteria. *J Agric Food Chem* 2007;**55**:2115–2120.

97 Wu H, Jin Y, Luo MB, Bi SP. A simple and sensitive flow-injection on-line preconcentration coupled with hydride generation atomic fluorescence spectrometry for the determination of ultra-trace lead in water, wine, and rice. *Anal Sci* 2007;**23**:1109–1112.

98 Matos-Reyes MN, Cervera ML, Campos RC, de la Guardia M. Determination of arsenite, arsenate, monomethylarsonic acid and dimethylarsinic acid in cereals by hydride generation atomic fluorescence spectrometry. *Spectrochim Acta B* 2007;**62**:1078–1082.

99 Saygi KO, Melek E, Tuzen M, Soylak M. Speciation of selenium(IV) and selenium(VI) in environmental samples by the combination of graphite furnace atomic absorption spectrometric determination and solid phase extraction on Diaion HP-2MG. *Talanta* 2007;**71**:1375–1381.

100 Tuzen M, Uluozlu OD, Usta C, Soylak M. Biosorption of copper(II), lead(II), iron(III) and cobalt(II) on Bacillus sphaericus-loaded Diaion SP-850 resin. *Anal Chim Acta* 2007;**581**:241–246.

101 Chan KC, Yip YC, Chu HS, Sham WC. High-throughput determination of seven trace elements in food samples by inductively coupled plasma-mass spectrometry. *J AOAC Int* 2006;**89**:469–479.

102 Lemos VA, da Silva DG, de Carvalho AL, de Andrade Santana D, dos Santos Novaes G, dos Passos AS. Synthesis of amberlite XAD-2-PC resin for preconcentration and determination of trace elements in food samples by flame atomic absorption spectrometry. *Microchem J* 2006;**84**: 14–21.

103 Santelli RE, Bezerra MA, de Santana OD, Cassella RJ, Ferreira SLC. Multivariate technique for optimization of digestion procedure by focussed microwave system for determination of Mn, Zn and Fe in food samples using FAAS. *Talanta* 2006;**68**:1083–1088.

104 Lemos VA, Moreira Gama E, da Silva Lima A. On-line preconcentration and determination of cadmium, cobalt and nickel in food samples by flame atomic absorption spectrometry using a new functionalized resin. *Microchim Acta* 2006;**153**:179–186.

105 Azevedo LV, Rodrigues VD, Galvao NC, Eca RM, Selis SM, Terumi YR. Preconcentration systems using polyurethane foam/Me-BDBD for determination of copper in food samples. *Microchim Acta* 2006;**153**:193–201.

106 Zhou XC, Wang LR, Zeng Y, Lu GH, Zhen P. Determination of magnesium in foods by single-sweep polarography. *J AOAC Int* 2006;**89**:782–785.

107 Singh V, Garg AN. Availability of essential trace elements in Indian cereals, vegetables and spices using INAA and the contribution of spices to daily dietary intake. *Food Chem* 2006;**94**:81–90.

108 Lee HS, Cho YH, Park SO *et al*. Dietary exposure of the Korean population to arsenic, cadmium, lead and mercury. *J Food Compos Anal* 2006;**19**:S31–S37.

109 Rivero-Huguet M, Huertas R, Francini L, Vila L, Darre E. Concentrations of As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Pb, and Zn in Uruguayan rice determines by atomic absorption spectrometry. *Atom Spectrosc* 2006;**27**:48–55.

110 Momen AA, Zachariadis GA, Anthemidis AN, Stratis JA. Development and validation of routine analysis methods for the determination of essential, nonessential, and toxic

minor and trace elements in cereal and cereal flour samples by inductively coupled plasma-atomic emission spectrometry. *J AOAC Int* 2005;**88**:1797–1810.

111 Heinemann RJB, Fagundes PL, Pinto EA, Penteado MVC, Lanfer-Marquez U. Comparative study of nutrient composition of commercial brown, parboiled and milled rice from Brazil. *J Food Compos Anal* 2005;**18**:287–296.

112 Peng JD, Liu SP, Deng CY. Separation and determination of trace amounts of aluminium(III), vanadium(V), iron(III), Copper(II) and nickel(II) with CALKS and PAR by RP-HPLC. *Anal Sci* 2005;**21**:259–262.

113 Laparra JM, Velez D, Barbera R, Farre R, Montoro R. Bioavailability of inorganic arsenic in cooked rice: practical aspects for human health risk assessments. *J Agric Food Chem* 2005;**53**:8829–8833.

114 Anzano JM, Ruiz-Gil M. Comparison of microwave acid digestion with the wet digestion and ashing methods for the determination of Fe, Mn, and Zn in food samples by flame AAS. *Atom Spectrosc* 2005;**26**:28–33.

115 Sanz E, Munoz-Olivas R, Camara C. A rapid and novel alternative to conventional sample treatment for arsenic speciation in rice using enzymatic ultrasonic probe. *Anal Chim Acta* 2005;**535**:227–235.

116 Sirichakwal PP, Puwastien P, Polngam J, Kongkachuichai R. Selenium content of Thai foods. *J Food Compos Anal* 2005;**18**:47–59.

117 Fang GZ, Pan JM. Spectrophotometric determination of lead in environmental and biological samples by flow injection microcolumn preconcentration and separation using DBMCSA chromogenic agent. *Chem Anal (Warsaw, Pol.)* 2005;**50**:925–934.

118 Ha J, Sun HW, Kang WJ, Liang SX. Continuous flow chemical vapor generation for the determination of cadmium in foodstuffs by atomic absorption spectrometry using derivative signal processing. *Microchim Acta* 2005;**150**:277–282.

119 d'Amato M, Forte G, Caroli S. Identification and quantification of major species of arsenic in rice. *J AOAC Int* 2004;**87**:238–243.

120 Chen SZ, Lu DB, Xu QY. Electrothermal vaporization inductively coupled plasma atomic emission spectrometry for direct multielement analysis of solid food sample with slurry sampling. *Can J Anal Sci Spectrosc* 2004;**49**:290–295.

121 Liu YW, Yang D, Chang XJ, Guo Y, Din BJ, Meng SM. Direct spectrophotometric determination of trace cadmium(II) in food samples with 2-acetylmercaptophenyldiazoaminoazobenzene (AMPDAA). *Microchim Acta* 2004;**147**:265–271.

122 Tuzen M, Soylak M. Column system using diaion HP-2MG for determination of some metal ions by flame atomic-absorption spectrometry. *Anal Chim Acta* 2004;**504**: 325–334.

123 Kohlmeyer U, Jantzen E, Kuballa J, Jakubik S. Benefits of high resolution IC-ICP-MS for the routine analysis of inorganic and organic arsenic species in food products of marine and terrestrial origin. *Anal Bioanal Chem* 2003;**377**:6–13.

124 Li PC, Jiang SJ. Electrothermal vaporization inductively coupled plasma-mass spectrometry for the determination of chromium, copper, cadmium, mercury and lead in rice flour. *Anal Chim Acta* 2003;**495**:143–150.

125 Chen LC, Yang FM, Xu J *et al*. Determination of selenium concentration of rice in China and effect of fertilization of selenite and selenate on selenium content of rice. *J Agric Food Chem* 2002;**50**:5128–5130.

126 Heitkemper DT, Vela NP, Stewart KR, Westphal CS. Determination of total and speciated arsenic in rice by ion chromatography and inductively coupled plasma mass spectrometry. *J Anal Atom Spectrom* 2001;**16**:299–306.

127 Yasui A, Shindoh K. Determination of the geographic origin of brown-rice by trace-element composition. *Bunseki Kagaku* 2000;**49**:405–410.

128 Liu M, Liu HS, Wang NF, Wang XY. Determination of fifteen trace rare-earth elements in rice by microwave digestion ICP MS. *Guangpuxue Yu Guangpu Fenxi* 1998;**18**:583–586.

129 Pourimani R, Abasnejad K, Ghanbarzadeh K, Zare MR, Kamali M. Determining the amount of Br, Na and K in six wheat samples with neutron activation analysis (NAA) method in Arak, I.R. Iran. *J Radioanal Nucl Chem* 2013;**295**:163–166.

130 Golbedaghi R, Jafari S, Khajavi F, Yaftian MR, Azadbakht R. Preconcentration and determination of Pb(II), Cu(II) and Cd(II) ions on octadecyl silica membrane disk modified with 2-mercapto-benzoimidazole by flame atomic absorption spectrometry. *Anal Method* 2012;**4**:2318–2322.

131 Gholivand MB, Geravandi B, Parvin MH. Anodic stripping voltammetric determination of iron(II) at a carbon paste electrode modified with dithiodianiline (DTDA) and gold nanoparticles (GNP). *Electroanalysis* 2011;**23**:1345–1351.

132 Lemos VA, David GT. An on-line cloud point extraction system for flame atomic absorption spectrometric determination of trace manganese in food samples. *Microchem J* 2010;**94**:42–47.

133 Shi RL, Zou CQ, Rui YK, Zhang XY, Xia XP, Zhang FS. Application of ICP-AES to detecting nutrients in grain of wheat core collection of China. *Guangpuxue Yu Guangpu Fenxi* 2009;**29**:1104–1107.

134 Ferreira HS, Santos ACN, Portugal LA, Costa ACS, Miro M, Ferreira SLC. Pre-concentration procedure for determination of copper and zinc in food samples by sequential multi-element flame atomic absorption spectrometry. *Talanta* 2008;**77**:73–76.

135 Sergeev GM, Shlyapunova EV, Pozdnyakova MA. Selective extraction-photometric redox determination of low concentrations of sulfur(IV), selenium(IV), tellurium(IV), and arsenic(III). *J Anal Chem (Zh Anal Khim)* 2007;**62**:416–423.

136 Melucci D, Locatelli C. Sequential voltammetric determination of trace metals in meals. *Microchem J* 2007;**85**: 321–328.

137 Skrbic B, Durisic-Mladenovic N, Cvejanov J. Principal component analysis of trace elements in Serbian wheat. *J Agric Food Chem* 2005;**53**:2171–2175.

138 Locatelli C. Overlapping voltammetric peaks: an analytical procedure for simultaneous determination of trace metals. Application to food and environmental matrices. *Anal Bioanal Chem* 2005;**381**:1073–1081.

139 Ajtony Z, Szoboszlai N, Bella Z, Bolla S, Szakal P, Bencs L. Determination of total selenium content in cereals and bakery products by flow injection hydride generation graphite furnace atomic absorption spectrometry applying in-situ trapping on iridium-treated graphite platforms. *Microchim Acta* 2005;**150**:1–8.

140 Cubadda F, Raggi A. Determination of cadmium, lead, iron, nickel and chromium in selected food matrices by plasma spectrometric techniques. *Microchem J* 2005;**79**: 91–96.

141 Locatelli C, Torsi G. Simultaneous square-wave anodic-stripping voltammetric determination of chromium, lead, tin, antimony, copper and zinc in presence of reciprocal interference: application to meal matrices. *Microchem J* 2004;**78**:175–180.

142 Locatelli C. Heavy metals in matrices of food interest: sequential voltammetric determination at trace and ultra-trace levels of copper, lead, cadmium, zinc, arsenic, selenium, manganese and iron in meals. *Electroanalysis* 2004;**16**:1478–1486.

143 Locatelli C, Torsi G. Analytical procedures for the simultaneous voltammetric determination of heavy metals in meals. *Microchem J* 2003;**75**:233–240.

144 Al-Dayel O, Al-Kahtani S. Elemental content in wheat products of Al-Qusim region, Saudi Arabia using the INAA technique. *J Radioanal Nucl Chem* 2002;**252**:605–609.

145 Cubadda F, Raggi A, Testoni A, Zanasi F. Multielemental analysis of food and agricultural matrices by inductively coupled plasma mass spectrometry. *J AOAC Int* 2002;**85**: 113–121.

146 Stadlober M, Sager M, Irgolic KJ. Effects of selenate supplemented fertilisation on the selenium level of cereals: identification and quantification of selenium compounds by HPLC-ICP-MS. *Food Chem* 2001;**73**:357–366.

147 Yavuz E, Tokalioglu S, Sahan H, Patat S. Ultralayered Co_3O_4 as a new adsorbent for preconcentration of Pb(II) from water, food, sediment and tobacco samples. *Talanta* 2013;**115**:724–729.

148 Pytlakowska K, Feist B. The use of Chromazurol S in the presence of a mixture of cationic and non-ionic surfactants for the spectrophotometric determination of iron in cereals. *J Anal Chem (Zh Anal Khim)* 2011;**66**:139–143.

149 Afkhami A, Abbasi-Tarighat M, Khanmohammadi H. Simultaneous determination of Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} ions in foodstuffs and vegetables with a new Schiff base using artificial neural networks. *Talanta* 2009;**77**: 995–1001.

150 Poitevin E, Nicolas M, Graveleau L, Richoz J, Andrey D, Monard F. Improvement of AOAC official method 984.27 for the determination of nine nutritional elements in food products by inductively coupled plasma-atomic emission spectroscopy after microwave digestion: single-laboratory validation and ring trial. *J AOAC Int* 2009;**92**:1484–1518.

151 Nyarko BJB, Akaho EHK, Fletcher JJ, Zwicker B, Chatt A. Simultaneous determination of short-to-medium lived nuclides in Ghanaian food items using INAA and Compton suppression counting. *J Radioanal Nucl Chem* 2006;**270**: 243–248.

152 Koplik R, Borkova M, Bicanova B, Polak J, Mestek O, Kominkova J. Speciation analysis of elements in cereal flours by liquid chromatography-inductively coupled plasma mass spectrometry. *Food Chem* 2006;**99**:158–167.

153 Delgado-Vasallo O, Peña JL, Martínez ESM *et al*. Photoacoustic determination of iron in corn meal. *Anal Sci* 2003;**19**:599–602.

154 Munoz O, Diaz OP, Leyton I *et al*. Vegetables collected in the cultivated Andean area of northern Chile: total and inorganic arsenic contents in raw vegetables. *J Agric Food Chem* 2002;**50**:642–647.

155 Sun GX, Williams PN, Zhu YG *et al*. Survey of arsenic and its speciation in rice products such as breakfast cereals, rice crackers and Japanese rice condiments. *Environ Int* 2009;**35**:473–475.

156 Funes-Collado V, Morell-Garcia A, Rubio R, López-Sánchez JF. Study of selenocompounds from selenium-enriched culture of edible sprouts. *Food Chem* 2013;**141**: 3738–3743.

157 Citak D, Tuzen M. Cloud point extraction of copper, lead, cadmium, and iron using 2,6-diamino-4-phenyl-1,3,5-triazine and nonionic surfactant, and their flame atomic absorption spectrometric determination in water and canned food samples. *J AOAC Int* 2012;**95**:1170–1175.

158 Marahel F, Ghaedi M, Shokrollahi A, Montazerozohori M, Davoodi S. Sodium dodecyl sulfate coated poly (vinyl) chloride: an alternative support for solid phase extraction of some transition and heavy metals. *Chemosphere* 2009;**74**:583–589.

159 Santos WPC, Hatje V, Lima LN *et al*. Evaluation of sample preparation (grinding and sieving) of bivalves, coffee and cowpea beans for multi-element analysis. *Microchem J* 2008;**89**:123–130.

160 Sanchez M, Landsberger S, Braisted J. Evaluation of 40 K in food by determining total potassium using neutron activation analysis. *J Radioanal Nucl Chem* 2006;**269**: 487–490.

161 Agrawal YK, Sharma KR. Speciation, liquid–liquid extraction, sequential separation, preconcentration, transport and ICP-AES determination of Cr(III), Mo(VI) and W(VI) with calix-crown hydroxamic acid in high purity grade materials and environmental samples. *Talanta* 2005;**67**: 112–120.

162 Dugo G, La Pera L, Lo Turco V, Palmieri RM, Saitta M. Effect of boiling and peeling on manganese content of some vegetables determined by derivative anodic stripping chronopotentiometry (dASCP). *Food Chem* 2005;**93**:703–711.

163 Yebra MC, Cancela S. Continuous ultrasound-assisted extraction of cadmium from legumes and dried fruit samples coupled with online preconcentration-flame atomic absorption spectrometry. *Anal Bioanal Chem* 2005;**382**:1093–1098.

164 Wang XM, Chen YC, Xie LQ, Fan ZL, Wu TY. Flame atomic absorption spectrophotometry for measurement of iron, manganese and zinc contents of wheat seeds. *Guangpuxue Yu Guangpu Fenxi* 2004;**24**:1467–1469.

165 Cabrera C, Lloris F, Giménez R, Olalla M, López MC. Mineral content in legumes and nuts: contribution to the Spanish dietary intake. *Sci Total Environ* 2003;**308**:1–14.

166 Miyamoto Y, Kajikawa A, Zaidi JH, Nakanishi T, Sakamoto K. Minor- and trace-element determination of food spices and pulses of different origins by NAA and PAA. *J Radioanal Nucl Chem* 2000;**243**:747–765.

167 Pedrero Z, Encinar JR, Madrid Y, Camara C. Identification of selenium species in selenium-enriched Lens esculenta plants by using two-dimensional liquid chromatography-inductively coupled plasma mass spectrometry and (^{77}Se) selenomethionine selenium oxide spikes. *J Chromatogr A* 2007;**1139**:247–253.

168 Koplik R, Borkova M, Mestek O, Kominkova J, Suchanek M. Application of size-exclusion chromatography-inductively coupled plasma mass spectrometry for fractionation of element species in seeds of legumes. *J Chromatogr B* 2002;**775**:179–187.

169 Koplík R, Mestek O, Komínková J, Borková M, Suchánek M. Effect of cooking on phosphorus and trace elements species in peas. *Food Chem* 2004;**85**:31–39.

170 Zhu Y, Hioki A, Chiba K. Distribution of the elements in cotyledon, embryonic axis, and testa of peanut seeds obtained by ICP-MS with microwave acid digestion. *Anal Sci* 2013;**29**:1027–1033.

171 Phan-Thien KY, Wright GC, Lee NA. Inductively coupled plasma-mass spectrometry (ICP-MS) and -optical emission spectroscopy (ICP-OES) for determination of essential minerals in closed acid digestates of peanuts (Arachis hypogaea L.). *Food Chem* 2012;**134**:453–460.

172 Momen AA, Zachariadis GA, Anthemidis AN, Stratis JA. Use of fractional factorial design for optimization of digestion procedures followed by multi-element determination of essential and non-essential elements in nuts using ICP-OES technique. *Talanta* 2007;**71**:443–451.

173 Anzano JM, Gónzalez P. Determination of iron and copper in peanuts by flame atomic absorption spectrometry using acid digestion. *Microchem J* 2000;**64**:141–145.

174 Plessi M, Bertelli D, Monzani A, Simonetti MS, Neri A, Damiani P. Dietary fiber and some elements in nuts and wheat brans. *J Food Compos Anal* 1999;**12**:91–96.

175 Shaltout AA, Castilho INB, Welz B *et al*. Method development and optimization for the determination of selenium in bean and soil samples using hydride generation electrothermal atomic absorption spectrometry. *Talanta* 2011;**85**:1350–1356.

176 Carey AM, Lombi E, Donner E *et al*. A review of recent developments in the speciation and location of arsenic and selenium in rice grain. *Anal Bioanal Chem* 2012;**402**:3275–3286.

177 Rasmussen RR, Qian YT, Sloth JJ. SPE HG-AAS method for the determination of inorganic arsenic in rice: results from method validation studies and a survey on rice products. *Anal Bioanal Chem* 2013;**405**:7851–7857.

178 Guzmán Mar JL, Reyes LH, Rahman GMM, Kingston HMS. Simultaneous extraction of arsenic and selenium species from rice products by microwave-assisted enzymatic extraction and analysis by ion chromatography-inductively coupled plasma-mass spectrometry. *J Agric Food Chem* 2009;**57**:3005–3013.

179 Paproski RE, Le XC. Boric acid-assisted anion-exchange chromatography for separating arsenic species. *Anal Chim Acta* 2004;**526**:69–76.

180 Bai J, Lu XF, Le XC, Yu B. Speciation analysis of arsenic in food samples with high performance liquid chromatography-inductively coupled-plasma-mass spectrometry. *Se Pu* 2003;**21**:545–548.

181 Wuilloud RG, Kannamkumarath SS, Caruso JA. Speciation of nickel, copper, zinc, and manganese in different edible nuts: a comparative study of molecular size distribution by SEC-UV-ICP-MS. *Anal Bioanal Chem* 2004;**379**:495–503.

CHAPTER 23
Bread and bakery products

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Abstract: Important micronutrients present in cereals include vitamins, minerals, antioxidants and phytochemicals. However, processing may decrease or increase the levels of mineral elements in grains and also modify the bioavailability of these components. Bread products vary widely around the world, as do their basic and optional ingredients and production techniques. Optional ingredients can be added to improve processing or to produce specialty and novelty breads, which often have an increased nutritional value. Wheat (*Triticum aestivum*) is the most important crop for breadmaking because of its supreme baking performance in comparison with all other cereals. In recent years there has been a great deal of interest in highly nutritious minor cereals, ancient crops and pseudocereals in breadmaking applications. Consumers are increasingly concerned about the contents of the food in their diets and are shifting away from acquiring nutrients via fortified foods and turning to products that are naturally high in vitamins/minerals and those that have been blended with other foods to create even higher nutrient levels. In this chapter, the role of bread and cereal-based baked goods as carriers of microconstituents with special emphasis on the alleviation of mineral deficiencies is reviewed. The mineral content and mineral bioaccessibility and bioavailability of cereal-based baked goods are revised, and the influence of processing, mainly milling and breadmaking, discussed. The mineral content of major cereal-based goods across the continents is compiled, and the technological, sensory and legal issues of mineral fortification are considered. New ingredients and reconsideration of ancient cereals and pseudocereals, processing steps and the application of new/emerging technologies such as high hydrostatic pressure in breadmaking are hot topics in cereal science research for developing baked goods with extra nutritional value, and with texture, appearance and sensory acceptability that can compete with regular wheat breads. Research on the influence of these processes and ingredient innovations on the mineral composition, availability and fortification of finished cereal-based goods remains basically unexplored, and is strongly encouraged.

Keywords: bread, baked goods, mineral composition, mineral bioavailability, mineral fortification

23.1 Introduction

The nearly ubiquitous consumption of cereals worldwide gives cereals an important position in international human nutrition. Cereals and cereal-based goods are multifaceted staple foods for the worldwide population as carriers of macronutrients and micronutrients or as tailored foods for specific targeted groups. Besides the high starch content as energy source, cereals provide dietary fibre, nutritious protein and lipids rich in essential fatty acids. Important micronutrients present in cereals include vitamins, minerals, antioxidants and phytochemicals. However, processing may decrease or increase the level of mineral elements in grains and also modify the bioavailability of these components. Bread products vary widely around the world, as do their basic and optional ingredients and production techniques. Optional ingredients can be added to improve processing or to produce specialty and novelty breads which often have increased nutritional value [1]. Wheat (*Triticum aestivum*) is the most important crop for breadmaking because of its supreme baking performance in comparison with all other cereals. In recent years there has been a great deal of interest in highly nutritious minor cereals, ancient crops and pseudocereals in breadmaking applications. Consumers are increasingly concerned about the contents of the food in their diets and are shifting away from acquiring nutrients via fortified foods and

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turning to products that are naturally high in vitamins/minerals and those that have been blended with other foods to create even higher nutrient levels [2–5].

In this chapter, the role of bread and cereal-based baked goods as carriers of microconstituents with special emphasis on the alleviation of mineral deficiencies is reviewed. The mineral content and mineral bioaccessibility and bioavailability of cereal-based baked goods are revised, and the influence of processing, mainly milling and breadmaking, discussed. The mineral content of major cereal-based based goods across the continents is compiled, and the technological, sensory and legal issues of mineral fortification are considered.

23.2 Bread and cereal-based baked goods as carriers of microconstituents: special emphasis on alleviation of mineral deficiencies

Micronutrient malnutrition (MNM) is widespread in the industrialized nations but is even more prevalent in the developing regions of the world. In addition to the obvious and direct health effects, the existence of MNM has profound implications for economic development and productivity, particularly in terms of the potentially huge public health costs and the loss of human capital formation. Worldwide, the three most common forms of MNM are iron, vitamin A and iodine deficiency. Together, these affect at least one-third of the world's population, the majority of whom are in developing countries. Of the three, iron deficiency is the most prevalent. It is estimated that just over 2000 million people are anaemic, just under 2000 million have inadequate iodine nutrition, and 254 million preschool-aged children are vitamin A deficient [6].

Cereals contain about 1.5–2.5% minerals [7]. The mineral in highest concentration (16–22% of total ash content) in all cereals is phosphorus which is mostly associated with calcium and magnesium phytates [1]. Wheat, rye and oats are classified as rich sources of phosphorus (200–1200 mg per 100 g) while barley is considered a moderate source (100–200 mg per 100 g). Potassium and sodium are minerals of concern in healthcare. The potassium levels are high in wheat, rye, barley and oats, but no cereal grain is considered to be a high or even moderate dietary source of sodium (before processing). Wheat, rye, barley and oats are also classified as moderate sources of calcium (100–200 mg per 100 g), magnesium (100–200 mg per 100 g), iron (1–5 mg per 100 g), zinc (1–5 mg per 100 g) and copper (0.1–1 mg per 100 g). Besides these, a large number of other elements are present in trace quantities. Wheat is an important dietary source of selenium, an essential micronutrient for humans with antioxidant, anticancer and antiviral effects [8]. Cereal grains are important sources of minerals, but also contain 1–4% phytic acid (PA), also known as myoinositol hexakisphosphate (IP6), which is considered an anti-nutritional factor [9]. PA has high chelating activity which may decrease the bioavailability of minerals [7].

Although cereals (especially milled and processed) may be considered a moderate source of minerals, cereal-based foods (especially bread) are proven to serve as good vehicles for both endogenous and exogenous (fortified) mineral delivery in the diet (Tables 23.1, 23.2, 23.3, 23.4 and 23.5).

23.3 Mineral content and bioavailability in cereal-based baked goods: influence of processing

Mineral deficiencies, especially of iron, calcium and zinc, have a negative effect on human health and may lead to conditions such as iron-deficiency anaemia, rickets, osteoporosis, and diseases of the immune system. To maintain mineral balance, the amount of mineral that is available to be absorbed is much more important than the intake. Bread and bakery products made with cereal flours are a staple food in many countries and are therefore of global importance in international nutrition. Processing is a prerequisite for manufacturing attractive cereal-based products and must initially render the food in a suitable form with good palatability. Processing steps, especially milling and breadmaking, may change the levels of the bioactive compounds in grains and also modify bioavailability of these compounds. In addition, storage can also alter the bioavailability of nutrients in cereals or cereal-based products [1].

The objective of milling is to separate the bran and germ from the starchy endosperm so that the endosperm can be ground into flour. The aleurone layer, which is rich in protein, minerals and vitamins, usually breaks away with the outer layer of the bran in the milling process thus contributing significantly to the nutritional

Table 23.1 Mineral amounts per 100-g serving (percent daily values for adults or children aged 4 years or older, and based on a 2000 calorie reference diet) of different breads.

Mineral	Ca (mg)	Fe (mg)	Mg (mg)	P (mg)	K (mg)	Na (mg)	Zn (mg)	Cu (mg)	Mn (mg)	Se (µg)	F (µg)
French or vienna (includes sourdough)	44 (4%)	3.63 (20%)	28 (7%)	114 (11%)	128 (4%)	513 (27%)	0.93 (6%)	0.136 (7%)	0.524 (26%)	27.1 (39%)	—
Cornbread, prepared from recipe, made with low-fat (2%) milk	249 (25%)	2.50 (14%)	25 (6%)	169 (17%)	147 (4%)	658 (27%)	0.60 (4%)	0.051 (3%)	0.118 (6%)	10.1 (14%)	—
Italian	78 (8%)	2.94 (16%)	27 (7%)	103 (10%)	110 (3%)	613 (24%)	0.86 (6%)	0.191 (10%)	0.464 (23%)	27.2 (39%)	—
Multi-Grain (includes wholegrain)	103 (10%)	2.50 (14%)	78 (19%)	228 (23%)	230 (7%)	381 (17%)	1.70 (11%)	0.282 (14%)	2.025 (101%)	32.9 (47%)	—
Oatmeal	66 (7%)	2.70 (15%)	37 (9%)	126 (13%)	142 (4%)	599 (25%)	1.02 (7%)	0.209 (10%)	0.940 (47%)	24.6 (35%)	—
Pita, whole-wheat	15 (1%)	3.06 (17%)	69 (17%)	180 (18%)	170 (5%)	444 (22%)	1.52 (10%)	0.290 (14%)	1.740 (87%)	44.0 (63%)	—
Bread, protein (includes gluten)	124 (12%)	4.15 (23%)	65 (16%)	185.8 (19%)	322 (9%)	404 (23%)	1.82 (12%)	0.417 (21%)	1.472 (74%)	32.9 (47%)	—
Bread, pumpernickel	68 (7%)	2.87 (16%)	54 (14%)	178 (18%)	208 (6%)	596 (28%)	1.48 (10%)	0.287 (14%)	1.305 (65%)	24.5 (35%)	—
Bread, rye	73 (7%)	2.83 (16%)	40 (10%)	125 (12%)	166 (5%)	603 (27%)	1.14 (8%)	0.186 (9%)	0.824 (41%)	30.9 (44%)	51.0
Bread, wheat	138 (14%)	3.52 (19%)	46 (12%)	153 (15%)	182 (5%)	519 (22%)	1.18 (8%)	0.161 (8%)	1.192 (56%)	28.8 (41%)	—
Reduced-calorie, rye	76 (8%)	3.10 (17%)	22 (5%)	78 (8%)	98 (3%)	513 (17%)	0.66 (4%)	0.133 (7%)	0.453 (23%)	27.9 (40%)	—
Reduced-calorie, wheat	163 (17%)	2.91 (16%)	43 (10%)	149 (15%)	150 (5%)	332 (11%)	1.35 (8%)	0.171 (9%)	1210 (61%)	30.4 (44%)	0

Source: US Department of Agriculture, Agricultural Research Service. 2012. USDA National Nutrient Database for Standard Reference, Release 25. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl, accessed March 2013 [21]; Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes. Elements. Food and Nutrition Board. Institute of Medicine. National Academies. http://www.nap.edu, accessed March 2013.

Table 23.2 Dietary reference intakes (DRIs): recommended dietary allowances (RDA) and adequate intakes (AI) for selected mineral elements.

Group	Ca (mg/day)	Cu (mg/day)	F (mg/day)	Fe (mg/day)	Mg (mg/day)	Mn (mg/day)	P (mg/day)	Se (mg/day)	Zn (mg/day)	K (g/day)	Na (g/day)
Infants 0–6 months	200*	200*	0.01*	0.27*	30*	0.003*	100*	15*	2*	0.4*	0.12*
6–12 months	260*	220*	0.5*	11	75*	0.6*	275*	20*	3	0.7*	0.37*
Children 1–3 years	700	340	0.7*	7	80	1.2*	460	20	3	3.0*	1.0*
4–8 years	1000	440	1*	10	130	1.5*	500	30	5	3.8*	1.2*
Males 9–13 years	1.3	700	2*	8	240	1.9*	1.25	40	8	4.5*	1.5*
14–18 years	1.3	890	3*	11	410	2.2*	1.25	55	11	4.7*	1.5*
19–30 years	1000	900	4*	8	400	2.3*	700	55	11	4.7*	1.5*
31–50 years	1000	900	4*	8	420	2.3*	700	55	11	4.7*	1.5*
51–70 years	1000	900	4*	8	420	2.3*	700	55	11	4.7*	1.3*
> 70 years	1.2	900	4*	8	420	2.3*	700	55	11	4.7*	1.2*
Females 9–13 years	1.3	700	2*	8	240	1.6*	1.25	40	8	4.5*	1.5*
14–18 years	1.3	890	3*	15	360	1.6*	1.25	55	9	4.7*	1.5*
19–30 years	1000	900	3*	18	310	1.8*	700	55	8	4.7*	1.5*
31–50 years	1000	900	3*	18	320	1.8*	700	55	8	4.7*	1.5*
51–70 years	1.2	900	3*	8	320	1.8*	700	55	8	4.7*	1.3*
> 70 years	1.2	900	3*	8	320	1.8*	700	55	8	4.7*	1.2*
Pregnancy											
14–18 years	1.3	1000	3*	27	400	2.0*	1.25	60	12	4.7*	1.5*
19–30 years	1000	1000	3*	27	350	2.0*	700	60	11	4.7*	1.5*
31–50 years	1000	1000	3*	27	360	2.0*	700	60	11	4.7*	1.5*
Lactation											
14–18 years	1.3	1.3	3*	10	360	2.6*	1.25	70	13	5.1*	1.5*
19–30 years	1000	1.3	3*	9	310	2.6*	700	70	12	5.1*	1.5*
31–50 years	1000	1.3	3*	9	320	2.6*	700	70	12	5.1*	1.5*

* All values are RDAs, except those followed by an asterisk which are AIs. An RDA is the average daily dietary intake level sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a group.

Source: Otten *et al.* [22].

Table 23.3 Mineral amounts per 100-g serving of different pasta products.

Nutrient	Ca (mg)	Fe (mg)	Mg (mg)	P (mg)	K (mg)	Na (mg)	Zn (mg)	Cu (mg)	Mn (mg)	Se (µg)
Pasta corn, cooked	1	0.25	36	76	31	0	0.63	0.064	0.153	2.8
Rice, white, with pasta, cooked	8	0.94	12	37	42	568	0.28	0.020	0.209	
Pasta, fresh-refrigerated, plain, cooked	6	1.14	18	63	24	6	0.56	0.093	0.224	
Pasta, fresh-refrigerated, spinach, cooked	18	1.11	24	57	37	6	0.63	0.080	0.316	
Pasta, homemade, made with egg, cooked	10	1.16	14	52	21	83	0.44	0.056	0.183	
Pasta, homemade, made without egg, cooked	6	1.13	14	40	19	74	0.37	0.060	0.193	

Source: US Department of Agriculture, Agricultural Research Service. 2012. USDA National Nutrient Database for Standard Reference, Release 25. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl, accessed March 2013 [21].

Table 23.4 Mineral amounts per 100-g serving of different bakery and confectionery products.

Mineral	Ca (mg)	Fe (mg)	Mg (mg)	P (mg)	K (mg)	Na (mg)	Zn (mg)	Cu (mg)	Mn (mg)	Se (µg)	F (µg)
Bagel, wheat	20	2.76	51	142	165	439	1.10	0.175	1430	28.7	24.1
Biscuits, plain or buttermilk, frozen, baked	49	3.30	17	430	224	942	0.48	0.083	0.392	18.8	
Cake, sponge, prepared from recipe	42	1.58	9	100	141	228	0.59	0.056	0.167	18.5	
Cake, yellow, dry mix, regular, enriched	211	2.08	10	310	47	728	0.27	0.072	0.191	3.0	
Cookies, chocolate wafers	31	4.01	53	132	210	690	1.09	0.463	0.696	5.7	
Cookies, oatmeal, refrigerated dough, baked	35	2.38	32	116	163	327	0.71	0.122	0.953	10.1	
Cookies, sugar, refrigerated dough, baked	90	1.84	8	187	163	321	0.27	0.041	0.289	3.5	
Crackers, wheat, regular	81	4.69	46	257	207	787	1.73	0.214	2062	12.5	
Croissants, butter	37	2.03	16	105	118	467	0.75	0.080	0.330	22.7	
Danish pastry, cinnamon, enriched	71	1.96	19	107	125	414	0.72	0.100	0.362	17.0	
Danish pastry, fruit, unenriched (includes apple, cinnamon, raisin, strawberry)	46	0.75	15	89	83	354	0.54	0.065	0.253		
Doughnuts, cake-type, plain (includes unsugared, old-fashioned)	25	3.00	16	261	113	450	0.68	0.073	0.290	10.3	
English muffins, wheat	178	2.87	37	107	186	353	1.07	0.148	1001	29.2	
Pancakes, plain, prepared from recipe	219	1.80	16	159	132	439	0.56	0.049	0.200	14.9	
Pie, apple, commercially prepared, enriched flour	11	0.45	7	24	65	201	0.16	0.046	0.182	1.0	
Pie crust, cookie-type, chocolate, ready crust	32	4.30	40	120	187	503	2.10	0.770	1833	2.5	

Source: US Department of Agriculture, Agricultural Research Service. 2012. USDA National Nutrient Database for Standard Reference, Release 25. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl, accessed March 2013 [21].

Table 23.5 Mineral composition (per 1000 g) of wheat bread and multigrain (oat/rye/buckwheat/wheat) breads a (15 : 15 : 15 : 55), b (20 : 20 : 20 : 40) and c (25 : 25 : 25 : 25).

Minerals	Wheat	Blend A	Blend B	Blend C
Total (g)	3.36[a]	3.85[b]	3.96[c]	4.06[cd]
Ca (mg)	247[b]	222[a]	214[a]	206[a]
Cu (mg)	0.1[a]	0.1[a]	0.2[b]	0.2[b]
Fe (mg)	0.7[a]	1.1[b]	1.4[c]	1.6[d]
Mg (mg)	24.9[a]	33.1[b]	35.9[c]	38.6[d]
Mn (mg)	0.3[a]	0.8[b]	0.9[c]	1.1[d]
K(mg)	62.8[a]	117.4[b]	135.6[c]	153.9[d]
Na (mg)	241.9[d]	210.4[c]	199.9[b]	189.4[a]
Zn (mg)	0.5[a]	0.9[b]	1.0[c]	1.1[cd]

Within each row, values with different letters differ significantly from each other ($P \leq 0.05$).
Source: Angioloni and Collar [2]. Reproduced with permission of Wiley.

quality of the bran fraction. Flours differ in their extraction rate, which is defined as the proportion of flour by weight derived by milling from a known quantity of grains. White flour is generated when the extraction rate is 75% or less. If the extraction rate exceeds 80%, the flour will contain bran particles; if the flour extraction rate approaches 100%, a wholemeal flour is produced. The milling process removes many important nutrients when refined flour is produced [10]. Refining wheat flour removes the bran and the germ, decreasing essential nutrients to levels less than half of those naturally occurring in whole wheat for some mineral elements: 16% (Mg), 18% (Mn), 24% (Zn), 26% (K), 30% (Fe), 31% (P), 38% (Cu), 40% (Na), 44% (Ca) and 48% (Se) [11]. As a result of milling the palatability and the caloric content are increased but the nutritional value of the product is decreased. The breadmaking process consists of three major stages: mixing, fermentation and baking. During breadmaking, the content of PA decreases due to the action of phytases such as myo-inositol-hexakis (dihydrogenphosphate) phosphohydrolase in the dough, enzymes capable of liberating covalently bound phosphate groups from the inositol ring, thus decreasing or eliminating the anti-nutritional effect of PA [12]. Lower myo-inositol phosphate esters have a lower capacity to bind minerals [13]. Phytate-degrading enzymes exist in cereals, yeast and lactic acid bacteria isolated from sourdough [9]. Other factors such as particle size of the meals, pH, temperature, water content and fermentation time contribute to the phytate reduction. The reduction of PA has repeatedly been reported in yeast and sourdough processes. Although yeast fermentation minimizes the unfavourable effects of PA, sourdough bread is a better source of available minerals, especially magnesium, iron and zinc [9, 14].

Information on the presence of mineral elements in the diet typically refers to their total concentrations, usually measured by atomic absorption spectrometry (AAS), inductively coupled plasma (ICP) emission spectrometry, or ICP mass spectrometry. However, these methods do not allow measurement of the fraction that is bioaccessible to the human body. This fraction depends on the speciation of the elements, on the behaviour of organometallic species and complexes in the gastrointestinal tract, and on interactions with the food matrix [15]. Bioaccessibility, which can be defined as the amount of metal converted to soluble forms under gastrointestinal conditions, is typically evaluated using a sequential analysis with artificial gastric juice and intestinal juice, and the analysis of the soluble fraction. *In vitro* methods have been widely used to evaluate bioaccessibility of minerals and trace elements from different sort of food products. Khouzam *et al*. [15] evaluated the bioaccessibility of five essential micronutrients (Fe, Cu, Zn, Mn and Mo) from the Lebanese diet including bread, which contributes 136 g daily (10.3%) to total diet of the Lebanese population, and their contribution to the recommended daily allowance (RDA). The daily total intakes of elements from the analysed food products consumed by the Lebanese population lead to the conclusion that total consumption of nutrients as Cu (0.66 mg/day), Fe (6.6 mg/day), Mo (0.075 mg/day), Mn (5.9 mg/day) and

Zn (4.5 mg/day) is mainly provided by bread. *In vitro* methods characterize the concept of bioaccessibility and bioavailability: the former is the maximum soluble concentration of the target element in the simulated gastrointestinal solution after filtration or centrifugation, and the latter is the dialysable fraction of the element which can pass through a semi-permeable membrane with a specified pore size at equilibrium and non-equilibrium conditions. The *sine qua non* condition for an element to be bioaccessible is to be present in forms soluble under intestinal conditions. The experimental approach applied to verify this included two steps which imitated, first, extraction in an 'acidic stomach' using a gastric fluid (pepsin) and then extraction in a 'neutral small intestine' using an intestinal fluid (pancreatin, amylase and bile salts). Some soluble species may not pass through the intestinal membrane and thus are not bioaccessible. From an analytical point of view, quality control by demonstrating correspondence between the sum of the soluble fraction and the residue and the total element concentration is critical [15].

Ingredients used in different bakery products may have different effects on mineral bioavailability. Despite good correlations between *in vitro* dialysability and *in vivo* bioavailability, improvements have been obtained when the human colon carcinoma cell line has been incorporated into the methodology [16]. Several trace elements, such as iron, zinc, copper, manganese and molybdenum, are components of enzymes essential for life, and should be supplied to the human body, preferably from the diet. The availability of minerals in bakery products during processing was investigated by measuring solubility and dialysis, as well as mineral uptake and transport by Caco-2 cells after *in vitro* digestion [17]. Raw materials showed the highest amount of phytate, causing an important effect on the stage of processing. The solubility and dialysability of iron increased with fermentation, while calcium and zinc showed high variability depending on the product analysed. After baking, the dialysability of minerals increased with respect to the fermented dough in most cases. The highest uptake and transport efficiency of iron and calcium in cells corresponded to dough after fermentation of wheat flour with respect to the baked samples. For zinc, no differences were observed between fermented dough and after baking on uptake and transport efficiencies. This study showed that *in vitro* mineral availability of bakery products is influenced by the stage of processing and ingredients used [17].

Iron is a component of haemoglobin and various enzymes. Iron deficiency causes anaemia while excess may increase the risk of developing cancer or heart attacks. Iron is present in both divalent and trivalent forms; the former is known to be readily absorbed. Depending on the variety and on the source of information, concentrations of Fe in bread samples fall within different ranges: 41.9–47.0 μg/g [15], 25.0–41.5 μg/g [21] (**Table 23.1**), 18.2 ± 8.1 mg/kg [25]. The bioaccessible fraction was very low (11.8–12.8%) due the high contents of phytates. Phytates are known to bind Zn strongly, which is reflected by the very low bioaccessibility of this element (<10%) from bread despite its fairly high concentrations (24.9–34.8 μg/g). Phytates present in cereals and seeds can bind Zn^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+} and other divalent cations, whereas bioaccessibility of Zn^{2+} has been reported to be the most impaired in humans because of the formation of insoluble salts or even co-precipitation of Zn as a Zn–Ca–phytate complex, which is enhanced by high Ca intake from milk. Data on the bioaccessibility of these elements from foods are scarce, and for Mo non-existent. In bread, Cu, Mo and Mn show, in general, much higher bioaccessibility than Fe and Zn. The bioaccessible fraction of Cu was 49.2–62.4% from bread samples [15]. The manganese bioaccessible fraction in bread was 21.71–27.66%, and correlated negatively with the PA content, while the entirety of Mo present in bread is very bioaccessible (82.16–98.05%). Zinc deficiency is one of the most prevalent nutritional problems. According to World Health Organization (WHO) cut-offs, zinc in Baggett and Roghani breads had good bioavailability (50–55%), while other breads had moderate (30–35%) and flour had low (10–15%) zinc bioavailability [15].

The sourdough process, partial baking and freezing technology have impacted the nutritional value of breads compared with straight/direct traditional breadmaking processes [18]. The concentration of calcium was significantly lower in liver of rats fed with direct sourdough and part-baked frozen improved breads in comparison with rodents fed straight bread. Animals fed diets enriched with part-baked frozen conventional bread had higher phosphorus content in liver in comparison with rats fed straight breads. Rodents fed straight bread had the highest level of magnesium and iron in liver in comparison with the remaining experimental groups. The highest level of calcium in femoral bones of rats was

shown in the group fed part-baked frozen conventional bread in comparison with animals fed straight bread.

The effect of fermentation, germination and heating on the levels of some mineral elements was studied in Sudanese kisra bread and hulu-mur drink prepared from the two sorghum cultivars, dabar and fetarita, consecutively [19]. Fermentation caused no significant effect ($P<0.01$) on the mineral contents of kisra or hulu-mur. Addition of spices to hulu-mur dough caused significant increases ($P<0.01$) in strontium (80%), calcium (60%) and iron (35%). Germination of fetarita grains caused significant increases ($P<0.01$) of zinc (90%), lead (65%) and molybdenum (58%). Baking of kisra and hulu-mur did not cause any significant loss ($P<0.01$) in the contents of minerals.

The effect of technological processing on the contents of eight minerals (calcium, copper, iron, magnesium, phosphorus, potassium, selenium and zinc) was investigated in pasta making [20]. Analyte concentrations in whole grains, semolina, pasta and cooked pasta were determined by ICP mass spectrometry. Conventional roller milling significantly reduced the content of each mineral in durum wheat grains. However, concentration losses as a consequence of milling differed widely among elements, from 16% for Se to 66% for Mg and Zn on a dry weight basis. Retention of elements after milling followed the order Se>Ca>Cu>P>K>Fe>Mg>Zn. Pasta making had little effect on element concentrations in semolina. Cooking caused an increase in the calcium content of pasta, whereas the concentrations of the other elements were either unchanged or slightly reduced (0–18% on a dry weight basis) except potassium, which showed a decrease of 74%. Commercial pasta samples showed concentrations of minerals similar to those of the experimental samples, except selenium which was higher due to the use of imported wheat with higher levels of selenium in industrial semolina production. Overall, pasta appears to be a valuable source of several minerals, especially selenium, copper, magnesium and zinc (**Table 23.3**).

23.4 Mineral content of major cereal-based goods across the continents

Mineral amounts per 100-g serving of selected bread types (**Table 23.1**), pasta (**Table 23.3**) and bakery and confectionery products (**Table 23.4**) are compiled by the National Nutrient Database for Standard Reference [21]. Dietary reference intakes (DRIs), RDAs and adequate intakes (AI) for selected mineral elements (**Table 23.2**) are used to determine the contribution of the different cereal-based goods to daily mineral requirements [22].

In the study by Tahvonen and Kumpulainen [23], the mineral composition of six representative Finnish bread types was determined by flame AAS after digestion in concentrated HNO_3. The mineral element contents of the samples varied considerably within and between the different bread types: rye bread (wheat flour, 0–25%), Finnish coffee bread (wheat, low ash), French bread (wheat, low ash), whole wheat bread (high ash), mixed bread I (wheat, rye, and/or oat; mainly wheat), mixed bread II (flour components unknown; mainly wheat). Among analysed samples, mineral contents varied as follows: Cu 1.5–3.2 mg/kg, Fe 32–41 mg/kg, Zn 9–20 mg/kg, Mn 8–20 mg/kg and Mg 0.32–0.79 g/kg. Rye bread had the highest mean element content, with the exception of Fe. French bread had the highest Fe content, due to Fe fortification of wheat flours. The range of Fe contents in wheat breads was wide (23–52 mg/kg), indicating that all bakeries do not use fortified flours.

A hull-less barley flour and flakes were incorporated into white and wholegrain wheat bread in quantities usually applied in practice [24]. The supplemented breads were high in fibre, zinc and selenium content. It was estimated that a 300-g daily portion of such breads could meet up to 40% of dietary recommended intakes for selenium. Supplemented breads were not found to pose a significant risk with regard to excessive intakes of heavy elements (Pb, Cd, As).

Demirözü *et al.* [25] determined the level of iron, copper, zinc, lead and cadmium in bread samples obtained from 20 bakeries in Ankara and Samsun, Turkey, by AAS. The mean values of iron, copper, zinc, lead and cadmium were 19.2±8.1 mg/kg dry weight, 2.1±1.0 mg/kg dry weight, 10.0±3.0 mg/kg dry weight, 86.8±176.0 mg/kg dry weight and 12.2±6.1 mg/kg dry weight, respectively. No significant differences were found in copper and lead levels in samples obtained from bakeries in general, and in copper and cadmium levels in samples from different provinces ($P<0.05$), while they were considered significant in iron, zinc and cadmium levels in samples in general, and in iron, zinc and lead levels in samples from different provinces ($P<0.05$).

Iskander *et al.* [26] collected 19 different bread varieties baked at 13 different locations in Egypt, from Aswan in the far south to Edfina on the Mediterranean Sea.

These breads were formulated with wheat and/or corn flour with or without other ingredients such as fenugreek or okra. A total of 16 elements were measured by instrumental neutron activation analysis (INAA). The overall average concentrations of these elements in the investigated breads were as follows (in μg/g): Br, 5.06; Ca, 326; Cl, 2062; Cr, 0.95; Co, 0.044; Fe, 54.0; K, 2086; Mg, 692; Mn, 11.6; Na, 1709; Rb, 1.6; Sb, 0.040; Sc, 0.01; Se, 0.28; and Zn, 11.5. No As was detected in any of the bread types investigated. Assuming an average daily intake of 500 g, bread delivers 15–35% of RDA for Ca, 29–69% for K, 62–100% for Mg, 1–100% for Na, 33–77% for Zn, 43–100% for Se, 73–100% for Cr, and 100% for Fe and Mn.

In a study by Angioloni and Collar [2], the suitability of minor/ancient cereals (rye, oat, Kamut wheat, spelt wheat) and pseudocereals (buckwheat) in breadmaking was assessed in single (100% wheat flour replacement) and multigrain (20–44% of wheat flour replacement) matrices. The research allowed identification of the qualitative (oat, rye, buckwheat) and quantitative (up to 75% of wheat flour replacement) grains in mixed matrices providing enhancement of nutritional quality (higher protein content, higher mineral content, lower digestible starch, higher viscous fibre content, higher resistant starch content, source of antioxidants) and minimization of techno-functional impairment and sensory depreciation of the resulting breads. The main differences were observed in the contents of minerals in breads (total mineral content, 3.36–4.06 g/kg). Na, K, Mg and Ca were the minerals present in the highest concentrations in all samples, wheat bread included (**Table 23.5**). Maximum increases in the levels of minerals with respect to wheat bread were found in multigrain samples with maximum (75%) wheat flour replacement: (100%) Cu, (129%) Fe, (55%) Mg, (267%) Mn and (144%) K.

Over recent years, interest in aluminium has increased considerably as a result of new findings on its potentially toxic effects, it being implicated in a number of human pathologies including encephalopathy/dialysis dementia, Parkinson's disease and Alzheimer's disease [27]. Jalbani *et al*. [28] investigated the safety of branded and non-branded bakery products (bread and biscuits) consumed in Pakistan with respect to Al by comparing the estimated intake of Al with the value of Al permitted by FAO/WHO (1 mg/kg body weight) [29]. Samples of different branded and non-branded bread and biscuits were studied using electrothermal AAS (ETAAS) after microwave-assisted and conventional wet acid digestion methods. The mean Al concentrations of branded and non-branded biscuit samples were in the range 7.4–84.3 and 34.5–70.2 mg/kg, respectively, while analysis of branded and non-branded bread revealed the presence of Al at concentrations of 8.0–29.6 and 39.1–82.9 mg/kg, respectively. The authors observed that the levels of Al were significantly higher in non-branded bread, biscuits and in some branded products. The contribution of the bakery products to the daily intake of Al, based on the consumption of 250 g bread or biscuits per day, was found in the range 1.9–21.1, 8.62–17.6, 2.0–7.4 and 9.5–20.7 mg/day, from all branded and non-branded biscuit and bread, respectively. The limit of detection and limit of quantitation of Al were 0.5 and 5 mg/L, respectively.

The incorporation of amaranth flour in wheat flour matrices significantly increased mineral content of breads. Breads with amaranth have significantly higher amounts of phytates and lower myoinositol phosphates, which could predict low mineral bioavailability at high levels of substitution (30–40 g per 100 g). Mineral contents, both microelements and macroelements, were increased significantly by the wheat flour substitution [30]. The substitution of 40 g per 100 g increased the amount of Cu from 2.25 to 4.21 μg/g, Mn from 6.39 to 19.41 μg/g, Zn from 11.65 to 24.91 μg/g, Fe from 18.85 to 43.74 μg/g, Ca from 0.31 to 0.99 mg/g, Mg from 0.29 to 1.32 mg/g and K from 1.88 to 3.21 mg/g, respectively. However, the Na level decreased or remained unchanged by the substitution because it was included in the formulation as an ingredient (3.72–4.08 mg/g). Wholegrain breads are known to be richer sources of macroelements and microelements than breads made of refined flours [10]. The amounts of Cu, Zn, Fe and Mg in whole wheat bread are similar to the content in bread made with amaranth flour 30–40 g per 100 g, while the amounts of Mn, Ca and K are close to half [24]. The contribution to the DRIs (**Table 23.2**) for minerals from 250 g of bread with or without amaranth have been estimated. Control wheat bread contributes 38.3% of the Cu recommended for adults, whereas the breads incorporating amaranth contribute significantly increased intakes of this mineral (43.0–69.6%). Moreover, consumption of the control bread satisfies 42.5 or 54.3% of the Mn recommendation, whereas bread made with amaranth flour 20–30 g per 100 g could cover the requirements of

this microelement in adults. With regard to Zn, consumption of the control bread would provide only one-fifth (or less) of the daily requirement in adults, whereas the bread made with amaranth flour provided nearly 50% of these daily requirements in females. The same tendency was observed with Fe, where 20% flour substitution could supply more than 50% of the daily requirement of this mineral in males. The macronutrients followed the same trend. About 50% of the requirements of Mg and P could be covered by the inclusion of amaranth in the bread formulation (30–40 g per 100 g). However, P in cereal and pseudocereal whole flours corresponds almost exclusively to phytic phosphorus; it might not be bioavailable unless it is hydrolysed during fermentation by the action of the endogenous phytase in the cereal.

Skrbic and Filipcev [24] estimated that wholegrain-supplemented wheat breads prepared with the addition of high-oleic sunflower seed at various levels (8%, 12%, 16% flour basis) contribute to the corresponding DRIs in the range 33.7–40.8% (adults) for copper and 4.7–18.4% (males) and 6.4–25.3% (females) for zinc [24].

Erba *et al*. [31] determined trace elements (Zn, Fe, Cu and Mn) and minerals (Ca, Mg, P and K) concentrations in wholemeal flour of five einkorn accessions and one bread wheat cultivar, cropped in four different locations for 2 years. The major factors influencing mineral levels were year and genotype, as well as their interaction. Einkorn varieties exhibited higher Zn (7.18 ± 0.76 mg per 100 g dry matter), Fe (5.23 ± 0.47), Mn (4.65 ± 0.23), Cu (0.90 ± 0.08), Mg (151.2 ± 9.00) and P (541.1 ± 35.37) concentrations than bread wheat. The Mg concentration correlated positively with that of other bivalent cations (Zn and Ca). The relevant amount of trace elements consistently found in einkorn further confirms the potential of this cereal in human nutrition, either by direct consumption or by introgression of superior alleles into enhanced polyploid wheat cultivars.

23.5 Mineral-enriched cereal-based baked goods: a legal perspective of fortification

Calcium malabsorption is believed to aggravate negative calcium balance and contribute importantly to age-related bone loss. Although dairy products are considered a rich and bioavailable source of calcium, there are people who either avoid these products or do not consume them in adequate amounts. Therefore, there is a need for alternative food choices that can provide individuals with adequate calcium without the need for supplementation. Juma *et al*. [32] undertook a study to examine whether calcium bioavailability of a bread-based diet is comparable with that of a milk-based diet. Additionally, the calcium bioavailability of these two diets was compared with that of semi-purified diets with varying amounts of fat and protein. Calcium absorption was significantly lower in the milk and high-fat, high-protein groups in comparison with the bread and control groups. Skeletal growth was not compromised in any of the treatment groups as judged by femoral density and mineral content and tibial protein content. Despite similar energy intakes, the final mean body weight of animals fed the milk-based diet was significantly lower than that of the other groups. These results indicate that enriched bread can serve as a good source of bioavailable calcium. Additionally, data suggest that diets with higher fat content may interfere with calcium absorption.

Wheat flour was first fortified with calcium in the UK in 1943 in order to restore the calcium lost during milling. Today, it is compulsory to add 940–1560 mg calcium carbonate per kilogram to white and brown (but not wholegrain) flours milled in the UK. In the USA, the addition of calcium to flour has been optional since the early 1940s. Calcium sulfate, carbonate, chloride, phosphate, acetate or lactate are all suitable for fortification of wheat flours, but the oxide and hydroxide may require alterations in the pH of the dough for successful breadmaking [6].

The nutritional usefulness of iron fortification of wheat flour has been confirmed in a study in Thailand [33]. In this study the relative efficacy of electrolytic iron compared with ferrous sulfate was about 70% in women consuming fortified wheat flour cookies compared with 50% for hydrogen-reduced iron. Based on this evidence, adding double the amount of electrolytic iron or hydrogen-reduced iron as compared with ferrous sulfate should give an equivalent efficacy to ferrous sulfate. Ferrous sulfate and elemental iron powders have traditionally been used to fortify wheat and other cereal flours. Electrolytic iron remains the preferred elemental iron fortificant, although hydrogen-reduced iron could also be considered. In addition, recent evidence from rat studies suggests that carbonyl iron may

be as good as electrolytic iron as a fortificant, but human efficacy studies are still necessary to confirm this. Although ferrous sulfate has been successfully used for many years in Chile (where fortified flour is consumed within 6–8 weeks of purchase), and ferrous fumarate has been employed in Venezuela and throughout Central America, in other countries the addition of these iron compounds to wheat flours has caused rancidity. This problem could be overcome by using encapsulated forms to improve stability. Ferrous sulfate, and to a lesser extent ferrous fumarate, are also suitable fortificants for pasta which, because of its low moisture content, is less susceptible than wheat flour to the development of rancidity. Although potentially useful for some high-phytate flours, NaFeEDTA has not been used widely in any large-scale iron fortification programmes because of reports that it interferes with the bread fermentation process [34]. However, China is currently introducing NaFeEDTA to fortify wheat in several provinces, and so far there have been no recorded problems. Although ascorbic acid is often added to iron-fortified foods in order to enhance absorption, its usefulness in this respect in bread flours is limited by the fact that it is destroyed by the action of heat during baking. Ascorbic acid is nevertheless frequently added to flours, not so much to enhance iron absorption but rather as a raising agent. In its guidelines on iron fortification of cereal-based staples, the SUSTAIN Task Force [35] recommended the use of ferrous sulfate in preference, followed by ferrous fumarate, and lastly electrolytic iron (but at twice the iron concentration of the other iron compounds). In order to ensure the successful fortification of wheat flour and wheat flour products, it may be necessary for individual countries to adopt different strategies to take account of differences in climate, wheat flour quality, processing methods and storage conditions, as well as differences in the main uses of flour (i.e. to make bread or other foods). Attempts have also been made to fortify maize flours with iron. Lime-treated (nixtamalized) corn masa, a staple used to make tortillas in much of Latin America, goes rancid when soluble iron compounds, such as ferrous sulfate, are added to it. Further colour and texture changes occur during the preparation of tortillas. The difficulties are further compounded by the fact that iron absorption from corn masa is strongly inhibited by its high phytate and high calcium content. For these reasons, iron fortification of maize flours has not been widely adopted, except in a number of Latin American countries where the consumption of maize is high. In Venezuela, for example, ferrous fumarate mixed with elemental iron is used to fortify maize flours. In view of its highly inhibitory nature (especially if it is not degermed), the Pan American Health Organization (PAHO) recently recommended the use of either NaFeEDTA or ferrous fumarate (at twice the amount) for maize flour fortification [34]. These recommendations have yet to be put into practice. Whether they are appropriate for maize meal that is used to prepare porridge also needs to be evaluated. For maize flours that are not high in PA (e.g. degermed) and are not lime-treated, the same iron compounds as those recommended for the fortification of white wheat flour can be considered.

The importance of coeliac disease as a possible cause of iron-deficiency anaemia is increasingly being recognized [36]. It is estimated that coeliac disease may account for 3–5% of the prevalence of iron-deficiency anaemia [37]. Fortified or enriched gluten-free products are rare, but it has been suggested that development of such products would improve the quality of the diet. Fortification is an effective approach for increasing dietary iron intake, provided that certain conditions apply [38]. It is therefore within adopted practice to fortify gluten-free products. Successful fortification requires an iron compound that is adequately absorbed and does not affect the sensory properties of the products. Kiskini *et al*. [39] produced gluten-free bread fortified with iron (GFB-Fe), using selected iron compounds (ferric pyrophosphate, ferric pyrophosphate with emulsifiers, NaFeEDTA, electrolytic iron, ferrous gluconate, ferrous lactate and ferrous sulfate), to test sensory characteristics of the GFB-Fe (feel-mouth texture, crumb colour, aroma and taste) and to compare iron dialysability of various iron compounds in GFB-Fe. They found that most acceptable products were those fortified with ferric pyrophosphate with emulsifiers and ferric pyrophosphate. Ferrous dialysable iron (ferrous iron with molecular mass <8000 Da, an index for prediction of iron bioavailability) was measured under simulated gastrointestinal conditions. Ferrous dialysable iron in GFB-Fe fortified with ferric pyrophosphate with emulsifiers, NaFeEDTA, ferrous bis-glycinate, ferrous gluconate or ferrous sulfate was higher than that in GFB-Fe fortified with electrolytic iron, ferrous lactate or ferric pyrophosphate ($P<0.05$). The attempt to produce iron-fortified GFB was successful; the bread produced had satisfactory

sensory and nutritional characteristics. This encourages further research for developing such products and for testing them in humans, particularly gluten-sensitive individuals.

From a technical point of view, bread is a good vehicle for iodine and has been shown to be an effective way of ensuring a constant supply of dietary iodine. It has been used in a few European countries where bread is a staple food, such as Russia [40], and in Tasmania. The main carrier for iodine in the Netherlands is the salt added to bread (i.e. baker's salt), which has been enriched with iodine since 1942. In recent years, the potassium iodide content of Dutch baker's salt has been increased.

Recent studies have shown that the absorption of zinc from cereal products fortified with zinc oxide is as good as that from those fortified with the more soluble zinc sulfate [41], presumably because the oxide is soluble in gastric acid. However, zinc absorption from the oxide may be poor in individuals with low stomach acid secretion. Zinc absorption from food is dependent on the amount of zinc consumed and the ratio of phytate to zinc in the meal being consumed. According to recent estimates by the International Zinc Nutrition Consultative Group (IZiNCG), when zinc intake is just adequate to meet the physiological requirements for absorbed zinc, in adult men about 27% of the zinc content is absorbed from diets having a phytate/zinc molar ratio of less than 18, which drops to about 19% when the phytate/zinc molar ratio is greater than 18 (i.e. high phytate). The corresponding zinc absorption rates for adult women are 35% and 26%, respectively [42]. When zinc intake is greater than the critical level needed to meet requirements, the fractional absorption becomes progressively less, although the net absorption of zinc increases slightly. In one study involving healthy well-nourished adults from the USA, zinc absorption from the sulfate (or the oxide) added to a low-phytate bread meal was about 14% (total zinc content, 3.1–3.7 mg per meal) compared with around 6% from the same fortificants added to a high-phytate wheat porridge meal (total zinc content, 2.7–3.1 mg per meal) [43]. The addition of Na_2EDTA or NaFeEDTA to cereal flours inhibits the action of yeast during the breadmaking process, so these compounds would be of limited use, at least in cereal flours. The fortification of wheat flour with relatively high levels of zinc (as zinc acetate) did not affect the baking or organoleptic properties of the bread dough [44]. Likewise, the addition of zinc 60 or 100 mg/kg wheat flour (as zinc sulfate or zinc oxide) did not change the acceptability of bread [45]. Encapsulation of zinc compounds is possible but has not been considered to date. This would, however, be a convenient way to mask the unpleasant taste of some zinc compounds.

In recent research [46], the retention and speciation of selenium in flour and bread was determined following experimental applications of selenium fertilizers to a high-yielding UK wheat crop. Flour and bread were produced using standard commercial practices. Total selenium was measured using ICP mass spectrometry (ICP-MS) and the profile of selenium species in the flour and bread were determined using high-performance liquid chromatography (HPLC) ICP-MS. The selenium concentration of flour ranged from 30 ng/g in white flour and 35 ng/g in wholemeal flour from untreated plots to more than 1800 ng/g in white and more than 2200 ng/g in wholemeal flour processed from grain treated with selenium (as selenate) at the highest application rate of 100 g/ha. The relationship between the amount of selenium applied to the crop and the amount of selenium in flour and bread was approximately linear, indicating minimal loss of Se during grain processing and bread production. On average, application of selenium at 10 g/ha increased total selenium in white and wholemeal bread by 155 and 185 ng/g, respectively, equivalent to 6.4 and 7.1 lg selenium per average slice of white and wholemeal bread, respectively. Selenomethionine accounted for 65–87% of total extractable selenium species in Se-enriched flour and bread; selenocysteine, Se-methylselenocysteine, selenite and selenate were also detected. Controlled agronomic biofortification of wheat crops for flour and bread production could provide an appropriate strategy to increase the intake of bioavailable selenium.

23.6 Conclusions and future prospects

Because the awareness of a healthy lifestyle is increasing, breads containing wholegrain, multigrain or other functional ingredients will become more important in the bakery industry and on the market. New ingredients and reconsideration of ancient cereals and pseudocereals, processing steps and application of new/emerging technologies such as high hydrostatic pressure in breadmaking are hot topics in cereal science research to develop baked goods with extra nutritional value, and

with texture, appearance and sensory acceptability that can compete with regular wheat breads. Research on the influence of these processes and ingredient innovations on the mineral composition, availability and fortification of the finished cereal-based goods remains basically unexplored and is strongly encouraged.

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Abbreviations

AAS: Atomic absorption spectrometry
AI: Adequate intake
DRI: Dietary reference intake
ETAAS: Electrothermal atomic absorption spectrometry
GFB: Gluten-free bread
HPLC: High-performance liquid chromatography
ICP: Inductively coupled plasma
IP6: Myo-inositol hexakisphosphate
MNM: Micronutrient malnutrition
MS: Mass spectrometry
PA: Phytic acid
PAHO: Pan American Health Organization
RDA: Recommended dietary allowance

References

1 Dewettinck K, Van Bockstael F, Kühne B, Van de Walle D, Courtens TM, Gellynck X. Nutritional value of bread: influence of processing, food interaction and consumer perception. *J Cereal Sci* 2008;**48**:243–257.
2 Angioloni A, Collar C. Nutritional and functional added value of oat, Kamut, spelt, rye and buckwheat versus common wheat in breadmaking. *J Sci Food Agric* 2011;**91**: 1283–1292.
3 Angioloni A, Collar C. Polyphenol composition and 'in vitro' antiradical activity of single and multigrain breads. *J Cereal Sci* 2011;**53**:90–96.
4 Angioloni A, Collar C. High legume–wheat matrices: an alternative to promote bread nutritional value meeting dough viscoelastic restrictions. *Eur Food Res Technol* 2012;**234**: 273–284.
5 Angioloni A, Collar C. Suitability of oat, millet and sorghum in breadmaking. *Food Bioprocess Technol* 2013;**6**:1486–1493.
6 Allen L, de Benoist B, Dary O, Hurrell R. (eds) *Guidelines on Food Fortification with Micronutrients*. World Health Organization, Food and Agricultural Organization, Geneva, 2006.
7 Bock MA. Minor constituents of cereals. In: Kulp K, Ponte JG Jr (eds) *Handbook of Cereal Science and Technology*, 2nd edn. Marcel Dekker, New York, 2000, pp. 479–504.
8 Lyons G, Ortiz-Monasterio I, Stangoulis J, Graham R. Selenium concentration in wheat grain: is there sufficient genotypic variation to use in breeding? *Plant Soil* 2005;**269**:369–380.
9 Lopez HW, Duclos V, Coudray C *et al*. Making bread with sourdough improves mineral bioavailability from reconstituted whole wheat flour in rats. *Nutrition* 2003;**19**:524–530.
10 Collar C. Novel high-fibre and whole grain bread. In: Hamaker BR (ed.) *Technology of Functional Cereal Products*. Woodhead Publishing Ltd, Cambridge, 2008, pp. 184–214.
11 The Whole Grains Council (2004) www.wholegrainscouncil.org.
12 Gobbetti M, De Angelis M, Corsetti A, Di Cagno R. Biochemistry and physiology of sourdough lactic acid bacteria. *Trends Food Sci Technol* 2005;**16**:57–69.
13 Leenhardt F, Levrat-Verny MA, Chanliaud E, Rémésy C. Moderate decrease of pH by sourdough fermentation is sufficient to reduce phytate content of whole wheat flour through endogenous phytase activity. *J Agric Food Chem* 2005;**53**:98–102.
14 Chaoui A, Faid M, Belahsen R. Making bread with sourdough improves iron bioavailability from reconstituted fortified wheat flour in mice. *J Trace Elem Med Biol* 2006;**20**:217–220.
15 Khouzam RB, Pohl P, Lobinski R. Bioaccessibility of essential elements from white cheese, bread, fruit and vegetables. *Talanta* 2011;**86**:425–428.
16 Perales S, Barberá R, Lagarda MJ, Farré R. Bioavailability of calcium from milk-based formulas and fruit juices containing milk and cereals estimated by in vitro methods (solubility, dialysability, and uptake and transport by caco-2 cells). *J Agric Food Chem* 2005;**53**:3721–3726.
17 Frontela C, Ros G, Martínez C. Phytic acid content and 'in vitro' iron, calcium and zinc bioavailability in bakery products: the effect of processing. *J Cereal Sci* 2011;**54**:173–179.
18 Kopec A, Pysz M, Borczak B *et al*. Effects of sourdough and dietary fibers on the nutritional quality of breads produced by bake-off technology. *J Cereal Sci* 2011;**54**:499–505.
19 Mahgoub SEO, Ahmed BM, Ahmed MMO, El Agib ENAA. Effect of traditional Sudanese processing of kisra bread and hulu-mur drink on their thiamine, riboflavin and mineral contents. *Food Chem* 1999;**67**:129–133.
20 Cubadda F, Aureli F, Raggi A, Carcea M. Effect of milling, pasta making and cooking on minerals in durum wheat. *J Cereal Sci* 2009;**49**:92–97.
21 US Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 25. Nutrient Data Laboratory. http://www.ars.usda.gov/ba/bhnrc/ndl (accessed March 2013).

22 Otten JJ, Hellwig JP, Meyers LD. *Dietary Reference Intakes DRI. The Essential Guide to Nutrient Requirements.* The National Academies Press, Washington, DC, 2006. Available at www.nap.edu

23 Tahvonen R, Kumpulainen J. Levels of selected elements in Finnish breads. *J Food Compos Anal* 1994;**7**:83–93.

24 Skrbic B, Filipcev B. Nutritional and sensory evaluation of wheat breads supplemented with oleic-rich sunflower seed. *Food Chem* 2008;**108**:119–129.

25 Demirözü B, Saldamli I, Gürsel B, Uçak A, Cetinyokus F, Yüzbasi N. Determination of some metals which are important for food quality control in bread. *J Cereal Sci* 2003;**37**:171–177.

26 Iskander FY, Davis KR, Ashour H, Hassan HF. Aluminium content of Egyptian breads. *Food Chem* 1990;**35**:197–208.

27 Agency for Toxic Substances and Disease Registry. Toxicological profile for aluminum, September 2008. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=191&tid=34.

28 Jalbani N, Kazi TG, Jamali MK, Arain BL, Afridi HI, Baloch A. Evaluation of aluminum contents in different bakery foods by electrothermal atomic absorption spectrometer. *J Food Compos Anal* 2007;**20**:226–231.

29 International Programme on Chemical Safety. *Toxicological Evaluation of Certain Food Additives and Contaminants*. WHO Food Additive Series Vol. 24. WHO, Geneva, 1989.

30 Sanz-Penella JM, Wronkowska M, Soral-Smietana M, Haros M. Effect of whole amaranth flour on bread properties and nutritive value. *LWT Food Sci Technol* 2013;**50**:679–685.

31 Erba D, Hidalgo A, Bresciani J, Brandolini A. Environmental and genotypic influences on trace element and mineral concentrations in whole meal flour of einkorn (*Triticum monococcum* L. subsp. *monococcum*). *J Cereal Sci* 2011;**54**:250–254.

32 Juma S, Sohn E, Arjmandi BH. Calcium-enriched bread supports skeletal growth of young rats. *Nutr Res* 1999;**19**:389–399.

33 Zimmermann MB, Winichagoon P, Gowachirapant S *et al*. Comparison of the efficacy of wheat-based snacks fortified with ferrous sulfate, electrolytic iron, or hydrogen-reduced elemental iron: randomized, double-blind, controlled trial in Thai women. *Am J Clin Nutr* 2005;**82**:1276–1282.

34 Dary O. Lessons learned with iron fortification in Central America. *Nutr Rev* 2002;**60**:S30–S33.

35 SUSTAIN Task Force. *Guidelines for Iron Fortification of Cereal Food Staples*. SUSTAIN, Washington, DC, 2001. Available at www.sustaintech.org.

36 Hershko C, Lahad A, Kereth D. Gastropathic sideropenia. *Best Pract Res Clin Haematol* 2005;**18**:363–380.

37 Grisolano SW, Oxentenko AS, Murray JA, Burgart LJ, Dierkhising RA, Alexander JA. The usefulness of routine small bowel biopsies in evaluation of iron deficiency anemia. *J Clin Gastroenterol* 2004;**38**:756–760.

38 Hurrell R. Fortification: overcoming technical and practical barriers. *J Nutr* 2002;**132**:806S–812S.

39 Kiskini A, Argiri K, Kalogeropoulos M *et al*. Sensory characteristics and iron dialyzability of gluten-free bread fortified with iron. *Food Chem* 2007;**102**:309–316.

40 Gerasimov G *et al*. Bread iodization for iodine deficient regions of Russia and other newly independent states. *IDD Newsletter* 1997;**13**:12–13. http://www.iccidd.org/.

41 López de Romana D, Lonnerdal B, Brown KH. Absorption of zinc from wheat products fortified with iron and either zinc sulfate or zinc oxide. *Am J Clin Nutr* 2003;**78**:279–283.

42 Holt C, Brown KH (eds) Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr Bull* 2004;**25**:S94–S203.

43 Hurrell RF, Reddy MB, Juillerat MA, Cook JD. Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. *Am J Clin Nutr* 2003;**77**:1213–1219.

44 Kiliç I, Ozalp I, Coŝkun T *et al*. The effect of zinc-supplemented bread consumption on school children with asymptomatic zinc deficiency. *J Pediatr Gastroenterol Nutr* 1998;**26**:167–171.

45 López de Romana D, Brown KH, Guinard JX. Sensory trial to assess the acceptability of zinc fortificants added to iron-fortified wheat products. *J Food Sci* 2002;**67**:461–465.

46 Hart DJ, Fairweather-Tait SJ, Broadley MR *et al*. Selenium concentration and speciation in biofortified flour and bread: retention of selenium during grain biofortification, processing and production of Se-enriched food. *Food Chem* 2011;**126**:1771–1778.

CHAPTER 24
Edible fats and oils

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Abstract: This chapter presents a review of the scientific literature concerning the determination of the mineral contents of edible fats and oils. The search was carried out by means of the Scopus and Google Scholar internet search engines without publishing date restrictions, and produced 84 references comprising book chapters and journal articles. Detection techniques and sample preparation are discussed in detail. As expected, the majority of the articles refer to the use of atomic spectrometric techniques such as atomic absorption and emission and plasma source mass spectrometry. Sample preparation techniques range from simple dilution in organic solvent to various wet-ashing approaches including microwave-assisted digestion and formation of emulsions and microemulsions, either batch-wise or by means of flow injection analysis. Dry ashing is also reported. Based on this review, it can be concluded that the determination of the mineral contents of edible oils and fats may be relevant from the technological and toxicological points of view but not from the nutritional one. It should also be noted that almost all the references report the determination of total element regardless of its speciation.

Keywords: edible oils, edible fats, determination, minerals, elements

24.1 Introduction

Edible fats and oils are either products of natural origin (vegetal or animal, often referred to as lipids) or industrialized products where natural lipids are chemically or physically modified in order to obtain the desired properties. They include such diverse products as sunflower oil, lard, butter and margarines. The occurrence and relative importance of different oils and fats of vegetable and animal origin depends heavily on the geographical area and on the culture of the people inhabiting it. However, some vegetable oils are widely used across the world, for instance those from sunflower or olive. Others are of more restricted use within smaller areas. Cultural globalization has contributed to the diffusion of specific oils and fats as a part of the extended interest in world cuisine. Detailed information on the origin, characteristics and usage of different edible oils and fats can be found in several books on food chemistry [1–4].

From a chemical point of view, they consist basically of triglycerides (also called triacyglycerols), which comprise esters of 1,2,3-propanetriol (glycerine) and up to three fatty acid residues, which may ('simple' triglycerides) or may not ('mixed' triglycerides) be identical [1–4]. The fatty acids determine the chemical and physical properties of the oil or fat and are also responsible for its nutritional characteristics. Considerable emphasis is currently given to this subject, as the presence of saturated fatty acids, as well as the *cis–trans* isomerism presented by unsaturated fatty acids, has been linked with the biosynthesis of cholesterol in the human body, thus raising the issue of health problems associated with this substance. The group of substances other than triglycerides, known as the unsaponifiable fraction, amount to less than 3% and are composed mostly of free fatty acids and other lipids [1, 2]. The difference between fats and oils arise from their physical properties; fats are semi-solid at room temperature, while oils are liquid, although presenting high viscosities. Thus the boundary between the two is not clearly defined.

Minerals present in edible oils and fats may come from different sources. In the case of oils and fats of vegetable origin, a significant fraction is due to the soil where the plant was grown. During life, plants absorb a number of nutrients and a fraction of them, present in those parts

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used for the extraction of the oil, may accompany the product. Similarly, oils and fats of animal origin reflect the composition of the feeds which composed the diet of the animal during its lifetime. Edible oils and fats sold commercially are to some extent industrial products, and the manufacturing process may affect the mineral content. For some of them, such as virgin oils, the industrial process is minimal, consisting basically of mechanical extraction possibly followed by filtration. Others may suffer more complex processes involving extraction in hot organic solvents or chemical reactions (refining) intended to neutralize strong tastes or other defects. Some products such as butter, included in the category for the purpose of this monograph, are artificial products that do not exist as such in nature but are manufactured from natural oils and fats plus several additives such as emulsifiers, stabilizers, colorants, flavouring agents and preservatives. Both butter and margarines are water-in-oil (W/O) emulsions; however, while butter is manufactured from milk fat, margarines are most often manufactured from vegetable oils, which in turn have been hydrogenated catalytically (usually employing nickel as catalyst) in order to transform part of the polyunsaturated fatty acids to saturated fatty acids. Solvents, chemicals, additives and even the industrial reactors and containers used for storage can be sources of contaminants affecting the levels of trace elements in the product beyond those originally present in the natural raw materials.

Minerals in foods play a significant role in human nutrition. However, interest in the mineral contents of edible oils and fats does not derive from their nutritional importance as their contribution is minimal, but rather from the toxic properties some of the elements have and from the negative effects which others may have from the technological point of view. Examples of the former include the contents of lead, cadmium or arsenic in edible vegetable oils, and of nickel in margarines. On the other hand, levels of copper, iron and other heavy elements are routinely determined in edible oils because they act as catalysts in chemical processes that lead to the appearance of oxidative rancidity [1]. A newer trend in food analysis concerns the use of information on levels of certain trace elements for detection of adulteration. However, little could be found on the determination of elements for this purpose in oils and fats.

While a number of books and reviews have been written about oils and fats and the analysis thereof, the subject of the mineral contents of these foods has not yet been dealt with extensively. A significant fraction of the references in the literature were published during the 1980s and 1990s, probably as a consequence of the growing availability at that time of analytical techniques such as flame atomic absorption spectrometry (FAAS), electrothermal atomic absorption spectrometry (ETAAS) and inductively coupled plasma optical emission spectrometry (ICP-OES) for the determination of elements in difficult matrices. The current availability of robust techniques for the determination of trace elements in a number of samples has stimulated research on this subject. Also, the growing interest in the analysis of biodiesel is noteworthy, given that this fuel is obtained from vegetable oils, and biodiesel itself manifests some properties similar to those of edible oils. Thus from a methodological point of view both can be treated in a similar manner. A recent review on the topic by Lepri *et al.* [5] is a good example of this trend.

For the preparation of this chapter the following products were considered: vegetable edible oils, natural fats of vegetable or animal origin, and manufactured products including butter and margarines.

24.2 Sample preparation

24.2.1 General considerations

The subject of sample preparation for the analysis of food samples has been covered by different monographs and publications [6, 7]. However, edible oils and fats have not received extensive coverage as to their preparation for analysis.

With regard to sample handling and preparation for trace element determination, oils and fats are probably among the most difficult matrices. They have in common their high hydrophobicity, which is at odds with traditional practices in inorganic analysis which were designed for aqueous media. On the other hand, oils are intrinsically highly viscous samples, a characteristic that may hinder sample handling and some of the analytical unitary operations such as volume measurements and mixing, as well as direct sample introduction for instance in atomic absorption or emission spectrometers due to the characteristics of the nebulizers used in FAAS and ICP-OES as well as the difficulties presented by autosamplers in handling such samples.

These physicochemical characteristics are shared by a different group of samples, namely lubricating oils and fats (derived either from petroleum or synthetic) as well

as petroleum itself and its derivatives. This fact allows for similar strategies in their handling, especially in connection with the use of atomic spectrometric techniques, and experience gained in the analysis of one group of samples may be useful for facing the challenges posed by others.

For the determination of trace elements the two classical strategies for samples of edible oils and fats are mineralization or dilution with an appropriate solvent. The first strategy produces a processed sample that is totally inorganic in nature, amenable to classical inorganic analysis and appropriate for introduction to atomic spectrometers. The second strategy usually involves the use of an organic solvent compatible with the sample, and can be used for introduction of the sample into an atomic spectrometer, but at the price of loss of sensitivity.

In order to attain quantitative measurement, samples may be quantified by weight instead of by volume, thus avoiding one of the difficulties that arises from the high viscosity. Dilutions can be made with appropriate organic solvents. However, if samples are to be mixed with aqueous reagents, the use of organic solvents does not always solve the problems posed by hydrophobicity as many organic solvents (e.g. pentane, hexane, methyl isobutyl ketone or even kerosene) are also hydrophobic, thus forcing the search for solvent mixtures of intermediate polarity. Nevertheless, direct dilution with organic solvents is a simple and effective way of preparing the sample for introduction to a spectrometer for AAS or ICP-OES. However, the dilution involved entails loss of sensitivity and thus limits the usefulness of this approach to those elements with concentrations high enough to fall within the working range of the spectrometric technique. Another problem arises from the need to use organic elements standards for calibration. These are sometimes difficult to find and their solubility should be carefully evaluated. Some authors have resorted to using organic elements standards sold for the analysis of lubricating oils.

24.2.2 Application of flow techniques to the analysis of edible oils and fats

After the first papers presenting the concept of flow injection analysis (FIA) were published in the 1970s [8, 9], the advantages that this novel strategy offered for the analysis of such difficult matrices as edible oils did not go unnoticed; research groups mainly from Greece and Spain applied it for the determination of several important parameters in olive oil [10–20]. Nevertheless, in this early work the application of FIA to the determination of trace elements was uncommon [21].

In the following decades, new flow techniques were devised and developed, including sequential injection analysis (SIA) [22, 23], multicommutated flow analysis (MCFA) [24, 25], lab-on-valve (LOV) [26, 27], multisyringe flow analysis (MSFA) [28, 29] and multipumping flow analysis (MPFA) [30, 31]. However, despite the availability of various flow techniques with different characteristics and advantages, so far FIA remains virtually the only flow technique successfully applied to the analysis of edible oils, either for sample pretreatment [32, 33] or for the complete analytical process [10–21]. The possibility of an easy coupling or *hyphenation* between flow systems and atomic spectrometers [34] opens a wide array of options for the determination of elements in these kinds of samples. Apart from FIA, however, little can be found in the literature concerning the application of newer flow techniques to the analysis of edible oils and fats. While an increasing number of papers are published each year dealing with the determination of different metals and non-metallic inorganic analytes by means of SIA, MCFA, MPFA and MSFA, the focus of most of these papers is not the analysis of edible oils or fats. A noticeable exception is the proposal by Pinto *et al*. [35] for the use of SIA for handling without pretreatment of oil samples. FIA and other flow techniques provide unmatched flexibility. Most analytical unit operations can be implemented in a flow system, so that operations such as sample handling and introduction, dilutions, chemical reactions, separation and preconcentration can be carried out. A number of different detection systems are amenable to coupling with flow systems. In this sense it is surprising that flow techniques do not have a more significant presence in the analysis of oils and fats.

24.2.3 Application of emulsions and microemulsions to the analysis of edible oils and fats

An entirely different and promising strategy for facilitating the handling of oils with water and aqueous solvents is the generation and use of emulsions and microemulsions [36]. This technique has been investigated and applied to the analysis of edible oils in batch [37–41] and also by means of flow injection [42–45]. The success of the latter approach makes this technique

complementary with flow injection and other flow techniques. Emulsions provide a suitable way for transporting insoluble hydrophobic samples such as oils in an aqueous stream. Since the contact surface between the two phases is largely increased, it also facilitates chemical reactions between the hydrophobic oil and oxidizing acids and other reagents present in the aqueous phase.

24.2.4 Problems associated with mineralization

Mineralization of samples of edible oils and fats by either dry ashing [46] or wet chemistry [47] is an extremely difficult task. The composition of these samples is almost totally organic and thus their oxidation is extremely difficult. If wet ashing procedures are attempted, hydrophobicity and viscosity hinder the mixture of the aqueous (oxidizing) and oily phases, the latter floating around unreacted on top of the former.

Dry ashing, on the other hand, is a relatively straightforward technique. However, when this technique of mineralization is carried out in the classical way in an open system (e.g. by means of an open crucible heated in a muffle furnace) satisfactory results are often not obtained. More volatile elements such as As, Se, Pb and Cd can be lost during ashing [48]. Also, contamination can easily occur [48]. Losses and contamination can be kept under control if appropriate precautions are taken and this simple technique is capable of producing satisfactory results for less volatile elements such as Fe, Cu, Ca, Mg, K, Na, Mn or Ni. For instance, Garrido *et al*. [49] determined concentrations of Na, K, Ca, Mg, Cu, Zn and Fe in Spanish edible oils. Samples were dry-ashed in a muffle furnace first at 300 °C, then at 400 °C for 2 hours and finally at 500 °C overnight. Ashes were dissolved in HCl and determinations carried out by FAAS. However there are a number of reasons for preferring those strategies based on closed systems for the mineralization of these samples.

Saleh *et al*. [50] investigated the use of open and semi-closed systems for the determination of Fe and Cu in palm oil, finding better precision and higher recoveries when the semi-closed system was employed. Sun [51] reported the use of a low-temperature oxygen plasma for the mineralization of salad oil, aiming at the determination of Cu and Fe by ETAAS. Nevertheless, as discussed in the paper, this ashing technique although highly effective is very slow, and is employed preferably when few samples are to be processed.

Wet ashing is a traditional approach employed with variable success for the mineralization of edible oil samples. One of the oldest and simplest ways is by means of a hot plate. This approach was used by Mohammed *et al*. [52] for the determination of Cd, Cr, Zn, Cu, Fe, K, Mg and Ca in argan oils by ICP-OES, with the goal of detecting adulteration. Mineralization was carried out with nitric acid and hydrogen peroxide. However, the authors do not discuss the mineralization method further.

Juranovic *et al*. [53] determined the contents of Ca, Cd, Mg, Mn, Ti, Zn, Co, Cu, Fe, K, Mo, Na, Ni, Pb, V, Al, Cr and P in pumpkin seed oil and pumpkin seeds by ICP-OES. These authors also employed open- and closed-vessel (steel bomb) nitric acid–peroxide wet ashing, along with closed-system microwave digestion, which was used as the reference method. Both open- and closed-vessel digestions carried out in the traditional way were lengthy (12 hours). With the exception of Zn, the authors found lower results (up to 50%) when using open-vessel digestion, although the authors state that differences were not statistically significant, so they could not be attributed to analyte loss. Nevertheless, recoveries for sulfur were below 50% for either method of mineralization and thus the authors do not report results for this element. Roca *et al*. [54] determined Ca, Mg, Mn, Zn, Se and Cr in olive oils by FAAS (Ca, Mg) and ETAAS (Mn, Zn, Se and Cr). Wet mineralization was carried out in a digestion block with nitric acid using V_2O_5 as catalyst.

Currently, microwave radiation in chemical analysis [55] has found widespread use as a tool not only for sample mineralization via wet chemistry, but also for a number of laboratory unitary operations such as drying, ashing, dissolution and extraction, giving rise to the new and wider concepts of microwave-assisted chemistry and microwave-enhanced chemistry. Sample mineralization via wet digestion in closed systems is currently an established technique; the availability of commercial microwave digestion systems with pressure control has greatly reduced the risks associated with the use of closed systems in operations which involve the generation of gases and thus an increase in pressure inside the reaction vessels.

The use of microwave radiation has also been extended to flow analysis systems [56, 57]. This combination presents several advantages, such as reduced analysis time, less consumption of sample and reagents (hence less chemical residues) and reduced risks of sample contamination. However, commercial microwave

digestion systems do not usually allow the introduction of samples and reagents in flow, so this alternative remains mainly in the sphere of research laboratories.

Several papers [58–62] present results for mineral contents obtained by using closed-system microwave digestion. A mixture of concentrated nitric acid and hydrogen peroxide was often used as reagent [58–62]. In one case [62], samples were pre-reacted with sulfuric and nitric acids before submitting them to digestion in a microwave oven. Other authors [63, 64] report the use of concentrated nitric acid only. One of the most obvious advantages found when using closed-system microwave digestion is the short time needed and high recoveries obtained.

An entirely different approach for sample preparation was applied by Buldini *et al*. [65]. These authors applied saponification followed by oxidative ultraviolet (UV) photolysis to remove the organic matrix of edible vegetable oils and fats, which were then submitted to ion chromatography for the determination of inorganic species. The times involved in sample preparation were 30 min for saponification and 1 hour for UV photolysis.

24.2.5 Sample dilution

As explained above, the high viscosity of oily samples produces difficulties in transport of the sample to the atomizer in atomic spectrometers, and hinders nebulization in FAAS and ICP-OES spectrometers. These difficulties can sometimes be circumvented by dilution in an appropriate organic solvent. This approach has the advantage of simplicity. It may solve, at least partially, the issue of sample transport, and may be used when the detection technique is not hindered by the presence of the organic matrix (both the sample itself and the solvent), as in FAAS and ICP-OES. However, it negatively affects both detectability and sensitivity, and in order to address the solubility issues caused by the solvent on the standards, calibrations should be made with organic standards, which are unfortunately not as easily available as inorganic ones. Thus organic standards sold for the analysis of lubricating oils and petroleum derivatives are often used. Dilution in organic solvent has also been used for sample introduction in the graphite furnace for ETAAS. In this case matrix elimination is carried out as part of the analytical cycle in the furnace. The issue of sensitivity and detectability loss can at least partially be remediated by augmenting the volume of sample introduced to the atomizer or by resorting to multiple injections. However, organic standards are still necessary.

Chaves *et al*. [66] used 1-propanol as solvent for the determination of Ca, Cu, Fe, K, Mg, Na, P, S and Zn by ICP-OES in vegetable oil samples. Karadjova *et al*. [67], Martin-Polvillo *et al*. [68], Farhan [69] and Nash *et al*. [70] applied this strategy of sample dilution for the determination of trace metals by ETAAS. The first group used 1,4-dioxane as solvent for the determination of Al, Cd, Cr, Cu, Fe, Mn, Ni and Pb in olive oil, while the second group used 4-methyl-2-pentanone (methyl isobutyl ketone, MIBK) for the determination of Al, Cr, Cu, Fe, Ni and Pb in sunflower and olive oils. Egg lecithin was added as matrix modifier for the determination of Pb. The same solvent was used by Farhan for the determination of Cu, Fe, Cr, Ni, Pb, Zn, Mg and Ca in edible oils by FAAS, while in an older work Nash *et al*. [70] reported diluting cooking oils, margarines and solid shortenings with it for the determination of Ni, Cr, Cu and Fe. In an earlier publication, Dijkstra and Meert [71] diluted samples with MIBK for introduction to a direct-current plasma for determination of P and other elements.

Table 24.1 summarizes the different techniques used for sample preparation.

24.3 Analytical techniques

From an analytical point of view, classical techniques such as volumetry or gravimetry do not provide appropriate detectability for the low concentration levels of most minerals present in edible oils and fats, and thus are virtually useless in this context. Molecular absorption spectrometry via chemical reactions with formation of metal complexes absorbing in the UV/visible region could conceivably be used for certain elements and has found some use in the past; however more complex sampling preparation involving mineralization is usually needed in order to present the elements in a form suitable for the reaction as well as to eliminate interferences originating in the matrix.

The atomic spectrometric techniques (including atomic mass spectrometry) are by far the most useful for the determination of minerals in foods, including oils and fats [72]. One of their main strengths is the high selectivity they exhibit; however, they do not provide speciation information unless special provisions are taken, such as coupling them to separative techniques.

Table 24.1 Sample preparation techniques reported in the literature.

Procedure	Comments	Analytes	Sample	Reference
Dry ashing		Na, K, Ca, Mg, Cu, Zn, Fe	Rapeseed, seeds, sunflower, corn, soya and olive oils	[49]
		Fe, Cu	Palm oil	[50]
	Oxygen plasma ashing	Fe, Cu	Salad oil	[51]
Wet ashing in hot plate	$HNO_3 + H_2O_2$	Cd, Cr, Zn, Cu, Fe, K, Mg, Ca	Argan oil, roasted and unroasted	[52]
	$HNO_3 + V_2O_5$	Ca, Mg , Mn, Zn, Se, Cr	Olive oil	[54]
	HNO_3, $HNO_3 + HClO_4$	Hg, Zn, Se, As, Cd, Pb, Cu	Herring oil	[80]
Wet ashing, microwave digestion	$HNO_3 + H_2O_2$	Al, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Ti, V, Zn	Pumpkin seeds and pumpkin seed oil	[53]
Microwave digestion	$HNO_3 + H_2O_2$, pre-reacted with H_2SO_4	Cu, Pb, Ni	Corn soybean and canola oil	[62]
	HNO_3	Be, Mg, Ca, Sc, Cr, Mn, Fe, Co, Ni, As, Se, Sr, Y, Cd, Sb, Sm, Eu, Gd	Virgin olive oil	[63]
		As, Ca, Cu, Cr, Fe, Mg, Mn, Se, Zn	Nut oil	[64]
	$HNO_3 + H_2O_2$	Fe, Mn, Zn, Cu, Pb, Co, Cd, Na, K, Ca, Mg	Olive oil, hazelnut oil, sunflower oil, margarine, butter and corn oil	[58]
		Cd, Cr, Cu, Mn, Pb	Vegetable oils	[59]
		Ca, Fe, Mg, Na, Al, Cu, Co, Cr, K, Ni, Mn, Pb	Olive, pumpkin seed, sunflower, sesame seed, hazelnut, grape, soya, rice oil	[60]
		Ca, Fe, Mg, Na, Zn, Al, Co, Cu, K, Mn, Ni, Cr, Pb	Olive oil	[61]
Dilution in suitable solvent	1-propanol	Ca, Cu, Fe, K, Mg, Na, P, S, Zn	Biodiesel, soybean oil	[66]
	1,4-dioxane	Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb	Olive oil	[67]
	Methyl isobutyl ketone	Al, Cr, Cu, Fe, Ni, Pb	Sunflower and olive oils	[68]
		Cu, Fe, Cr, Ni, Pb, Zn, Mg, Ca	Crude soybean, alkali refining, bleaching, hydrogenated and deodorized	[69]
		Ni, Cr, Cu, Fe	Margarine, butter (solid shortening) and cooking oil	[70]
		Pb, Fe, Cu, Cd, Ni, P	Sunflower oil	[71]
Emulsions and microemulsions		Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, Mg, Mn, Ni, Pb, Tl, Zn	Olive, sunflower and corn oil	[44]
		Cu, Fe, Ni, Zn	Soybean, olive and sunflower oil	[41]
		Ag, As, Ba, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Ti, Tl, V	Rice oil, canola oil, sunflower oil, corn oil, soy oil, olive oil, light margarine, regular margarine and butter	[76]
		Al, Ba, Bi, Ca, Cu, Mg, Mn, Na, Pb, Sn	Virgin olive oil	[43]
		Na, K, Ca, Mg, Zn, Fe, Cr, Ni, As, Pb, Cd, Cu, Mn	Soybean, olive and colza	[38]
		Cd, Co, Cr, Cu, Ni, Mn	Olive and soybean oil, margarine and butter	[37]
Miscellaneous	Milling and homogenization	Se, I	Pumpkin oil	[78]
	Saponification and UV photolysis	Total Cl, P and S; and Fe, Cu, Ni, Zn, Co, Pb, Cd	Olive, peanut, soybean and sunflower oils; margarine	[51]
	No sample preparation	Cu, Fe	Olive oil	[21]
		As, Ba, Ce, Co, Cr, Cs, Eu, Fe, Hg, K, Na, Rb, Sb, Sc, Se, Sr, Zn	Almond, sunflower, peanut, sesame, linseed, soy, corn and olive oils; margarine	[77]
	Extraction and derivatization	Organotin compounds	Edible oils	[79]

Atomic absorption spectrometry employing atomization either by flame (FAAS) or by graphite furnace is widely available and used for the majority of the metallic and semi-metallic elements. Flame atomization is cheaper and easier to use, but provides limited sensitivity and higher detection and quantification limits. ETAAS, usually implemented with a graphite furnace (sometimes referred to as GF-AAS), is a microanalytical technique that allows determination of concentrations in the parts per billion (ppb) range, and often in the parts per trillion (ppt) range, for a large number of metals and semi-metals. Also some manufacturers provide instruments capable of working with solid samples. However, it is a slow technique not easily amenable to simultaneous multi element determination.

Flame AAS was used by Ieggli *et al*. [38] for the determination of Na, K, Ca, Mg, Zn and Fe in vegetable oils, margarine and butter after sample emulsification, and by Jiménez *et al*. [43] for the determination of Na, Ca and Mg in olive oils. Sample transport and introduction to the nebulizer were solved by both groups by formation of emulsions, either off-line or on-line in an FIA system.

The use of FAAS was also reported by Carbonell *et al*. [21] (reversed FIA system with standard additions), Roca *et al*. [54] and Farhan [69]. High-resolution continuum source flame atomic absorption spectrometry (HR-CS-FAAS) is a modern technique where traditional hollow-cathode lamps are replaced by a continuum spectrum xenon lamp, and the monochromator and photomultiplier are replaced by an echelle polychromator and a CCD or other bidimensional detector. This new technique is reported to allow fast sequential multi-element determinations, without the hassles of lamp changing characteristic of traditional AAS spectrometers. Nunes *et al*. [41] have applied this technique for the determination of Cu, Fe, Ni and Zn in vegetable oil samples. As reported above for this work, sample preparation was based on the formation of microemulsions.

Nevertheless, the use of FAAS is scarce, and the majority of the papers found relate to the use of ETAAS and/or ICP-OES as spectrometric techniques. A number of workers report the use of ETAAS, for instance Karadjova *et al*. [67], Martin-Polvillo *et al*. [68], Farhan [69] and Nash *et al*. [70], who report dispersing the samples in 4-methyl-2-pentanone as discussed above. Roca *et al*. [54] determined Mn, Zn, Se and Cr in olive oils by ETAAS after mineralizing the sample by wet ashing. Others such as Ieggli *et al*. [38], Mendil *et al*. [58], Juranovic Cindric *et al*. [60], Zeiner *et al*. [61] and Allen *et al*. [62] used microwave digestion prior to submitting the sample to ETAAS. A different approach was adopted by Matos Reyes and Campos [73] who employed direct-sampling ETAAS using a spectrometer fitted with an accessory designed for solid sampling. The samples (about 0.5 mg) were weighed out directly onto the sample platform prior to introduction in the furnace. The authors report using an aqueous standards calibration curve.

Although AAS is most frequently used for the determination of metals and semi-metals, it can be used for the determination of some non-metals. A specific case is the determination of phosphorus reported by Zakharov *et al*. [74]. Determination of this element by ETAAS is proposed considering the advantages relative to conventional spectrophotometric methods on the basis of higher speed and simplicity. The authors discuss the difficulties of handling oil samples directly with an autosampler, and adopt several recommendations from a previous IUPAC collaborative study [75]. However, the paper is focused on the development of the method, and they do not report results obtained from real samples but rather from spiked samples used for validation.

Atomic emission spectrometry is another useful technique implemented by means of inductively coupled plasma as atomization–excitation source (ICP-OES). It presents several advantages, such as an extended linear range and the possibility of simultaneous multi-element determination, which several equipment manufacturers include as standard in their spectrometers. However, detection and quantification limits, although acceptably low for many elements, are not appropriate for the determination of the lowest concentrations of trace elements in the ppb (μg/g) range. This technique also requires that sample be introduced as a liquid stream, and thus samples of oils and fats should be prepared accordingly, as was the case for FAAS. Nevertheless, this technique is extensively applied to the determination of trace elements in edible oils and fats [17, 37, 44, 52, 53, 60, 61, 64, 66, 71], applying different procedures for sample introduction, with or without mineralization.

Some elements such as As, Se and Sb are usually determined by atomic spectrometric techniques (AAS, ICP-OES or AFS) via the formation of volatile derivatives, hydrides being the most usually preferred. Mercury, on the other hand, is usually determined by generation of the elemental species and measurement of its atomic absorption or atomic fluorescence. In the

case of samples of edibles oils and fats, this approach has not received wide acceptance, probably as a consequence of the difficulties originating in the characteristics of the matrix. As an exception, Chang and Jiang [45] reported the determination of As, Cd and Hg in emulsified vegetable oil by ICP-MS after flow injection chemical vapour generation.

Plasma source atomic mass spectrometry (ICP-MS) has gained wide acceptance because of its much lower detection and quantification limits. Here, the ICP is used as an ion source for the mass spectrometer. Together ETAAS and ICP-MS are the most frequently used techniques for elements present at or below ppb levels. Papers reporting the use of ICP-MS for the analysis of edible oils and fats include those by Jiménez *et al*. [42] Benincasa *et al*. [63] and Llorent-Martínez *et al*. [76].

Other miscellaneous techniques (nuclear, chromatographic, electroanalytical and spectroscopic) have also been reported in the literature although are far less used than atomic spectrometric techniques, with few papers published so far. Instrumental neutron activation analysis (INAA) is a non-destructive multi-element nuclear technique capable of low detection limits even with small samples. The main disadvantage in comparison with other analytical techniques is the need for a specific infrastructure, which is both scarce and expensive. Iskander [77] reported the determination of As, Ba, Ce, Co, Cr, Cs, Eu, Fe, Hg, K, Na, Rb, Sb, Sc, Se, Sr and Zn in almond, sunflower, peanut, sesame, linseed, soy, corn and olive oils, as well as margarine samples. No sample preparation was necessary prior to analysis. A slightly different technique, radiochemical neutron activation analysis (RNAA), was used by Kreft *et al*. [78] for the determination of iodine and selenium. A minimum of sample preparation was necessary.

Forsyth *et al*. [79] reported the determination of organotin compounds in edible oils by gas chromatography (GC) coupled with atomic absorption spectrometry. Butyl-tin, cyclohexyl-tin, octyl-tin and phenyl-tin compounds were extracted with 0.05% tropolone in 0.04 mol/L HCl in methanol, methylated and separated by GC using AAS as detection technique. The authors reported the determination of monooctyl-tin and dioctyl-tin in the range 5.7–26.9 ng/g and 23.2–118.4 ng/g, respectively, in the samples analysed.

Buldini *et al*. [65] applied ion chromatography to the determination of Fe, Cu, Ni, Zn, Co, Pb and Cd, as well as total Cl, P and S, in edible vegetable oils and fats. The organic matrix was removed by saponification followed by oxidative UV photolysis as discussed above. The authors report that Cl, P and S can only be determined as total amounts, without speciation. The rest of the elements could be determined although it was not possible to determine all of them with the same set of chromatographic conditions. Results obtained for metals were within ±3% of those found using voltammetric techniques.

Electroanalytical techniques have also been used for determination of elements in edible oil samples. Elson and Ackman [80] reported the use of differential pulse anodic and cathodic stripping voltammetry for the determination of Zn and Se, respectively, in samples of herring oil at different stages of refining and partial hydrogenation. Dugo *et al*. [81] determined Cd(II), Cu(II), Pb(II) and Zn(II) in peanut, sunflower, soy, maize, rice, grape seed and hazelnut oil by derivative potentiometric stripping analysis. Sample preparation consisted of an extraction with a hot mixture of hydrochloric acid and hydrogen peroxide. Wahdat *et al*. [82] used inverse voltammetry for the determination of Pb, Cu and Cd in linseed, soybean, sunflower and olive oil. Sample preparation consisted of the addition of a 5% solution of tetramethylammonium hydroxide pentahydrate in methanol. Wong *et al*. [83] applied differential-pulse anodic-stripping voltammetry to the determination of Cu in palm oil, while Fung and Fung [84] used potentiometry with a Cu(II) ion-selective electrode for the same purpose.

The different analytical techniques used for the determination of elements in edible oils and fats are summarized in Table 24.2.

24.4 Mineral contents in oil and fats

Results reported for different elements and matrices are presented in Table 24.3. As can be seen, the analytical results presented by different authors vary widely if they are analysed on an element-by-element basis. This could be expected given the different types and origins of the samples. Also, different analytical techniques (with different detection and quantification limits) and different sample preparation methods can influence the results, introducing a significant source of analytical variability.

One obvious observation is that the few elements often reported at very high concentrations are the alkaline and alkaline earth elements. Iron is also often reported at high concentrations, although much lower

Table 24.2 Analytical techniques used for the determination of elements in edible oils and fats.

Analytical technique	Analytes	Samples	Reference
FAAS	Ca, Mg	Virgin olive, olive and olive-pomace oils	[54]
	Cu, Fe, Cr, Ni, Pb, Zn, Mg, Ca	Oils in different refining stages	[69]
	Al, Ba, Bi, Ca, Cu, Mg, Mn, Na, Pb, Sn	Virgin olive oil	[43]
	Cr, Ni, As, Pb, Cd, Cu, Mn	Soybean, olive and colza oils	[38]
	Cu, Fe	Olive oil	[21]
ETAAS	Mn, Zn, Se, Cr	Olive oil	[54]
	Ni, Cr, Cu, Fe	Margarine, butter (solid shortening) and cooking oil	[70]
	Cu, Pb, Ni	Corn, soybean and canola oils	[62]
	Al, Cu, Co, Cr, K, Ni, Mn, Pb	Olive, pumpkin seed, sunflower, sesame seed, hazelnut, grape, soya and rice oils	[60]
	Al, Co, Cu, K, Mn, Ni, Cr, Pb	Olive oil	[61]
	Pb, Co, Cd	Olive, hazelnut, sunflower and corn oils, margarine, butter	[58]
	Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb	Olive oil	[67]
	Al, Cr, Cu, Fe, Ni, Pb	Sunflower and olive oils	[68]
	Cr, Ni, As, Pb, Cd, Cu, Mn	Soybean, olive and colza oils	[38]
	P		[74]
	Cu, Ni	Vegetable oils (corn and soybean oils)	[73]
HR-CS-AAS	Cu, Fe, Ni, Zn	Soybean, olive and sunflower oils	[41]
ICP-OES	Cd, Cr, Zn, Cu, Fe, K, Mg, Ca	Argan oil, roasted and unroasted	[52]
	Al, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Ti, V and Zn	Pumpkin seeds and pumpkin seed oil	[53]
	Cu, Pb, Ni	Corn, soybean and canola oil	[62]
	Ca, Cu, Cr, Fe, Mg, Mn, Se, Zn	Nut oil	[64]
	Ca, Fe, Mg, Na	Olive, pumpkin seed, sunflower, sesame seed, hazelnut, grape, soya and rice oils	[60]
	Ca, Fe, Mg, Na, Zn	Olive oil	[61]
	Ca, Cu, Fe, K, Mg, Na, P, S, Zn	Biodiesel and soybean oil	[66]
	Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, Mg, Mn, Ni, Pb, Tl, Zn	Olive, sunflower and corn oils	[44]
	Cd, Co, Cr, Cu, Ni, Mn	Olive and soybean oil, margarine and butter	[37]
ICP-MS	Be, Mg, Ca, Sc, Cr, Mn, Fe, Co, Ni, As, Se, Sr, Y, Cd, Sb, Sm, Eu, Gd	Virgin olive oil	[63]
	Ag, As, Ba, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Ti, Tl, V	Ricel, canola, sunflower, corn, soy and olive oils, light margarine, regular margarine and butter	[76]
	Al, Ba, Bi, Ca, Cu, Mg, Mn, Na, Pb, Sn	Virgin olive oil	[43]
DCP-OES	Pb, Fe, Cu, Cd, Ni, P	Sunflower oil	[71]
GC-AAS	Organotin compounds	Edible oils	[79]
Instrumental neutron activation analysis	As, Ba, Ce, Co, Cr, Cs, Eu, Fe, Hg, K, Na, Rb, Sb, Sc, Se, Sr, Zn	Almond, sunflower, peanut, sesame, linseed, soy, corn and olive oils and margarine	[77]
Radiochemical neutron activation analysis	Se, I	Pumpkin oil	[78]
Ion chromatography, voltammetry	Total Cl, P, S, Fe, Cr, Ni, Zn, Co, Pb and Cd	Olive, peanut, soybean and sunflower oils, margarine	[65]
AAS (Cu, Cd, Pb, As) DPASV (Zn) DPCSV (Se) CVAAS (Hg)	Hg, Se, As, Zn, Cd, Pb, Cu	Herring oil at different industrial stages	[80]

Table 24.3 Maximum and minimum values reported for the elements in different types of oils and fats.

Element	Sample	Reported range (ng/g)
Al	Olive oil	1–1110
	Sunflower oil	22–100
As	Olive oil	1–93
	Sunflower oil	<110 to 164
	Soybean oil	<130*
	Corn oil	<100†
	Margarine	<1000†
	Butter	<56†
Ba	Olive oil	0.28–1800
	Sunflower oil	<1900†
	Soybean oil	<1700†
	Corn oil	<1800†
	Corn oil	<1500†
Be	Olive oil	<0.12 to 0.182
	Pomace-olive oil, sunflower oil, corn oil	<0.12 to 70†
Bi	Olive oil	0.01–1.48
Ca	Olive oil	<50 to 76,000
	Sunflower oil	400–174,200
	Soybean oil	<200 to 28,500
	Corn oil	1060–71,000
	Margarine	22,600†
	Butter	125,500†
Cd	Olive oil	<10 to 150
	Sunflower oil	1–3760
	Soybean oil	<84*
	Corn oil	<90†
	Margarine	<50*
	Butter	<83†
Ce	Olive oil	<32†
	Sunflower oil	<49†
	Soybean oil	<69†
	Corn oil	<32†
	Margarine	<56†
Cl	Olive oil	35.8†
	Sunflower oil	35†
	Margarine	795,000†
Co	Olive oil	0.056–16,000
	Sunflower oil	10–540
	Soybean oil	<50*
	Corn oil	16–500
	Margarine	9–530
Cr	Olive oil	<75,000*
	Sunflower oil	<72*
	Soybean oil	<100*
	Corn oil	<75†
	Margarine	<73†
	Butter	<14†
Cs	Olive oil	<6000†
	Sunflower oil	<8†
	Soybean oil	<7†
	Corn oil	<6†
	Margarine	<5†
Cu	Olive oil	<1 to 4510
	Sunflower oil	<1.3 to 1100
	Soybean oil	<5 to 1620
	Corn oil	ND to 70
	Margarine	48–90
	Butter	32†
Eu	Olive oil	<5000*
	Sunflower oil	<4†
	Soybean oil	<3†
	Corn oil	<5†
	Margarine	<2†
Fe	Olive oil	89.3–139,000
	Sunflower oil	55–105,300
	Soybean oil	<60 to 21,200
	Corn oil	220–52,000
	Margarine	378–10,600
	Butter	9860†
Gd	Olive oil	0.003–0.047
Hg	Olive oil	<5†
	Sunflower oil	<8†
	Soybean oil	<9000†
	Corn oil	<5†
	Margarine	<6†
K	Olive oil	<1 to 47,200
	Sunflower oil	240–5930
	Soybean oil	250–189,000
	Corn oil	60–47,400
	Margarine	50,100–1,114,000
	Butter	117,700†
Mg	Olive oil	9.5–5300
	Sunflower oil	51–7040
	Soybean oil	60–33,800
	Corn oil	90–20,800
	Margarine	18,300†
	Butter	8400†
Mn	Olive oil	<1 to 296
	Sunflower oil	<2 to 120
	Soybean oil	<7†
	Corn oil	1640†
	Margarine	120†
	Butter	20†

(Continued)

Table 24.3 (Continued)

Element	Sample	Reported range (ng/g)
Na	Olive oil	75–38,030
	Sunflower oil	<370 to 34,800
	Soybean oil	<370 to 5170
	Corn oil	820–12,900
	Margarine	13,200–9,870,000
	Butter	46,000†
Ni	Olive oil	<1 to 2260
	Sunflower oil	<5 to <1900
	Soybean oil	41–2740
	Margarine	50.5–51
	Butter	590†
P	Olive oil	<10†
	Sunflower oil	1100†
	Soybean oil	1500–530,250
	Margarine	12,938†
Pb	Olive oil	<0.02 to 50
	Sunflower oil	<1 to <49
	Soybean oil	<16 to 1500
	Corn oil	ND†
	Margarine	10 to <50
	Butter	80†
Rb	Olive oil	<200†
	Sunflower oil	<250†
	Soybean oil	<440†
	Corn oil	<200†
	Margarine	<310†
S	Olive oil	<10†
	Soybean oil	92–817,000
	Margarine	250†
Sb	Olive oil	<14†
	Sunflower oil	<1500†
	Soybean oil	<0.12†
	Corn oil	<14,000†
	Margarine	<9000 to 15,000
Sc	Olive oil	<0.5 to 748
	Sunfower oil	<500†
	Soybean oil	<9.5†
	Corn oil	<500†
	Margarine	<9000 to 15,000
Se	Olive oil	<10.2 to 179
	Sunflower oil	<66,000†
	Soybean oil	<54†
	Corn oil	<57,000†
	Margarine	<41,000†
Sm	Olive oil	0.019–0.226
Sn	Olive oil	0.22–4.6
Sr	Olive oil	<4100
	Sunflower oil	<1800†
	Soybean oil	<5600†
	Corn oil	<4100†
	Margarine	<3900†
Y	Olive oil	<0.12 to 0.331
Zn	Olive oil	10–4300
	Sunflower oil	400–3400
	Soybean oil	25–9100
	Corn oil	50–3080
	Margarine	64–2710
	Butter	1360†

In order to facilitate comparison, all values are given in ng/g. All values presented as '<' were reported by the authors as either 'ND' (not detected) or as lower than the quantification limit. In those cases where the value of the detection limit was not given, 'ND' is presented.

*Either the maximum value or both maximum and minimum values were reported as '<', so only the higher limit is reported here.

†Only one result reported.

than, for instance, sodium or potassium. However, for all of them the results reported cover very wide ranges, thus making difficult to draw general conclusions.

Other observation is that, as expected, edible oils of vegetable origin are the most frequently analysed matrix in the category, while margarines and butter are less frequently reported. Furthermore, excluding butter, results obtained from fats of animal origin are virtually non-existent. No publications could be found concerning the determination of elements in edible animal fats.

24.5 Some conclusions and perspectives

Based on the literature reviewed, it can be concluded that the determination of trace elements in edible oils and fats is a subject which focuses interest from the technological and toxicological viewpoints, although based on the number of publications found this interest is clearly minor compared with other foods. Considering

the context of the papers reviewed, and contrary to expectations, the determination of elements in these matrices does not seem to be of interest from the nutritional point of view, something that can be explained on the basis of the low levels found for most elements.

With the exception of just one paper [79], all publications deal with results for total element contents, with no apparent interest in speciation analysis. It seems reasonable to think that this situation may change in the future, as has happened with other foods where speciation analysis is often carried out.

Abbreviations

AAS: Atomic absorption spectrometry
AFS: Atomic fluorescence spectrometry
ETAAS: Electrothermal atomic absorption spectrometry
FAAS: Flame atomic absorption spectrometry
FIA: Flow injection analysis
GC: Gas chromatography
HR-CS-FAAS: High-resolution continuum source flame atomic absorption spectrometry
ICP-MS: Inductively coupled plasma mass spectrometry
ICP-OES: Inductively coupled plasma optical emission spectrometry
INAA: Instrumental neutron activation analysis
LOV: Lab-on-valve
MCFA: Multicommutated flow analysis
MIBK: Methyl isobutyl ketone
MPFA: Multipumping flow analysis
MSFA: Multisyringe flow analysis
RNAA: Radiochemical neutron activation analysis
SIA: Sequential injection analysis
UV: Ultraviolet

References

1 Belitz HD, Grosch W, Schieberle P. *Food Chemistry*, 4th edn. Springer-Verlag, Heidelberg, 2009, chapter 14.
2 Coultate TP. *Food. The Chemistry of Its Components*, 4th edn. Royal Society of Chemistry, Cambridge, 2002, chapter 4.
3 Fennema OR. *Food Chemistry*, 3rd edn. Marcel Dekker, New York, 1996, chapter 5.
4 Akoh CS, Min DB (eds) *Food Lipids. Chemistry, Nutrition, and Biotechnology*, 2nd edn. Marcel Dekker, New York, 2002.
5 Lepri FG, Chaves ES, Vieira MA *et al*. Determination of trace elements in vegetable oils and biodiesel by atomic spectrometric techniques: a review. *Appl Spectrosc Rev* 2011;**46**:175–206.
6 Ihnat M. Sample preparation for food analysis. In: Mester Z, Sturgeon R (eds) *Sample Preparation for Trace Element Analysis*. Elsevier, Amsterdam, 2003, pp. 765–855.
7 Kebbekus BB. Preparation of samples for metals analysis. In: Mitra S (ed.) *Sample Preparation Techniques in Analytical Chemistry*. John Wiley & Sons, Inc., Hoboken, NJ, 2003, pp. 227–267.
8 Ruzicka J, Hansen EH. Flow injection analyses. Part I. A new concept of fast continuous flow analysis. *Anal Chim Acta* 1975;**78**:145–157.
9 Stewart KK, Beecher GR, Hare PE. Rapid analysis of discrete samples: the use of nonsegmented, continuous flow. *Anal Biochem* 1976;**70**:167–173.
10 Thomaidis NS, Georgiou CA. Edible oil analysis by flow injection. *Lab Autom Inf Manage* 1999;**34**:101–114.
11 Nouros PG, Georgiou CA, Polissiou MG. Automated flow injection spectrophotometric non-aqueous titrimetric determination of the free fatty acid content of olive oil. *Anal Chim Acta* 1997;**351**:291–297.
12 Nouros PG, Georgiou CA, Polissiou MG. Direct parallel flow injection multichannel spectrophotometric determination of olive oil peroxide value. *Anal Chim Acta* 1999;**389**:239–245.
13 Nouros PG, Georgiou CA, Polissiou MG. Determination of olive oil 2-thiobarbituric acid reactive substances by parallel flow injection. *Anal Chim Acta* 2000;**417**:119–124.
14 Thomaidis NS, Georgiou CA. Direct parallel flow injection multichannel spectrophotometric determination of olive oil iodine value. *Anal Chim Acta* 2000;**405**:239–245.
15 Ruíz A, Ayora Cañada MJ, Lendl B. A rapid method for peroxide value determination in edible oils based on flow analysis with Fourier transform infrared spectroscopic detection. *Analyst* 2001;**126**:242–246.
16 Cañada MJA, Medina AR, Lendl B. Determination of free fatty acids in edible oils by continuous-flow analysis with FT-IR spectroscopic detection. *Appl Spectrosc* 2001;**55**:356–360.
17 Labrinea EP, Thomaidis NS, Georgiou CA. Direct olive oil anisidine value determination by flow injection. *Anal Chim Acta* 2001;**448**:201–206.
18 Minioti KS, Georgiou CA. High throughput flow injection bioluminometric method for olive oil antioxidant capacity. *Food Chem* 2008;**109**:455–461.
19 Filiz Ayyildiz H, Kara H, Sherazi STH. A novel approach for determination of free fatty acids in vegetable oils by a flow injection system with manual injection. *Lipids* 2011;**46**:1181–1190.
20 Gerasimenko EO, Tur'yan YaI. Automated flow pH-method for the determination of total free fatty acids content in edible oils. *Food Chem* 2012;**132**:1562–1565.
21 Carbonell V, Mauri AR, Salvador A, de la Guardia M. Direct determination of copper and iron in edible oils using flame atomic absorption spectrometry. *J Anal Atom Spectrom* 1991;**6**:581–584.

22 Ruzicka J, Marshall GD. Sequential injection: a new concept for chemical sensors, process analysis and laboratory assays. *Anal Chim Acta* 1990;**237**:329–343.
23 Lenehan CE, Barnett NW, Lewis SW. Sequential injection analysis. *Analyst* 2002;**127**:997–1020.
24 Reis BF, Giné MF, Zagatto EAG, Lima JLFC, Lapa RA. Multicommutation in flow analysis. Part 1. Binary sampling: concepts, instrumentation and spectrophotometric determination of iron in plant digests. *Anal Chim Acta* 1994;**293**:129–138.
25 Rocha FRP, Reis BF, Zagatto EAG, Lima JLFC, Lapa RAS, Santos JLM. Multicommutation in flow analysis: concepts, applications and trends. *Anal Chim Acta* 2002;**468**:119–131.
26 Ruzicka J. Lab-on-valve: universal microflow analyzer based on sequential and bead injection. *Analyst* 2000;**125**: 1053–1060.
27 Wang J, Hansen EH. Sequential injection lab-on-valve: the third generation of flow injection analysis. *Trends Anal Chem* 2003;**22**:225–231.
28 Miró M, Cerdà V, Estela JM. Multisyringe flow injection analysis: characterization and applications. *Trends Anal Chem* 2002;**21**:199–210.
29 Segundo MA, Magalhaes LM. Multisyringe flow injection analysis: state-of-the art and perspectives. *Anal Sci* 2006;**22**:3–8.
30 Lapa RAS, Lima JLFC, Reis BF, Santos JLM, Zagatto EAG. Multi-pumping in flow analysis: concepts, instrumentation, potentialities. *Anal Chim Acta* 2002;**466**:125–132.
31 Lima JLFC, Santos JLM, Dias ACB, Ribeiro MFT, Zagatto EAG. Multi-pumping flow systems: an automation tool. *Talanta* 2004;**64**:1091–1098.
32 Zagatto EAG, Dias EAB. In-line sample preparation in flow analysis. In: Arruda MAZ (ed.) *Trends in Sample Preparation*. Nova Science Publishers, New York, 2007, pp. 197–232.
33 Burguera JL, Burguera M. Pretreatment of oily samples for analysis by flow injection-spectrometric methods. *Talanta* 2011;**83**:691–699.
34 Sanz-Medel A. *Flow Analysis with Atomic Spectrometric Detectors*. Elsevier, Amsterdam, 1999.
35 Pinto PCAG, Saraiva MLMFS, Lima JLFC. A flow sampling strategy for the analysis of oil samples without pretreatment in a sequential injection analysis system. *Anal Chim Acta* 2006;**555**:377–383.
36 Burguera JL, Burguera M. Analytical applications of emulsions and microemulsions. *Talanta* 2012;**96**:11–20.
37 De Souza RM, Mathias BM, Da Silveira CLP, Aucélio RQ. Inductively coupled plasma optical emission spectrometry for trace multi-element determination in vegetable oils, margarine and butter after stabilization with propan-1-ol and water. *Spectrochim Acta B* 2005;**60**:711–715.
38 Ieggli CVS, Bohrer D, Do Nascimento PC, de Carvalho LM. Flame and graphite furnace atomic absorption spectrometry for trace element determination in vegetable oils, margarine and butter after sample emulsification. *Food Addit Contam* 2011;**28**:640–648.
39 Robaina NF, Brum DM, Cassella RJ. Application of the extraction induced by emulsion breaking for the determination of chromium and manganese in edible oils by electrothermal atomic absorption spectrometry. *Talanta* 2012;**99**: 104–112.
40 Bakircioglu D, Kurtulus YB, Yurtsever S. Comparison of extraction induced by emulsion breaking, ultrasonic extraction and wet digestion procedures for determination of metals in edible oil samples in Turkey using ICP-OES. *Food Chem* 2013;**138**:770–775.
41 Nunes LS, Barbosa JTP, Fernandes AP *et al*. Multi-element determination of Cu, Fe, Ni and Zn content in vegetable oils samples by high-resolution continuum source atomic absorption spectrometry and microemulsion sample preparation. *Food Chem* 2011;**127**:780–783.
42 Jiménez MS, Velarte R, Castillo JR. On-line emulsions of olive oil samples and ICP-MS multi-elemental determination. *J Anal Atom Spectrom* 2003;**18**:1154–1162.
43 Jiménez MS, Velarte R, Gómez MT, Castillo JR. Multielement determination using on-line emulsion formation and ICP-MS/FAAS for the characterization of virgin olive oils by principal component analysis. *Atom Spectrosc* 2004;**25**:1–12.
44 Anthemidis AN, Arvanitidis V, Stratis JA. On-line emulsion formation and multi-element analysis of edible oils by inductively coupled plasma atomic emission spectrometry. *Anal Chim Acta* 2005;**537**:271–278.
45 Chang Y-T, Jiang S-J. Determination of As, Cd and Hg in emulsified vegetable oil by flow injection chemical vapour generation inductively coupled plasma mass spectrometry. *J Anal Atom Spectrom* 2008;**23**:140–144.
46 Hoenig M. Dry ashing. In: Mester Z, Sturgeon R (eds) *Sample Preparation For Trace Element Analysis*. Elsevier, Amsterdam, 2003, pp. 235–255.
47 Matusiewicz H. Wet digestion methods. In: Mester Z, Sturgeon R (eds) *Sample Preparation For Trace Element Analysis*. Elsevier, Amsterdam, 2003, pp. 193–223.
48 Knapp G, Schramel P. Sources of analyte contamination and loss during the analytical process. In: Mester Z, Sturgeon R (eds) *Sample Preparation For Trace Element Analysis*. Elsevier, Amsterdam, 2003, pp. 23–45.
49 Garrido MD, Frias I, Diaz C, Hardisson A. Concentrations of metals in vegetable edible oils. *Food Chem* 1994;**511**: 237–243.
50 Saleh MI, Murray RS, Chin CN. Ashing techniques in the determination of iron and copper in palm oil. *J Am Oil Chem Soc* 1988;**65**:1767–1770.
51 Sun H-J. A rapid method for the determination of trace Cu and Fe in edible salad oil by graphite furnace atomic absorption spectroscopy. *J Am Oil Chem Soc* 1989;**66**:549-552.
52 Mohammed FAE, Bchitou R, Bouhaouss A *et al*. Can the dietary element content of virgin argan oils really be used for adulteration detection? *Food Chem* 2013;**136**:105–108.
53 Juranovic I, Breinhoelder P, Steffan I. Determination of trace elements in pumpkin seed oils and pumpkin seeds by ICP-AES. *J Anal Atom Spectrom* 2003;**18**:54–58.

54 Roca A, Cabrera C, Lorenzo ML, López MC. Niveles de calcio, magnesio, manganeso, cinc, selenio y cromo en aceites de oliva comercializados en Andalucía. *Grasas y Aceites* 2000;**51**: 393–399.

55 Kingston HM, Haswell SJ (eds) *Microwave-Enhanced Chemistry: Fundamentals, Sample Preparation, and Applications*. American Chemical Society, Washington, DC, 1997.

56 Burguera M, Burguera JL. Microwave-assisted sample decomposition in flow analysis. *Anal Chim Acta* 1998;**366**:63–80.

57 Chen L, Song D, Tian Y, Ding L, Yu A, Zhang H. Application of on-line microwave sample-preparation techniques. *Trends Anal Chem* 2008;**27**:151–159.

58 Mendil D, Uluözlüa ÖD, Tüzen M, Soylak M. Investigation of the levels of some element in edible oil samples produced in Turkey by atomic absorption spectrometry. *J Hazard Mater* 2009;**165**:724–728.

59 Bakkali K, Ballesteros E, Souhail B, Ramos Martos N. Determinación de trazas metálicas en aceites vegetales de España y Marruecos mediante espectroscopía de absorción con cámara de grafito después de la digestión en horno de microondas. *Grasas y Aceites* 2009;**60**:490–497.

60 Juranovic Cindric I, Zeiner M, Steffan I. Trace elemental characterization of edible oils by ICP–AES and GFAAS. *Microchem J* 2007;**85**:136–139.

61 Zeiner M, Steffan I, Juranovic Cindric I. Determination of trace elements in olive oil by ICP-AES and ETA-AAS: a pilot study on the geographical characterization. *Microchem J* 2005;**81**:171– 176.

62 Allen LB, Siitonen PH, Thompson HC Jr. Determination of copper, lead, and nickel in edible oils by plasma and furnace atomic spectroscopies. *J Am Oil Chem Soc* 1998;**75**:477–481.

63 Benincasa C, Lewis J, Perri E, Sindon G, Tagarelli A. Determination of trace element in Italian virgin olive oils and their characterization according to geographical origin by statistical analysis. *Anal Chim Acta* 2007;**585**:366–370.

64 Moodley R, Kindness A, Jonnalagadda SB. Elemental composition and chemical characteristics of five edible nuts (almond, Brazil, pecan, macadamia and walnut) consumed in Southern Africa. *J Environ Sci Health B* 2007;**42**:585–591.

65 Buldini PL, Ferri D, Sharma JL. Determination of some inorganic species in edible vegetable oils and fats by ion chromatography. *J Chromatogr A* 1997;**789**:549–555.

66 Chaves ES, de Loos-Vollebregt MTC, Curtius AJ, Vanhaecke F. Determination of trace elements in biodiesel and vegetable oil by inductively coupled plasma optical emission spectrometry following alcohol dilution. *Spectrochim Acta B* 2011;**66**:733–739.

67 Karadjova I, Zachariadis G, Boskou G, Stratis J. Electrothermal atomic absorption spectrometric determination of aluminium, cadmium, chromium, copper, iron, manganese, nickel and lead in olive oil. *J Anal Atom Spectrom* 1998;**13**:201–204.

68 Martin-Polvillo M, Albi T, Guinda A. Determination of trace elements in edible vegetable oils by atomic absorption spectrophotometry. *J Am Oil Chem Soc* 1994;**71**:347–353.

69 Farhan FM. Variations of trace metal content of edible oils and fats during refining processes. *J Am Oil Chem Soc* 1988;**65**:1961–1962.

70 Nash AM, Mounts TL, Kwolek WF. Determination of ultra-trace metals in hydrogenated vegetable oils and fats. *J Am Oil Chem Soc* 1983;**60**:811–814.

71 Dijkstra AJ, Meert D. Determination of trace elements in oils by plasma emission spectroscopy. *J Am Oil Chem Soc* 1982;**59**:199–204.

72 Caroli S (ed.) *The Determination of Chemical Elements in Food: Applications for Atomic and Mass Spectrometry*. John Wiley & Sons, Inc., Hoboken, NJ, 2007.

73 Matos Reyes MN, Campos RC. Determination of copper and nickel in vegetable oils by direct sampling graphite furnace atomic absorption spectrometry. *Talanta* 2006;**70**: 929–932.

74 Zakharov YuA, Motygullin EK, Gil'mutdinov AKh. Direct determination of phosphorus in vegetable oils by electrothermal atomic absorption spectrometry. *J Anal Chem* 2000;**55**:649–652.

75 Hendrikse PW, Dieffenbacher A. Determination of phosphorus in oils and fats by direct graphite furnace atomic absorption spectrometry. *Pure Appl Chem* 1991;**63**: 1191–1198.

76 Llorent-Martínez EJ, Ortega-Barrales P, Fernández-de Córdova ML, Domínguez-Vidal ML, Ruiz-Medina A. Investigation by ICP-MS of trace element levels in vegetable edible oils produced in Spain. *Food Chem* 2011;**127**: 1257–1262.

77 Iskander FY. Determination of seventeen elements in edible oils and margarine by instrumental neutron activation analysis. *J Am Oil Chem Soc* 1993;**70**:803–805.

78 Kreft I, Stibilj V, Trkov Z. Iodine and selenium contents in pumpkin (Cucurbita pepo L.) oil and oil-cake. *Eur Food Res Technol* 2002;**215**:279–281.

79 Forsyth DS, Weber D, Dalglish K. The determination of organotin compounds in edible oils by gas chromatography-atomic absorption spectrometry. *Talanta* 1993;**40**:299–305.

80 Elson CM, Ackman RG. Trace metal content of a herring oil at various stages of pilot-plant refining and partial hydrogenation. *J Am Oil Chem Soc* 1978;**55**:616–618.

81 Dugo G, La Pera L, La Torre GL, Giuffrida D. Determination of Cd(II), Cu(II), Pb(II), and Zn(II) content in commercial vegetable oils using derivative potentiometric stripping analysis. *Food Chem* 2004;**87**:639–645.

82 Wahdat F, Hinkel S, Neeb R. Direct inverse voltammetric determination of Pb, Cu and Cd in some edible oils after solubilisation. *Fresenius J Anal Chem* 1995;**352**:393–394.

83 Wong KH, Fung YS, Fung KW. Determination of trace amounts of copper in palm oil by differential-pulse anodic-stripping voltammetry. *Analyst* 1980;**105**:30–36.

84 Fung YS, Fung KW. Potentiometric determination of copper in palm oil with a copper(II) ion-selective electrode. *Analyst* 1978;**103**:149–155.

CHAPTER 25
Elemental composition of sugar and honey

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Abstract: Sugar and honey contain various elements, mostly metals, at different concentrations. Major elements present in both products (i.e. Ca, K, Mg and Na) are of endogenous origin and can indirectly be derived from the soil and sugar-rich and melliferous plants. However, exogenous sources of elements in sugar and honey also have to be considered, i.e. environmental pollution as well as the technology and the processing used for retrieval of both products. These exogenous sources of elements may contribute to increases in the concentration of some minor elements (e.g. Al, Cu, Fe, Mn, Zn) and trace elements (i.e. As, Cd, Co, Cr, Hg, Ni, Pb, Sn). With reference to the element composition of sugar and honey, quality control and quality assurance of both products is of particular interest with regard to their ubiquity in cooking and the production of other beverages and food products. This chapter addresses both sources of elements in sugar and honey and the necessity of elemental analysis of both products. The presence of certain groups of elements in sugar and honey has been surveyed and their role and implications are discussed. In addition, the methodology based on spectrochemical methods and used for elemental analysis of sugar and honey is highlighted, including sample treatments and analytical techniques applied for measurement.

Keywords: sugar, honey, elements, sample treatment, sample preparation, atomic absorption spectrometry, optical emission spectrometry

25.1 Source of elements in sugar and honey

In the case of both white sugar and honey, two main sources of elements in these ubiquitous sweeteners can be distinguished, endogenous and exogenous. Since white sugar is a product of plant origin, processed from sugar beet or sugar cane, the chemical composition of the plant substrates from which it is produced has a great impact on the content of elements in by-products and final sugar products [1–8]. Indirectly, this endogenous source of elements is related to the types of soil on which both sugar-rich plants grow and their mineralization, the types of fertilizer applied, and the means of irrigation used during the vegetative stage of both plants [1–8]. In the case of honey, classified as a natural product of animal origin, the main endogenous source of elements are melliferous plants and the chemical composition of their produced resources, i.e. nectar, pollen and honeydew. These resources are harvested and pretreated by bees in beehives. In this way, as in the case of white sugar, elements in honey are indirectly derived from the soil where honey-producing plants grow [9–11]. During the vegetative stage of these honey-producing plants, species of elements are taken up by the root systems, pass to the nectar or other resources, and are finally transported to honey with the aid of bees [12]. In particular for honey, which is much better researched than sugar, there is much evidence that the content of major and minor elements is strongly affected by soil composition and is related to the geological and geochemical properties of the bees' forage area [13–24]. The botanical origin of honey-producing plants, the flower density, and the quantity and quality of resources produced by melliferous plants, especially of the flower nectar, also greatly contribute to the variation in concentrations of elements found in various types of honey [13, 21–31].

The large-scale industrial processing of sugar beet and sugar cane includes different operations and practices.

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All production stages involved in this processing can be regarded as the exogenous source of elements in sugar, and this has a considerable effect on quality [2–4, 7, 8]. Sugar juices remain in contact with mill machinery (e.g. boilers, containers, pipelines) for a long time. Changes in the pH of processing media, the passage of CO_2 and SO_2 through them and their evaporation are responsible for the corrosion of materials from which these components are made [5, 7]. In addition, various chemicals such as $Ca(OH)_2$, CaO, H_2SO_4, H_3PO_4, as well as bleaching and floating reagents are used in sugar production processes. They can also be regarded as another exogenous source of the contamination of sugar by-products and end products with elements [2, 5, 32–36]. As a result, different soluble and insoluble organic and organic pollutants are present in sugar. Roughly extracted juices of sugar beet and sugar cane commonly contain higher amounts of various endogenous elements, including Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Sr and Zn [5]. However, during subsequent production stages the content of these elements in extracted sugar juices (Table 25.1) tends to decrease and therefore the final products contain rather minimal concentrations of various elements [5, 6].

The concentrations of exogenous elements in sugar by-products arising at different stages of the sugar production process and in end products are higher compared with those in sugar juices [2, 5]. The content of this group of elements usually increases rapidly during the initial stages of processing and then remains virtually the same or increases during further sugar processing [2, 5]. It should also be remembered that the carbonation of sugar juices, their further purification and bleaching commonly lead to a large decrease in the concentration of Ca, Mg and other minor and trace elements [5, 7]. Interestingly, relatively low concentrations of Ca and Mg and other elements in white sugar are evidence of the exceptional effectiveness of the production stages involved [7].

Table 25.1 Stages of sugar production.

Sugar production stage	Description
Delivery, storage and pretreatment of beets or canes	Washing, slicing and shredding followed by crushing and pressing to obtain raw sugar juices
Purification of sugar juices	Liming, carbonation with CO_2 and sulfurization with SO_2 to remove insoluble solids containing undesirable organic and inorganic sugar constituents, polysaccharides and colorants as well as to destroy invert sugars, regulate pH and bleach sugar juices
Filtration and thickening of juices	Boiling and evaporation of water to concentrate juices and develop sugar crystals
Centrifugation of syrup juices	Separation of sugar crystals, granulation and packing

Source: based on Terrab *et al*. [25].

In the case of honey, different anthropogenic factors can be regarded as the exogenous source of elements (Figure 25.1). When the forage area of bees is polluted, pollutants can enter honey via melliferous plants that grow on contaminated soils and absorb contaminated water. Bees themselves, searching for resources in the polluted forage area, can carry pollutants from the surrounding environment into hives and change the quality of honey because they come into the contact with the air, plants, water and soil [14, 16, 17, 37–40]. When the forage area of bees covers industrial or urban areas with roads and highways, mines, steelworks or other kinds of mills located therein, the concentrations of certain elements (i.e. Al, Cd, Cu, Mn, Ni, Pb, Pd, Zn) in plant resources and honey from such apiculture areas can be higher compared with honeys from other areas due to the pollution by wastes, fumes and other exhausts [12, 16, 22, 39, 41, 42]. However, the degree of contamination of honey is recognized to be plant-dependent because various species of honey-producing plants have different abilities to take up and accumulate elements from the environment [28].

Finally, honey can be contaminated during its manufacture: collection, processing including extraction and centrifugation, followed by ripening, confection and storage. Here, the natural acidity of honey (pH 3–5) may result in the corrosion of beekeeping tools, mostly made of galvanized steel, aluminium and brass, as well as the containers used for the settling of honey, its storage and shipment [12, 14, 16, 22, 24, 29, 31, 39, 43, 44]. Materials used for the construction and finishing of beehives are also of importance and can be an additional source of contamination with trace elements and other contaminants [9–11]. Finally, improper beekeeping practices and tools can be responsible for the contamination of honey and elevated concentrations of several

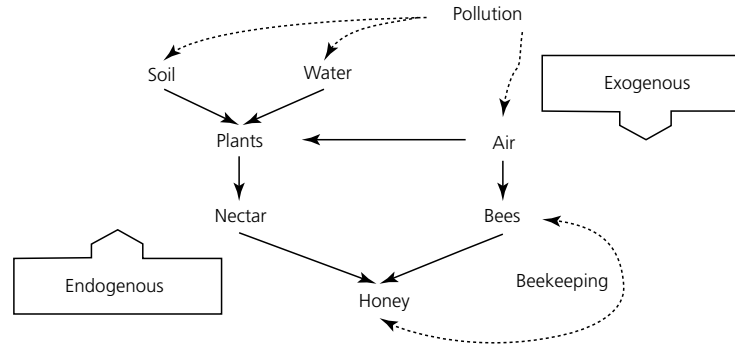


Figure 25.1 Endogenous and exogenous sources of elements in honey.

exogenous minor and trace elements (i.e. Al, Cd, Co, Cr, Cu Fe, Pb, Ni and Zn). In the same way, substances used for healing and feeding of the bee colony can contribute to an increase in the concentration of some major, minor and trace elements (Cd, Co, Fe, K, Mg, Mn, Na and Pb) [18].

25.2 Necessity for elemental analysis of sugar and honey

Since both sweeteners are typically used in baking and cooking or the sweetening of different food products and beverages, their quality in reference to chemical composition should be verified and assured. Bearing this in mind, examination for the presence of forbidden substances and compounds in sugar and honey appears to be obvious [1, 7, 9, 10, 16, 35, 39, 45–47]. When considering trace elements, the greatest awareness should be given to those elements that are toxic and harmful to human health, namely As, Cd, Hg and Pb. Although the legislation of the European Union regarding permissible levels of As, Cd, Hg and Pb does not include sugar and honey, national regulations are commonly considered in this matter and therefore acceptable limits for these elements in white sugar and honey are set to be within the range 0.1–1 μg/g [9–11, 33, 34, 48–50]. In general, the concentrations of As, Cd, Hg and Pb in sugar are quite low, and hence they pose no hazard to human health [2, 8, 32, 34, 49]. When considering that the total concentration of these elements is close to or lower than 5 μg/g and assuming their 100% intake by the human body, it appears that consumption of 100 g of sugar per week may contribute to less than a few percent or even less of the recommended daily allowance (RDA) and adequate intake (AI) [36].

In contrast, information on the concentrations of Ca, Cu, Fe, K, Mg, Na and Zn in sugar is quite important in view of the technological processes involved in its production [2, 8, 32–36]. Information about their content is helpful in evaluating the quality of sugar by-products derived from different stages of the sugar production process and the efficiency of the purification and filtration processes employed during juice extraction or the crystallization of sugar from molasses. In this way, elemental analysis is a critical part of quality control (QC) and quality assurance (QA), ensuring an end product of the highest safety and quality [34, 48]. Hence, it is of particular interest and significance for the whole sugar industry. In addition, the type and content of elements present in sugar is taken into account in the event of its further application in the manufacturing and processing of other food products and beverages [34, 35, 51].

In the case of honey, essential elements (Ca, Cu, Fe, K, Mg, Mn and Na) are commonly present at much higher concentrations than in sugar and hence may have a certain dietary value [9, 10, 12, 47, 52]. Therefore, information on the content of these elements seems to be important for consumers in view of the potential effects of honey on their health. For individuals who consume higher quantities of honey, this could be especially significant when they treat this product as a valuable supplementary source of major and minor elements [9, 10, 18]. In other cases, typical consumption of honey in the population is rather low [18, 53, 54]. As a result, the RDA of essential elements associated with a moderate intake of honey does not exceed a few percent and usually is even less [53]. Nevertheless, the consumption of honey should not raise any concern about its wholesomeness, safety and quality and therefore elemental analysis can guarantee the safety of this

food product in reference to elements that are regarded as toxic when present in elevated amounts. Thus, when trace elements such Cd, Co, Hg, Ni and Pb are not detected in honey, it can indicate that no contamination of honey occurred during its processing and storage [10]. This can also be instructive feedback to beekeepers, who may improve their means of obtaining and processing of honey. In view of this, the elemental analysis of honey is also important for its QC and QA according to different food safety regulations.

However, the importance of elemental analysis of honey is reported to be more wide-ranging. The elements present in honey are recognized to be very good descriptors of their provenance because their concentrations are stable for a long period and highly distinctive for a given bee-foraging area, its geochemical and agrochemical conditions, floral characteristics and density, and climate [15, 20, 24, 43]. These factors are profoundly responsible for the great variance in concentrations of elements in honey. Since the content of elements is plant-dependent and mainly derived from the soil, the assessment of concentrations of different elements in honey can be used for classification with regard to botanical and geographical origins [13, 14, 16, 20, 21]. Such analyses of the total content of major, minor and trace elements are commonly combined with chemometric data-reduction and visualization methods, namely cluster analysis (CA), principal component analysis (PCA) and/or pattern recognition and classification methods such as linear discriminant analysis (LDA). This analytical tool is indeed a very good way to distinguish honey according to its botanical and geographical origins and for detecting potential fraud, i.e. falsely declared authenticity, adulteration through dilution, or the addition of syrups or the blending of different samples [15, 18–21, 23–25, 28, 29, 37, 43, 52, 55–58]. In the latter case, identification of the authenticity of honey via the distinctive and unique pattern of elements can be an important part of QC and QA, promoting natural and original products manufactured in a given region according to traditional procedures.

Honey is also considered a biomarker of environmental pollution, indicating the level of contamination of the air, water and soil in a relatively small territory [12, 16–18, 23, 24, 37, 38, 40–42, 54, 59, 60]. However, some authors question such a role for honey [39, 55]. Since honey contains relatively low concentrations of elements, and these elements vary greatly in their concentrations according to the origin of melliferous plants and with season, its usefulness as a bio-indicator can only be limited to cases of very heavy environmental pollution [60].

25.3 Presence of certain groups of elements in sugar and honey: their role and implications

Except for Ca, K, Mg and Na, it seems that white sugar contains rather low concentrations of other elements. Possible concentration ranges of different elements reported in the literature for sugars originating from different countries are given in Table 25.2.

Normally, the surplus of Ca ions, introduced during the purification of sugar juices with lime milk, and Mg ions is reduced by precipitation of these elements in the form of their carbonates at the carbonization stage [8]. Relatively high concentrations of Ca and Mg that remain may indicate that the purification process was incomplete, likely due to improper pH of the processed juices. Such sugars are of lower quality [7, 8].

Concentrations of minor and trace elements in sugar usually follow the order Fe > Zn ≈ Mn > Cu > Ni >>As ≈

Table 25.2 Concentrations (μg/g) of main, minor and trace elements in sugar produced and sold in different countries.

Main elements [2, 4–8, 32–35, 46, 61]
Ca 0.6–1.5 × 10^4
Na 0.06–1.1 × 10^3
K 2.9–2.0 × 10^4
P 4–70
Mg 0.1–2.0 × 10^3
Minor and trace elements [2–8, 32–35, 45, 46, 48, 49, 51, 62–64]
Al 0.004–0.09
Co <0.001–0.008
Fe 0.01–54.6
Mn 0.003–18.0
V 0.02–0.05
As 0.0001–0.0006
Cr 0.002–0.40
Hg <0.001–0.001
Ni <0.003–0.03
Zn 0.004–14.1
Cd <0.001–0.16
Cu 0.005–1.4
Li 0.001–0.008
Sn 0.0001–0.015

Co ≈ Pb ≈ Hg. It seems that minor elements of sugar, for example Cu, Fe and Zn, manifest the highest variability in concentration, mostly due to differences in the composition of raw materials (sugar cane, sugar beet) and the technological processes involved in sugar production, i.e. extraction, purification and filtration, as well as materials and equipment required [32, 33, 35, 51]. The majority of Fe in sugar is present in the form of insoluble species (50–80% of its total) [34, 35]. This can be associated with a corrosive character of processed media and the corrosion of machinery (i.e. tanks, containers) [2, 5, 8, 32]. Unfortunately, a high content of Fe increases the conductivity of the sugar ash and worsens the colour of sugar solutions and crystals [2, 32, 34, 35]. When above certain trade specifications, elevated concentrations of Fe in sugar may even lower its marker category and price [34, 35]. The content of Cu and Zn also shows great variability [34, 51]. Insoluble species of Cu may also contribute 50–80% of the total content of this element in sugar [34]. High quantities of Cu in sugar solutions and crystals, by analogy with Fe, are responsible for their unfavourable darker colour and lower market grade [5]. In case of Zn, it seems that the quality of processed substrates with reference to the original content of this element and the sugar production technology, related to materials and additives used, may critically influence the quality of sugar [2, 7, 8, 32–34]. The insoluble species of Zn remaining in the final product may vary the most; their contribution to the total content of this element is reported to be within 10–80% [34].

The content of As, Cd, Co, Hg, Ni and Pb is very low and mostly depends on the concentration of these elements originally present in sugar cane and sugar beet and on adverse processes accompanying sugar production, for example the corrosion of stainless steel and contamination with chemicals and additives applied [3, 4, 63, 64].

As for sugar, Ca, K, Mg and Na are present in the highest quantities in honey (see concentration ranges of different elements given in Table 25.3). As can be seen, the most abundant element is K, which can contribute almost 85% of the total concentration of all elements of honey [13, 16, 19–21, 23–26, 29, 50, 52, 53, 59, 65–69]. The next most abundant element in honey is Na, followed by Ca and Mg. The presence of the major elements (Ca, K, Mg and Na) as well as Li, Rb and Sr in honey is primarily related to its botanical and geographical origins. In the latter case, soil characteristics and agricultural practices in the territory available for bees are of considerable importance [15, 19–21, 24, 37, 52, 57, 58]. The presence of minor elements (up to 100 μg/g) and trace elements (levels of several micrograms per gram or lower), i.e. Al, Cd, Cu, Fe, Ni, Pb and Zn, can also be related to botanical and geographical origins, although elevated concentrations of these elements could be a result of contamination at the level of forage areas or apiaries and also of beekeeping practices [15, 19–21, 24, 37, 56–58].

It is acknowledged that the presence of certain elements and their concentrations affect the colour of honey [23, 47, 59, 69]. Dark-coloured honeys commonly contain higher amounts of different elements (i.e. Al, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, Ni and Zn) than pale and light-coloured honeys [20, 21, 23, 47, 69]. In particular, honeydew honeys are characterized by the highest sums of concentrations of various elements [16, 23, 25, 43, 50, 56]. Interestingly, the colour of dark honeys is strongly correlated with concentrations of Cd, Fe and Pb [69], while the colour of pale honeys with concentrations of Al and Mg [69]. Elements present in honey also affect its ash content and electrical conductivity [47, 59]. As a rule, those honeys which contain high concentrations of various elements exhibit high ash contents and electrical conductivities.

Table 25.3 Concentrations (μg/g) of main, minor and trace elements in honey produced and sold in different countries.

Main and minor elements [12–31, 37–39, 41–44, 47, 50, 52–59, 65–76]
Ca 3.0–9.0 × 10^2
Na 0.4–1.2 × 10^3
Zn <0.01–29.0
K 6.8–7.7 × 10^4
Fe <0.03–60.0
Mn <0.01–82.0
Mg <1.1–3.7 × 10^2
Li 0.02–35.4
Cu <0.02–7.8
Trace elements [15–19, 22, 23, 27–31, 37–44, 47, 54–57, 60, 68–77]
Al 0.004–28.7
Cr 0.001–2.0
Cd <0.001–0.6
Ni <0.01–3.4
Co 0.001–1.6
Pb 0.003–1.2

25.4 Methodology used for the elemental analysis of sugar and honey

The elemental analysis of sugar and honey, commonly undertaken by means of spectrochemical methods, is a complex task that normally includes the initial preparation of representative samples of both products, the analysis of resulting sample solutions, and the quality control and assessment of results achieved. Possible strategies for preparing samples of sugar and honey before measurement of element concentrations are given in Figure 25.2. High temperature dry or wet ashing is used to decompose the complex sugar-rich matrices of these samples and release elements into sample solutions. These sample treatments are time-consuming, include several stages and require attention and skills because they can be potential sources of losses or gains of elements.

25.4.1 Sample preparation

Dry ashing is a fairly common sample pretreatment of sugar and honey and is used to decompose the sample matrix and transfer all mineral components into solution. In the case of sugar, relatively high sample masses, up to 100 g [62], are required for decomposition so as to pre-concentrate elements of interest. The sample masses required for the dry ashing of honey, which at high temperature easily foams, are lower: 20 g [13, 26, 57, 75], 10 g [16, 20, 41, 44] or even 5 g [19, 25, 47, 55, 59, 65, 66, 69]. Selected ashing aides, such as concentrated HNO_3 or H_2SO_4 solutions, $Mg(NO_3)_2$ with HNO_3, or $Mg(NO_3)_2$, can be used in some procedures [16, 62]. Samples are ashed by heating in crucibles placed in muffle furnaces at 450–600°C for 1–3 days, depending on sample mass and the type of ashing aids used. Any non-combustible residues left have to be dissolved in concentrated HCl or HNO_3 solutions or their mixtures.

Although bulky samples of sugar and honey can be pretreated in this way, there are several disadvantages of the dry ashing procedure that are reported to be the source of serious errors in the analysis, namely losses of certain elements (As, Cd, Hg, Pb, Sn, Zn) due to sample overheating, the volatilization of element species formed at high temperatures and the retention of these species by the crucible material, or gains of elements due to contamination of samples during long-term decomposition. Hence, quite often other sample preparation procedures like wet ashing in open-vessel and closed-vessel microwave-assisted systems are applied to minimize the risk related to the loss of elements and the contamination of samples. The only drawback of these procedures is that relatively low quantities of sample (1–10 g) can be pretreated immediately prior to the analysis. For the decomposition of samples of sugar and honey the following oxidative reagents are used: HNO_3 with H_2SO_4 [2, 32, 67], HNO_3 with $HClO_4$ [8, 18, 33, 36], HNO_3 with H_2O_2 [3, 39, 45, 46, 48, 63, 64] or HNO_3

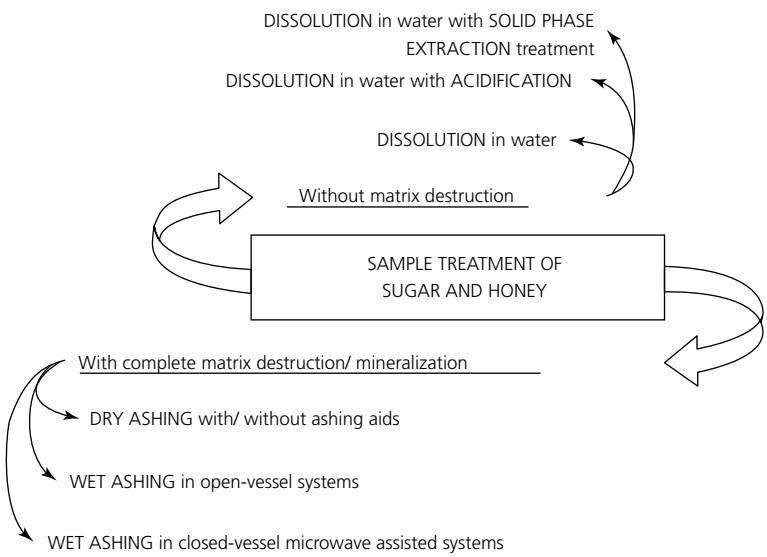


Figure 25.2 Strategies for sample treatment of sugar and honey before measurement of element concentrations.

alone [5, 6, 28]. Most such wet oxidative digestions are realized in open-vessel systems by heating samples with reagents in covered beakers on hot-plates. When using commercially available microwave-assisted digestion systems, decomposition of the organic matrix of sugar and honey samples is faster, losses of elements are eliminated and the risk of contamination of samples is much lower. However, sample masses required for this procedure are quite low, 0.1–1.0 g [3, 12, 14, 23, 27, 30, 31, 39, 48, 50, 52, 53, 63, 64, 70, 71]. Because of the vigorous course of such digestions, samples are usually initially dissolved in water or pre-digested before being subjected to the digestion procedure. With regard to the accuracy and precision of results, it is established that open-vessel digestions are in general comparable to digestions in closed-vessel microwave-assisted systems.

It is not surprising that analytical methods providing information on the elemental composition of sugar and honey samples using much simplified sample preparation procedures are also described in the literature. The development of such sample preparation procedures and methods of analysis was primarily stimulated by two reasons: (i) a willingness to eliminate the risk of contamination of samples and the loss of certain elements associated with decomposition and (ii) minimization of the time of sample treatment and analysis.

Dependable simplified methods for the analysis of sugar and honey samples described in the literature mainly include dissolution of samples in water [17, 37, 38, 40, 77], dissolution in water followed by acidification with HNO_3 in order to release elements of interest bound by the organic matrix [4, 15, 24, 51, 70], or dissolution in concentrated HNO_3 and H_2O_2 solutions followed by dilution with water [30, 73]. Resulting sample solutions are further measured using flame atomic absorption spectrometry (FAAS) [15, 24], graphite furnace atomic absorption spectrometry (GFAAS) [4, 30, 51, 73] and inductively coupled plasma optical emission spectrometry (ICP-OES) [17, 37, 38, 70]. Interesting procedures have been proposed [34, 61, 78–80], including the dissolution of relatively high sample masses in water and the subsequent treatment of resulting sample solutions with solid-phase extraction (SPE) columns filled with strongly acidic cation exchangers capable of retaining ions of Ca, Cu, Fe, K, Li, Mg, Mn, Na and Zn. Here, the application of specific sorptive materials guarantees the separation of elements of interest from the concomitant organic sample matrix (i.e. fructose, glucose, sucrose) and the pre-concentration of elements prior to sensitive measurements by FAAS or flame atomic emission spectrometry (FAES). A scheme of such sample treatment is given in Figure 25.3.

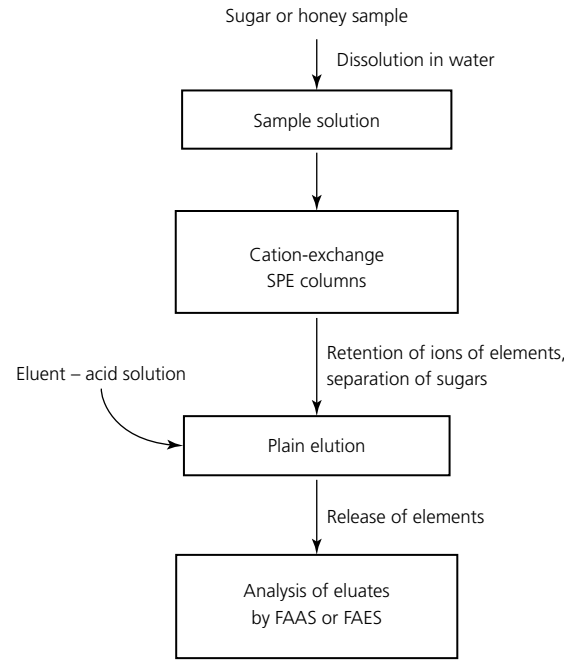


Figure 25.3 Scheme of solid-phase extraction procedure for separation of sugar-rich matrix and pre-concentration of elements.

No sample digestion is required in the case of the elemental analysis of sugar and honey samples by instrumental nuclear activation analysis (INAA) [36, 81, 82]. Dissolution in water is the only sample treatment in the case of the elemental analysis of sugar and honey samples by electrochemical methods. Here, resulting sample solutions can be adjusted to a proper pH, mixed with supporting electrolytes or purged with N_2 to remove O_2 dissolved [3, 48, 63, 64, 83].

25.4.2 Spectrometric methods of analysis

It is apparent from the literature that spectrochemical methods of analysis are commonly applied in the analysis of sugar and honey due to their universality, accessibility and good analytical performance. Among them, FAAS [2, 4, 5, 8, 12, 13, 15, 16, 18, 24, 26, 31–36, 39, 42, 44, 47, 50, 52, 53, 56–59, 61, 62, 67, 72, 74–76], GFAAS [3, 4, 8, 12, 22, 30, 31, 37, 39–41, 43, 45, 46, 48, 51–54, 60, 63, 71, 73, 77, 84] and ICP-OES [6, 14, 17,

19–21, 23, 25, 27, 28, 37, 38, 54–56, 65, 66, 68–70, 72] are the most commonly applied. FAAS is typically used for the determination of major and minor elements (i.e. Ca, Cu, Fe, K, Mg, Mn, Na, Zn); however, trace elements like Co, Cd, Ni, Pb and Sr [15, 18, 44, 57, 75] are also reported to be measured with this method. GFAAS is typically used for the determination of minor and trace elements (i.e. Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sn, V and Zn). Both methods are usually operated under instrumental conditions recommended by the manufacturer and they offer good analytical performance with regard to precision and accuracy of results and resistibility to matrix effects. To alleviate matrix effects related to the presence of sugars, samples of white sugar and honey are decomposed and analysed as resulting sample solutions. The calibration is made using simple standard solutions or standard solutions containing the same amounts of reagents applied at the sample decomposition stage.

ICP-OES is often applied for multi-element analyses of white sugar and honey. Using this method, many more elements (i.e. Ag, Al, Ba, Be, Ca, Cd, Ce, Co, Cr, Cu, Fe, Ga, K, La, Li, Mg, Mn, Na, Nb, Ni, P, Sb, Se, Sn, Sr, Ti, V and Zn) can be determined in a relatively short time. Because of the extended linear dynamic range and the relatively good detectability for most elements, ICP-OES is suitable for the determination of major as well as minor and trace elements.

Inductively coupled plasma mass spectrometry (ICP-MS) is used much more rarely for the elemental analysis of sugar and honey [14, 50]. Other non-spectrochemical analytical methods are also applied less commonly for the elemental analysis of white sugar and honey, such as stripping voltammetry and potentiometry techniques [3, 48, 63, 64, 83]. As compared with FAAS and GFAAS, costs of maintenance and operation of the latter methods are lower while analytical performance for certain electroactive elements (i.e. As, Cd, Co, Cu, Hg, Ni, Pb, Zn) is better, with regard to the sensitivity of measurements and detection limits of this group of elements. Another method that can be used for the analysis of sugar and honey samples is INAA [5, 6, 36, 81, 82]. Minimal sample handling is normally required in the case of multi-element analysis, although samples can also be ashed before measurement in order to pre-concentrate elements to be determined [5]. Nevertheless, INAA cannot be considered for routine analyses because special and expensive instrumentation, namely reactors for the irradiation of samples and a set of detectors, is needed.

25.5 Conclusions

Considering that sugar and honey are commonly used to sweeten other beverages and food products, determination of the elemental composition of these products can provide important information about their quality and safety. The elemental profile of sugar and honey seems to be a useful fingerprint of the regions where they originate and of the methods of pretreatment and processing. Hence, it can successfully be used for the purposes of classification and the distinction of various brands of sugar and honey or as evidence of the declared authenticity of these products. Information on the concentration of certain elements can also be important from the point of view of the processing of sugar and honey and can help in making decisions to improve their high quality. In the case of honey, monitoring of the concentrations of trace elements, after considering geochemical conditions, may also be helpful in the control of cumulative environmental pollution in a given territory.

Acknowledgements

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Abbreviations

AI: Adequate intake
CA: Cluster analysis
FAAS: Flame atomic absorption spectrometry
FAES: Flame atomic emission spectrometry
GFAAS: Graphite furnace atomic absorption spectrometry
ICP-MS: Inductively coupled plasma mass spectrometry
ICP-OES: Inductively coupled plasma optical emission spectrometry
INAA: Instrumental nuclear activation analysis

LDA: Linear discriminant analysis
PCA: Principal component analysis
QA: Quality assurance
QC: Quality control
RDA: Recommended daily allowance
SPE: Solid-phase extraction

References

1 Pohl P, Stecka H. Elemental composition of white refined sugar by instrumental methods of analysis. *Crit Rev Anal Chem* 2011;**41**:100–113.
2 Skrbic B, Gyura J. Iron, copper and zinc in white sugar from Serbian sugar beet refineries. *Food Control* 2007;**18**: 135–139.
3 Sanocho D, Deban L, Campos I, Pardo R, Gonzalez G. Determination of copper and arsenic in refined beet sugar by stripping voltammetry without sample pretreatment. *Analyst* 1998;**123**:743–747.
4 Skrbic B, Cupic S, Cvejanov J. Determination of heavy metals in beet sugar samples from Vojvodina province. *J Environ Protect Ecol* 2003;**4**:657–661.
5 Mohamed AE. Environmental variations of trace element concentrations in Egyptian cane sugar and soil samples (Edfu factories). *Food Chem* 1999;**65**:503–507.
6 Awadallah RM, Ismail SS, Mohamed AE. Application of multi-element clustering techniques of five Egyptian industrial sugar products. *J Radioanal Nucl Chem* 1995;**196**: 377–385.
7 De Bruijn JM, Bout M. Analytical approach to white sugar quality: anions, cations and their probable origin. *Zuckerindustie* 1999;**124**:532–535.
8 Skrbic B, Durisic-Mladenovic N, Macvanin N. Determination of metal contents in sugar beet (*Beta vulgaris*) and its products: empirical and chemometrical approach. *Food Sci Technol Res* 2010;**16**:123–134.
9 Pohl P. Determination of metal content in honey by atomic absorption and emission spectrometries. *Trends Anal Chem* 2009;**28**:117–128.
10 Pohl P, Sergiel I, Stecka H. Determination and fractionation of metals in honey. *Crit Rev Anal Chem* 2009;**39**:276–288.
11 Pohl P, Stecka H, Sergiel I, Jamroz P. Different aspects of the elemental analysis of honey by flame atomic absorption and emission spectrometry: a review. *Food Anal Method* 2012;**5**: 737–751.
12 Stankovska E, Stafilov T, Sajn R. Monitoring of trace elements in honey from the Republic of Macedonia by atomic absorption spectrometry. *Environ Monit Assess* 2008;**142**: 117–126.
13 Abu-Tarboush HM, Al-Kahtani HA, El-Sarrage MS. Floral-type identification and quality evaluation of some honey types. *Food Chem* 1993;**46**:13–17.
14 Caroli S, Forte G, Iamiceli AL, Galoppi B. Determination of essential and potentially toxic trace elements in honey by inductively coupled plasma-based techniques. *Talanta* 1999;**50**:327–336.
15 Hernandez OM, Fraga JMG, Jimenez AI, Jimenez F, Arias JJ. Characterization of honey from the Canary Islands: determination of the mineral content by atomic absorption spectrophotometry. *Food Chem* 2005;**93**:449–458.
16 Uren A, Serifoglu A, Sarikahya Y. Distribution of elements in honeys and effect of a thermoelectric power plant on the element contents. *Food Chem* 1998;**61**:185–190.
17 Matei N, Birghila S, Dobrinas S, Capota P. Determination of C vitamin and some essential trace elements (Ni, Mn, Fe, Cr) in bee products. *Acta Chim Slov* 2004;**51**:169–175.
18 Rashed MN, Soltan ME. Major and trace elements in different types of Egyptian mono-floral and non-floral bee honeys. *J Food Compos Anal* 2004;**17**:725–735.
19 Terrab A, Hernanz D, Heredia FJ. Inductively coupled plasma optical emission spectrometric determination of minerals in thyme honeys and their contribution to geographical discrimination. *J Agric Food Chem* 2004;**52**:3441–3445.
20 Nozal Nalda MJ, Bernal Yague JL, Diego Calva JC, Martin Gomez MT. Classifying honeys from the Soria Province of Spain via multivariate analysis. *Anal Bioanal Chem* 2005;**382**:311–319.
21 Fernandez-Torres R, Perez-Bernal JL, Bello-Lopez MA, Callejon-Mochon M, Jimenez-Sanchez JC, Guiraum-Perez A. Mineral content and botanical origin of Spanish honeys. *Talanta* 2005;**65**:686-691.
22 Yarsan E, Karacal F, Ibrahim IG, Dikmen B, Koksal A, Das YK. Contents of some metals in honeys from different regions in Turkey. *Bull Environ Contam Toxicol* 2007;**79**:255–258.
23 Pisani A, Protano G, Riccobono F. Minor and trace elements in different honey types produced in Siena County (Italy). *Food Chem* 2008;**107**:1553–1560.
24 Soares dos Santos J, Soares dos Santos N, Pires dos Santos ML, Novaes dos Santos S, de Jesus Lacerda JJ. Honey classification from semi-arid, Atlantic and transitional forest zones in Bahia, Brazil. *J Braz Chem Soc* 2008;**19**:502–508.
25 Terrab A, Gonzalez AG, Diez MJ, Heredia FJ. Mineral content and electrical conductivity of the honeys produced in Northwest Morocco and their contribution to the characterisation of unifloral honeys. *J Sci Food Agric* 2003;**83**: 637–643.
26 Rodriguez-Otero JL, Paseiro P, Simal J, Cepeda A. Mineral content of the honeys produced in Galicia (North-west Spain). *Food Chem* 1994;**49**:169–171.
27 Al-Khalifa AS, Al-Arify IA. Physicochemical characteristics and pollen spectrum of some Saudi honeys. *Food Chem* 1999;**67**:21–25.
28 Devillers J, Dore JC, Marenco M, Poirier-Duchene F, Galand N, Viel C. Chemometrical analysis of 18 metallic and non-metallic elements found in honeys sold in France. *J Agric Food Chem* 2002;**50**:5998–6007.

29 Bagci Y, Arslan D, Ozcan MM, Dursun N. Determination of the mineral content of bee honeys produced in Middle Anatolia. *Int J Food Sci Nutr* 2007;**58**:567–575.
30 Ajtony Z, Bencs L, Haraszi R, Szigeti J, Szoboszlai N. Study on the simultaneous determination of some essential and toxic trace elements in honey by multi-element graphite furnace atomic absorption spectrometry. *Talanta* 2007;**71**: 683–690.
31 Silici S, Uluozlu OD, Tuzen M, Soylak M. Assessment of trace element levels in rhododendron honeys of Black Sea Region, Turkey. *J Hazard Mater* 2008;**156**:612–618.
32 Skrbic B, Gyura I. Survey of some contaminants in white sugar from Serbian sugar beet refineries. *Food Addit Contam* 2006;**23**:31–35.
33 Skrbic B, Durisic-Mladenovic N. Toxic and essential trace elements in Serbian sugar beet, molasses and white sugar. *Zuckerindustrie* 2005;**130**:913–917.
34 Wojtczak M, Krol B Content of iron, copper and zinc in white sugar samples from Polish and other European sugar factories. *Food Addit Contam* 2002;**19**:984–989.
35 Wojtczak M. Content and composition of insoluble matter in white sugar. *Zuckerindustrie* 2006;**131**:567–571.
36 Waheed S, Rahman S, Gill KP. INAA and AAS of different products from sugar cane industry in Pakistan: toxic trace elements for nutritional safety. *J Radioanal Nucl Chem* 2009;**279**:725–731.
37 Rodríguez García JC, Iglesias Rodríguez R, Pena Crecente RM, Barciela García J, García Martín S, Herrero Latorre C. Preliminary chemometric study on the use of honey as an environmental marker in Galicia (northwestern Spain). *J Agric Food Chem* 2006;**54**:7206–7212.
38 Ioannidou MD, Zachariadis GA, Anthemidis AN, Stratis JA. Direct determination of toxic trace metals in honey and sugars using inductively coupled plasma atomic emission spectrometry. *Talanta* 2005;**65**:92–97.
39 Tuzen M, Silici S, Mendil D, Soylak M. Trace element levels in honeys from different regions of Turkey. *Food Chem* 2007;**103**:325–330.
40 Rodriguez Garcia JC, Barciela Garcia J, Herrero Latorre C, Garcia Martin S, Pena Crecente RM. Comparison of palladium–magnesium nitrate and ammonium dihydrogenphosphate modifiers for lead determination in honey by electrothermal atomic absorption spectrometry. *Food Chem* 2005;**91**:435–442.
41 Przybylowski P, Wilczynska A. Honey as an environmental marker. *Food Chem* 2001;**74**:289–291.
42 Bratu I, Georgescu C. Chemical contamination of bee honey: identifying sensor of the environment pollution. *J Cent Eur Agric* 2005;**6**:95–98.
43 Gonzalez Paramas AM, Gomez Barez JA, Garcia-Villanova RJ, Rivas Pala T, Ardanuy Albajar R, Sanchez Sanchez J. Geographical discrimination of honeys by using mineral composition and common chemical quality parameters. *J Sci Food Agric* 2000;**80**:157–165.
44 Erbilir F, Erdogrul O. Determination of heavy metals in honey in Kahramanmaras City, Turkey. *Environ Monit Assess* 2005;**109**:181–187.
45 Miller-Ihli NJ. Evaluation of graphite furnace atomic absorption method developed for the determination of lead in sugar. *J Agric Food Chem* 1995;**43**:923–927.
46 Miller-Ihli NJ. Graphite furnace atomic absorption method for the determination of lead in sugars and syrups. *J AOAC Int* 1994;**77**:1627–1630.
47 Osman KA, Al-Doghairi MA, Al-Rehiayani S, Helal MID. Mineral contents and physicochemical properties of natural honey produced in Al-Qassim region, Saudi Arabia. *J Food Agric Environ* 2007;**5**:142–146.
48 Sancho D, Vega M, Deban L, Pardo R, Gonzalez G. Determination of zinc, cadmium and lead in untreated sugar samples by anodic stripping voltammetry. *Analyst* 1997;**122**:727–730.
49 Dias VMC, Cardoso ASB. The determination of lead in sugar and sweets without digestion by electrothermal atomic absorption spectrometry (ETAAS) with a rhodium modifier. *Food Addit Contam* 2006;**23**:479–483.
50 Madejczyk M, Baralkiewicz D. Characterization of Polish rape and honeydew honey according to their mineral contents using ICP-MS and F-AAS/AES. *Anal Chim Acta* 2008;**617**:11-17.
51 Ronda F, Sancho D, del Alamo M, Gomez M. Direct determination of arsenic, cadmium, cobalt, copper, chromium, lead, tin and zinc in white sugar using graphite furnace atomic absorption spectrophotometry. *Zuckerindustrie* 2001;**126**: 208–212.
52 Conti ME, Stripeikis J, Campanella L, Cucina D, Tudino MB. Characterization of Italian honeys (Marche Region) on the basis of their mineral content and some typical quality parameters. *Chem Centr J* 2007;**1**:14. doi:10.1186/1752-153X-1-14.
53 Conti ME. Lazio region (central Italy) honeys: a survey of mineral content and typical quality parameters. *Food Control* 2000;**11**:459–463.
54 Frías I, Rubio C, Gonzalez-Iglesias T, Gutierrez AJ, Gonzalez-Weller D, Hardisson A. Metals in fresh honeys from Tenerife Island, Spain. *Bull Environ Contam Toxicol* 2008;**80**:30–33.
55 Fredes C, Montenegro G. Heavy metals and other trace elements contents in Chilean honey. *Cienc Investig Agrar* 2006;**33**:50–58.
56 Lachman J, Kolihova D, Miholova D, Kosata J, Titera D, Kult K. Analysis of minority honey components: possible use for the evaluation of honey quality. *Food Chem* 2007;**101**: 973–979.
57 Latorre MJ, Pena R, Pita C, Botana A, García S, Herrero C. Chemometric classification of honeys according to their type. II. Metal content data. *Food Chem* 1999;**66**:263–268.
58 Latorre MJ, Pena R, García S, Herrero C. Authentication of Galician (NW Spain) honeys by multivariate techniques based on metal content data. *Analyst* 2000;**125**:307–312.

59 Downey G, Hussey K, Kelly JD, Walshe TF, Martin PG. Preliminary contribution to the characterisation of artisanal honey produced on the island of Ireland by palynological and physico-chemical data. *Food Chem* 2005;**91**:347–354.
60 Conti ME, Botre F. Honeybees and their product as potential bioindicators of heavy metals contamination. *Environ Monit Assess* 2001;**69**:267–282.
61 Pohl P, Stecka H, Jamroz P. Solid phase extraction with flame atomic absorption spectrometry for determination of traces of Ca, K, Mg, Na in quality control of white sugar. *Food Chem* 2012;**130**:441–446.
62 Leblebici J, Volkan M. Sample preparation for arsenic, copper, iron and lead determination in sugar. *J Agric Food Chem* 1998;**46**:173–177.
63 Sancho D, Deban L, Campos I, Pardo R, Vega M. Determination of nickel and cobalt in refined beet sugar by adsorptive cathodic stripping voltammetry without sample pretreatment. *Food Chem* 2000;**71**:139–145.
64 Sancho D, Deban L, Barbosa F, Pardo R, Vega M. Determination of mercury in refined beet sugar by anodic stripping voltammetry without sample pretreatment. *Food Chem* 2001;**74**:527–531.
65 Terrab A, Recamales MAF, Hernanz D, Heredia FJ. Characterisation of Spanish thyme honeys by their physicochemical characteristics and mineral content. *Food Chem* 2004;**88**:537–542.
66 Terrab A, Heredia FJ. Characterisation of avocado (*Persea americana* Mill.) honeys by their mineral content and physicochemical characteristics. *J Sci Food Agric* 2004;**84**: 1801–1805.
67 Nanda V, Sarkar BC, Sharma HK, Bawa AS. Physicochemical properties and estimation of mineral content in honey produced from different plants in Northern India. *J Food Compos Anal* 2003;**16**:613–619.
68 Terrab A, Recamales MAF, Gonzalez-Miret ML, Heredia FJ. Contribution to the study of avocado honeys by their minerals content using inductively coupled plasma optical emission spectrometry. *Food Chem* 2005;**92**:305–309.
69 Gonzalez-Miret ML, Terrab A, Hernanz D, Recamales MAF, Heredia FJ. Multivariate correlation between color and mineral composition of honeys and by their botanical origin. *J Agric Food Chem* 2005;**53**:2574–2580.
70 Mendes TMFF, Baccan SN, Cadore S. Sample treatment procedures for the determination of mineral constituents in honey by inductively coupled plasma optical emission spectrometry. *J Braz Chem Soc* 2006;**17**:168–176.
71 Buldini PL, Cavalli S, Mevoli A, Lal Sharma J. Ion chromatographic and voltammetric determination of heavy and transition metals in honey. *Food Chem* 2001;**73**:487–495.
72 Kump P, Necemer M, Snajder J. Determination of trace elements in bee honey, pollen and tissue by total reflection and radioisotope X-ray fluorescence spectrometry. *Spectrochim Acta B* 1996;**51**:499–507.
73 Vinas P, Lopez-Garcia I, Lanzon M, Hernandez-Cordoba M. Direct determination of lead, cadmium, zinc, and copper in honey by electrothermal atomic absorption spectrometry using hydrogen peroxide as a matrix modifier. *J Agric Food Chem* 1997;**45**:3952–3956.
74 Lopez-Garcia I, Vinas P, Blanco C, Hernandez-Cordoba M. Fast determination of calcium, magnesium and zinc in honey using continuous flow flame atomic absorption spectrometry. *Talanta* 1999;**49**:597–602.
75 Yilmaz H, Yavuz O. Content of some trace metals in honey from south-eastern Anatolia. *Food Chem* 1999;**65**:475–476.
76 Kucuk M, Kolayli S, Karaoglu S, Ulusoy E, Baltaci C, Candan F. Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chem* 2007;**100**:526–534.
77 Rodriguez Garcia JC, Barciela Garcia J, Herrero Latorre C, Freire Rodriguez M, Garcia Martin S, Pena Crecente RM. Comparison of palladium–magnesium nitrate and ammonium dihydrogenphosphate modifiers for cadmium determination in honey samples by electrothermal atomic absorption spectrometry. *Talanta* 2003;**61**:509–517.
78 Stecka H, Pohl P. Pre-concentration of lithium prior to its determination in honey by flame optical emission spectrometry. *J Braz Chem Soc* 2011;**22**:677–683.
79 Pohl P, Stecka H, Jamroz P. Fast and interference free determination of calcium and magnesium in honeys by solid phase extraction followed by flame atomic absorption spectrometry. *J Braz Chem Soc* 2012;**23**:710–717.
80 Pohl P, Stecka H, Jamroz P. Solid phase extraction and sequential elution for pre-concentration of traces of Mn and Zn in analysis of honey by flame atomic absorption spectrometry. *Anal Method* 2012;**4**:125–131.
81 Pellerano RG, Unates MA, Cantarelli MA, Camina JM, Marchevsky EJ. Analysis of trace elements in multifloral Argentine honeys and their classification according to provenance. *Food Chem* 2012;**134**:578–582.
82 Almeida-Silva M, Canha N, Galinha C, Dung HM, Freitas MC, Sitoe T. Trace elements in wild and orchard honeys. *Appl Radiat Isotopes* 2011;**69**:1592–1595.
83 Munoz E, Palmero S. Determination of heavy metals in honey by potentiometric stripping analysis and using a continuous flow methodology. *Food Chem* 2006;**94**:478–483.
84 Miller-Ihli N, Greene FE. Direct determination of lead in sugars using graphite furnace atomic absorption spectrometry. *Atom Spectrosc* 1993;**44**:85–89.

CHAPTER 26
Meat

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Abstract: Meat, including offal, is an important source of essential minerals such as phosphorus, potassium, iron and selenium. The bioavailability of some of these minerals is also very much higher than that from vegetables sources. This chapter reviews the content and distribution of both macrominerals and microminerals in different edible animal species. The influence on the mineral composition of meat of factors such as age, gender, type of muscle and cut meat, and cooking process is also discussed. The content and distribution of toxic minerals (arsenic, cadmium, lead and mercury) are reviewed in relation to the European Union and international regulations that establish the maximum levels of these elements in different meat commodities and the tolerable intakes. Finally, the most frequently used sample pretreatment procedures and methods of analysis for macrominerals, microminerals and toxic minerals are presented and discussed, paying special attention to speciation analysis.

Keywords: meat, meat products, macrominerals, microminerals, toxic minerals

26.1 Introduction

Meat is defined in Regulation (EC) 853/2004 of the European Union [1] as 'the edible parts of domestic ungulates, poultry, lagomorphs, wild game, farmed game, small wild game and large wild game, including blood'. Offal is defined as 'fresh meat other than that of the carcase, including viscera and blood' (kidney, liver, heart, etc.). The most common sources of meat are domesticated animal species such as cattle, chickens and pigs, whose meats are named beef, poultry and pork, respectively. To a lesser extent, buffalo, sheep and goats are also consumed. In some countries other animal species such as llama, camel, horse, ostrich, game animals and crocodile are also eaten as meat. Pork is the most widely eaten meat worldwide, accounting for over 36% of world meat intake, followed by poultry and beef with about 33% and 24%, respectively (see http://www.fao.org/ag/againfo/themes/en/meat/backgr_sources.html). The definition of 'meat product' is also laid down in Regulation (EC) 853/2004: 'processed product resulting from the processing of meat or from the further processing of such processed products, so that the cut surface shows that the product no longer has the characteristics of fresh meat'. Meat products are the result of the need to preserve meat in ancient times. Taking into account the treatment of raw materials and the processing technologies used, it is possible to classify meat products into six broad groups [2]: fresh processed meat products (hamburger, kebab, etc.), cured meat cuts (bacon, cooked ham, raw ham, etc.), raw-cooked meat products (mortadella, frankfurter, etc.), precooked/cooked meat products (blood sausage, liver sausage, etc.), raw-fermented sausages (salami, etc.) and dried meat products (biltong, beef jerkey, etc.).

Meat has an important role in human nutrition because of its nutritive value, which is measured in terms of the major chemical components such as proteins, carbohydrates, fats, fatty acids and minerals. Minerals are transferred through the food chain to animals and humans, meat being one of the major sources of elements such as iron, phosphorus, zinc and selenium in the diet. Some of these minerals, those considered as essential elements with well-defined physiological functions, are present exclusively in meat or their bioavailability is much higher than that from vegetables. The concentration of the minerals in animal tissues depends mainly on the species of animal, the dietary concentration

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of the element, the absorption of the element and the concentration of other tissue minerals.

Despite being an excellent source of high-quality protein, vitamins and minerals, meat, especially red meat, is frequently considered an unhealthy food, mainly because it can be the principal source of fat and cholesterol and because of its reputation as a cancer promoter [3]. In the last few decades, many studies have reported an increase in the risk of developing colorectal cancer in consumers of red meat (mainly beef) and processed meat (mainly cured pork) [4], although the effect of meat consumption on cancer risk is a controversial issue. With the purpose of reducing the risk of cancer, in 2007 the World Cancer Research Fund proposed the recommendation to limit the consumption of red meat and to avoid processed meat intake [5].

26.2 Macrominerals

26.2.1 Content and distribution

Meat and meat products are good sources of most minerals. Muscle tissue, especially of red meat, is an important source of phosphorus and potassium, but is low in calcium and is a moderate source of magnesium. Offal, particularly liver, is also a good source of essential macrominerals, mainly potassium and phosphorus followed by sodium, magnesium and calcium [6, 7]. The levels of sodium in raw meat are not significant; however, meat products may contain high levels of this mineral because it is added as part of curing and preserving or as a flavor-enhancing ingredient. Table 26.1 shows the macromineral composition of different animal meats reported by studies carried out in different countries. As can be seen, the content of some macrominerals varies among meat of different species, although there are not generally many significant differences. Llama and alpaca stand out by their high content of potassium and phosphorus. As can be seen, animals such as donkey, llama, alpaca or iguana, which constitute an important source of meat production in some countries, have levels of macrominerals similar to those of beef, pork, lamb and poultry. According to the data in Table 26.1, in some cases there are also significant differences among the results reported by different authors on the mineral composition of the same animal meat. It is necessary to take into account that various factors, such as the diet of the animal, age, gender and hormones may cause variations in the mineral composition of the tissues [21]. Furthermore, differences in sampling methods, type of muscles sampled, cut of meat, genetics, production systems, different seasons, wide range of variability within individual animals, and mineral supplementation make valid comparisons of tissue mineral composition among different studies very difficult [6, 16]. Based on many reports, it appears that gender is not a determining factor in the concentration of macrominerals in meat and liver [6] and, in contrast, the content of macrominerals increases with age [6, 22].

On the other hand, it is important to consider the influence of cooking techniques on mineral content, while remembering that humans rarely eat raw meat. Several studies show that cooking significantly decreases the content of minerals in meat when it is cooked in water and that losses are due to the leaching of minerals into the broth [23]. In meat products, sodium is generally the major mineral, probably due to addition of salt during processing. As an example, levels of sodium of 1.8 and 0.994% have been found in Italian salami [24] and Spanish chorizo [25], respectively. The amounts of the different minerals in meat products vary enormously depending on their composition, and it is necessary to bear in mind that they contain not only meat or offal but other compounds such as starch, spices and additives.

26.2.2 Methods of analysis

The general steps in the pretreatment of meat after slaughter include pre-drying or defatting, ground by sieve screen and preservation of the prepared sample by freezing or drying to reduce or eliminate enzyme activity and microbial attack. The method of pretreatment depends on the sample matrix, nature and concentration of the minerals of interest and the instrumental technique employed for their determination. In general, most methods for determining the mineral content of meat and meat products involve atomic spectrometry techniques and therefore the samples have to be in solution. Sample pretreatment is usually performed by dry ashing and wet digestion, this latter being frequently microwave-assisted. Dry ashing is carried out in a muffle furnace at temperatures of 450–550 °C and has the advantages that no added reagent is necessary and that large amounts of sample can be processed [6, 13, 16, 25]. Afterwards, the residue is dissolved in a small volume of acid, usually HCl [13] or HNO_3 [6, 25]. Oxidative decomposition by wet digestion under ambient pressure or microwave-assisted involves the use of an acid or mixture of acids and oxidizing agents to

Table 26.1 Macromineral composition (mg/kg fresh muscle) of meats.

Meat	Reference	Ca	Mg	K	Na	P
Beef						
Australia	[8]	45	250	3630	510	2150
Iceland	[9]	61	168	2710	798	1840
South America	[10]	70	200	3500	610	1800
USDA	[11]	150	110	2830	460	2050
Pork						
USDA	[11]	170	230	3890	520	2110
Lamb						
Australia	[8]	72	280	3440	690	1940
Iceland	[9]	57	200	3200	461	2190
USDA	[11]	150	240	3270	750	1990
Chicken						
Venezuela	[12]	41.2	191.9	1890	515.5	1612
USDA	[11]	120	250	2290	770	1730
Rabbit						
Spain	[13]	87	270	3880	600	2370
USDA	[11]	130	190	3300	410	2130
Turkey						
USDA	[11]	110	270	2350	1180	1900
Quail						
USDA	[11]	130	250	2370	510	3070
Donkey						
Italy	[27]	86.5	248	3437	525	2129
Ostrich						
South Africa	[14]	80	220	2690	430	2130
Poland	[15]	47.6–60.3	233–250	2340–2560	320–390	2100–2280
USDA	[11]	60	220	3120	810	2140
Rhea						
Uruguay	[16]	18.9	150	2570	637	3840
Emu						
Canada	[17]	50	250	3100	470	2300
USDA	[11]	30	420	3000	1200	2360
Llama						
Peru	[18]	116	284	4471	1056	3794
Alpaca						
Peru	[18]	87.9	231	4117	918	3381
Peru	[19]	107	338	4190	884	2950
Buffalo						
Iran	[6]	87.4	239.7	3679.8	652	n/a
USDA	[11]	120	320	2970	530	1970
Venezuela	[20]	73.9	242.6	3512.4	661.3	2114.4
Iguana						
Venezuela	[12]	101.4	219.1	2661	893.1	2170

n/a, data unavailable or not reported.

dissolve the sample. Digestion under ambient pressure is frequently performed with HNO_3 [19], $HNO_3/HClO_4$ [7] or $HNO_3/HClO_4/H_2SO_4$ [6, 26]. In the case of microwave-assisted digestion, usually used for small amounts of samples, this is frequently performed with HNO_3/H_2O_2 [18, 27]. Another interesting method recently reported for sample treatment involves alkaline extraction with tetramethylammonium hydroxide (TMAH) [28], which has been demonstrated to be a good alternative to mineralization with conventional procedures.

Calcium, magnesium, sodium and potassium are usually determined by flame atomic absorption spectrometry (FAAS) [7, 16, 20, 26] and inductively coupled plasma atomic emission spectrometry (ICP-OES) [18, 19, 27]. In the case of sodium and potassium, determination by FAAS is carried out in emission mode; another technique frequently used is flame photometry [29]. The determination of phosphorus is carried out by a colorimetric method based on the use of molybdate–vanadate reagent [30]. Other alternative techniques for determination of macrominerals in meat and meat products include inductively coupled plasma mass spectrometry (ICP-MS) and neutron activation analysis (NAA). As an example, ICP-MS was applied to the analysis of calcium in reindeer [31] or calcium and magnesium in liver pâté [32] and NAA for determination of sodium, calcium and potassium in beef, pork and poultry [33].

26.3 Microminerals

26.3.1 Content and distribution

Meat, including liver, is an important source of essential microminerals such as iron, zinc, copper, manganese, selenium, cobalt, iodine and molybdenum due to the fact that some of them are exclusively present in meat or have a higher bioavailability than those from plant sources. Among them iron, selenium and zinc are the most important not only because of the significant concentrations in which they are found but also due to their high bioavailability, so that consumption of meat and liver (100 g/day) can cover up to 50% of their daily recommended intake (DRI) [4]. Table 26.2 shows the micromineral composition of different animal meats reported by studies carried out in different countries. As can be seen, the variation in the concentration of microminerals among different animal species is more significant than that for macrominerals (see Table 26.1). Lamb and beef are rich in iron and zinc. Other more exotic meats such as ostrich, emu, alpaca and llama also stand out by their high content of iron and zinc. On the other hand, rhea and emu seem to be good nutritional sources of selenium in comparison with other meats.

Iron

Iron is an essential mineral involved in gas exchange at the tissue and cellular levels via the oxygenation of haemoglobin in red cells and myoglobin in skeletal muscle. In addition, iron is incorporated in several enzymes involved in cellular energy metabolism and the immune system [4]. This mineral is present in meat and meat products as non-haem iron (nheFe) and haem iron (heFe), heFe being the most efficiently absorbed form from the diet [44]. A number of dietary components have been shown to increase nheFe absorption from meals, meat being among them. As can be seen in Table 26.2, iron concentration varies significant among species. From a nutritional point of view, it is necessary to take into account not only the total iron (tFe) content of an animal meat but also the percentage of heFe that it provides. Lombardi-Boccia *et al*. [45] found that the percentages of heFe in chicken, turkey, beef, lamb, pork, rabbit and ostrich meats were 38, 42, 87, 75, 62, 56 and 72%, respectively. Therefore beef and lamb are among the most iron-rich meat sources. Other factors such as meat cut [38] and type of muscle [16, 46] also influence the concentration of iron in raw meat. Cabrera *et al*. [47] evaluated seven different meat cuts from Hereford and Braford steers and found that the iron content was 16.4–48.2 mg/kg and 14.2–47.9 mg/kg, respectively. Therefore, selection of the meat cut used for the preparation of a particular meat-based food, for example baby food, could be of importance. Other studies have shown that there are also significant variations in the amounts of tFe and heFe among viscera [48], the spleen and the lungs having the highest concentration of heFe. This fact could be attributed to the diverse functions of viscera.

Thermal processes that meats undergo represent another important factor causing variation in their heFe content [45]. The usual moisture losses during the cooking process result in an increase in tFe content [23]. Nevertheless, heFe is converted, to varying degrees, into nheFe by heating, thus notably decreasing the heFe/nheFe ratio. In meat products there are obviously wide variations in iron content, depending on the type of

Table 26.2 Micromineral composition (mg/kg fresh muscle) of meats.

Meat	Reference	Zn	Fe	Mn	Cu	Se
Beef						
Australia	[8]	46	18	n/a	1.2	0.17
Iceland	[9]	39.1	17.1	n/a	n/a	0.038
Nigeria	[34]	23.5	n/a	n/a	1.60	n/a
Poland	[35]	34	23	0.11	1.2	n/a
UK	[36]	n/a	n/a	n/a	n/a	0.076
Italy	[38]	42.2	19.9	n/a	0.6	n/a
USDA	[11]	37.9	19.3	n/a	n/a	n/a
Pork						
UK	[36]	n/a	n/a	n/a	n/a	0.14
Nigeria	[34]	30.2	n/a	n/a	2.87	n/a
Spain	[39]	42.5	26.5	1.01	6.85	0.656
Poland	[40]	26	13	0.11	1.1	n/a
Italy	[38]	15.5	4.2	n/a	0.5	n/a
USDA	[11]	18.4	8.4	n/a	n/a	n/a
Lamb						
Australia	[8]	45	20	n/a	1.2	0.14
Iceland	[9]	28.1	15.2	n/a	0.96	0.085
UK	[36]	n/a	n/a	n/a	n/a	0.038
Australia	[37]	n/a	n/a	n/a	n/a	0.22
Italy	[38]	24.3	19.8	n/a	1.0	n/a
USDA	[11]	26.9	17.9	n/a	n/a	n/a
Chicken						
Venezuela	[12]	9.2	6.9	0.20	1.5	n/a
Nigeria	[34]	2.87	n/a	n/a	1.00	n/a
Ireland	[42]	n/a	n/a	n/a	n/a	0.115
UK	[43]	n/a	n/a	n/a	n/a	0.07
USDA	[11]	15.4	8.9	n/a	n/a	n/a
Rabbit						
Spain	[13]	10.9	5.56	0.33	0.78	n/a
Poland	[41]	18	15	0.12	0.49	n/a
USDA	[11]	15.7	15.7	n/a	n/a	n/a
Turkey						
Poland	[41]	12	4.91	0.094	0.31	n/a
USDA	[11]	18.4	8.6	n/a	n/a	n/a
Quail						
USDA	[11]	27.0	45.1	n/a	n/a	n/a
Donkey						
Italy	[27]	36.7	38.0	n/a	n/a	n/a
Ostrich						
South Africa	[14]	20	23	0.6	1	n/a
Poland	[15]	20.2–43.0	23.2–40.2	0.12–0.22	1.03–1.87	0.20–0.41
USDA	[11]	37.6	31.3	n/a	n/a	n/a
Rhea						
Uruguay	[16]	14.65	32.0	0.113	1.41	0.820

(Continued)

Table 26.2 (Continued)

Meat	Reference	Zn	Fe	Mn	Cu	Se
Emu						
Canada	[17]	36	50	0.30	2.30	1.10
USDA	[11]	35.0	45.0	n/a	n/a	n/a
Llama						
Peru	[18]	44.4	32.6	n/a	n/a	n/a
Alpaca						
Peru	[18]	38.7	30.3	n/a	n/a	n/a
Peru	[19]	44.4	26.9	0.15	1.01	n/a
Buffalo						
Iran	[6]	39.0	25.2	n/a	1.5	n/a
USDA	[11]	19.3	16.1	n/a	n/a	n/a
Venezuela	[20]	38.8	24.2	0.17	1.3	n/a
Iguana						
Venezuela	[12]	25.3	19.3	n/a	n/a	n/a

n/a, data unavailable or not reported.

meat used, the amount of non-meat ingredients, and the processing steps carried out in their manufacture [25].

Selenium

Selenium is an essential mineral of fundamental relevance to human biology due to different beneficial effects. Meat and offal are among the richest sources of selenium in the diet. Most of the selenium present in animal tissues is protein-bound and occurs in the form of two seleno-amino acids [49], selenocysteine (SeCys), which is considered to be biologically active, and selenomethionine (SeMet). The selenium content in foods, and particularly meat, is variable and greatly correlated with its concentration in the soil where animals are raised and plants are grown. It is well known that there is great variation in the content of selenium in soils from different regions [16]. In recent years, the levels of selenium in plasma or serum of European population have been decreasing, attributed mainly to a change in the origin of the wheat used for manufacture of bread and cereals products, which was imported predominantly from the USA and Canada (soils with high selenium content) but now comes mainly from Europe (soils with low selenium content). At the same time, many studies [43, 50] show that the levels of selenium in meat are also affected by the selenium content of soils, and therefore in feeds, and by its availability. In fact, the selenium content varies not only among animal species but also within the same animal species depending on its geographic origin. As an example, some authors [50] have found that the selenium concentration of meat produced in American countries is usually higher than that in meat produced in European countries. Notable differences have also been found for the concentrations of selenium between individual meat cuts [47].

Zinc

Zinc is required by the body for maintaining a healthy immune system, building proteins, triggering enzymes and creating DNA [51]. Meat and meat products are the main sources of zinc in the diet. Red meat, especially beef and lamb, is a rich and highly bioavailable source of zinc, while the zinc in plant foods has a lower bioavailability. Although less concentrated in this essential trace mineral, a good alternative to red meat is poultry. The meat cut significantly influences the zinc content. Lombardi *et al.* [38] found that zinc levels determined in five different cuts of beef on the Italian market ranged from 39.4 to 47.5 mg/kg wet tissue. Similar results were reported by Cabrera *et al.* [47], who analysed seven different cuts in Hereford and Braford meat and showed differences of twice or more in zinc concentrations among some cuts, with values ranging between 23 and 72.7 mg/kg wet tissue. Liver is also a rich source of zinc. Tajik *et al.* [6] found that buffalo liver had significantly more zinc than buffalo meat, 58.4 and 39.0 mg/kg,

respectively. These results agree with those reported by other authors for buffalo [52] and cattle and swine [53] liver.

26.3.2 Methods of analysis

Sample treatment for analysis of microminerals is carried out in a similar way to that for macrominerals and quantification is also frequently conducted by applying atomic spectrometry techniques (see section 26.2.2). Next, the particular cases of iron, selenium and zinc analysis are presented.

Iron

Determination of iron in meat and meat products includes the analysis of tFe, and heFe and nheFe forms. The sample pretreatment for determination of tFe is frequently performed by dry ashing [13, 25, 54] or wet digestion under ambient pressure with HNO_3 [19] or $HNO_3/HClO_4$ [7]. Microwave-assisted wet digestion is conducted in the usual way, with HNO_3, HCl or HNO_3/H_2O_2 [38, 46, 55]. Alkaline extraction with TMAH [56] is an alternative procedure that has been successfully applied to the analysis of processed meats. The quantification of tFe is then usually performed with FAAS [13, 25, 47]. ICP-OES [19, 46] and ICP-MS [31, 38, 50] are also used but generally when other macrominerals and/or microminerals are analysed together with iron. In this case, the known interference of Ar_2^+ and ArO^+ species on the determination of the ^{56}Fe isotope is avoided by using a dynamic reaction cell or dynamic collision cell [50, 57]. Recently, a rapid and highly selective spectrophotometric method [58] based on the reduction of Fe(III) to Fe(II) by ascorbic acid, on-line pre-concentration on a mini-column packed with polyurethane foam functionalized with *N*,*N*′-bis(salicylidene)-1,3-propanediamine (SPDA) and reaction with 2,4,6-tri(2′-pyridyl)-1,3,5-triazine (TPTZ) has been proposed for determination of tFe in bovine muscle reference material.

Haem iron is usually determined using an optimization of the Hornsey method for total pigment analysis [59], based on the extraction of the species in the form of hemin with acidified acetone solution and measurement of the absorption at 640 nm [16, 45, 60, 61]. The heFe content is then calculated with the factor 0.0882 µg iron per microgram hematin.

For the most part, the methods used for measurement of nheFe in tissues involve acid precipitation of protein in an aqueous tissue homogenate, with consequent release of iron. After centrifugation, solubilized iron is reduced with an appropriate agent (usually ascorbic or thioglycolic acid), bound to a chromogen and quantified by spectrophotometry [16, 62]. The most frequently used chromogen is ferrozine [63]. The quantification of nheFe can be done by following the described procedure, but finally quantifying iron by FAAS [48]. Generally, after determination of the nheFe form, heFe is quantified by difference between tFe and nheFe, and vice versa.

Finally, near-infrared (NIR) spectroscopy combined with multiple regression equations (MREs) for calibration has been successfully applied to determination of heFe and nheFe in raw muscle meats [63].

Selenium

The determination of total selenium in animal tissues requires that its organic forms be transformed into inorganic selenium. The decomposition will also destroy organic matter that may interfere with the detection method. Dry ashing is not recommended for this purpose due to the volatility of selenium. In the literature, the most popular methods for the individual determination of total selenium in animal tissues involve wet digestion, which is frequently performed with the mixtures $HNO_3/HClO_4$ [37, 64] or $HNO_3/HClO_4/H_2SO_4$ [37, 42, 65]. The main difficulty with wet oxidation is preventing the selenium from being volatilized. The presence of $HClO_4$ increases the oxidizing power of the acid mixture and shortens the digestion time. Concentrated HCl is usually avoided, since selenium forms volatile chloride adducts.

Hydride generation coupled to atomic absorption spectrometry (HG-AAS) is the technique most commonly used for the determination of total selenium content in meat and meat products [37, 42, 66], due to its minimal matrix interferences and relatively inexpensive instrumentation requirements. As Se(VI) is not quantitatively recovered in hydride generation, the application of this technique requires the previous reduction of Se(VI) to Se(IV), which is accomplished by heating the digested sample in HCl solution. Finally, the Se(IV) hydride is generated by treatment with $NaBH_4$. Total selenium content is also frequently determined by measuring the fluorescence of the complex formed with diaminonaphthalene (DAN) [37, 64], with previous extraction of the latter in cyclohexane. In this case, the use of H_2SO_4 is avoided in the digestion of sample since

this causes the precipitation of the DAN reagent. Tinggi *et al*. [37] compared the use of HG-AAS and spectrofluorimetry for determination of selenium in foodstuffs and found that spectrofluorimetry provided better detection limits (0.001 μg/g for spectrofluorimetry vs. 0.033 μg/g for HG-AAS). Although infrequent, hydride generation coupled to atomic fluorescence spectrometry (HG-AFS) has also been applied [65] for determination of pork, chicken and beef meat selected from the Greek market. The application of graphite furnace atomic absorption spectrometry (GF-AAS) for the determination of selenium in animal tissues is prone to matrix interferences, particularly from phosphates, and volatilization losses during atomization which require the use of matrix modifiers. Copper and magnesium nitrate salts are usually added for this purpose [47, 61]. ICP-MS [9, 41, 67] and ICP-OES [55] have been proposed for determination of the total selenium content together with other minerals. In this case, digestion of the sample is carried out with HCl, HNO_3 or HNO_3/H_2O_2, and frequently microwave-assisted.

Dietary selenium in the form of its amino acids SeCys and SeMet is absorbed to a greater extent than inorganic species. Therefore, the determination of these bound selenium compounds is necessary in order to understand its role in the quality, stability and nutritional value of meat. Bierla *et al*. [68] determined SeCys and SeMet by using high-performance liquid chromatography (HPLC) with ICP-MS detection (HPLC-ICP-MS). The sample treatment consisted of protein extraction with urea under reducing conditions (dithiothreitol), derivatization of SeCys and SeMe by carbamidomethylation with iodoacetamide (IAM) and quantitative proteolysis (protease/lipase). Before the chromatographic analysis, the mixture of both derivatized seleno-amino acids was purified by size-exclusion liquid chromatography (SEC). The coupling of SEC to ICP-MS also allows estimation of the molecular size and amounts of selenium-containing proteins and other compounds [69] and has been proposed for determination of soluble selenium compounds in two muscles from each of seven animal species (chicken, turkey, duck, ostrich, lamb, cattle and pig).

Zinc

As for analysis of iron, dry ashing [47, 61] and wet digestion with HNO_3, HCl or $HNO_3/HClO_4$ [7, 67] are the procedures frequently used in the decomposition of organic matter for determining zinc in meat. Digestion with HNO_3 or HNO_3/H_2O_2 microwave-assisted [17, 27, 38] is also a very popular pretreatment. Zinc is usually determined in meat together with other minerals of interest. Most of the methods reported in the literature are based on the use of FAAS [47, 54, 61], ICP-OES and ICP-MS [17, 27, 70].

26.4 Toxic minerals

Cadmium, lead, arsenic and mercury are the most frequently monitored toxic minerals in meat and, to a lesser extent, tin and aluminium. The main source of contamination by these metals in animals is water and feeding. Widespread contamination has frequently been reported in regions with long histories of smelting and mining, where high concentrations of heavy metals contaminate soils, vegetation and water [71]. Waste incineration and the disposal of sewage sludge onto land is another major source of contamination of soils. Contamination of meat with toxic minerals can also take place during its handling and processing, contact between meat and processing equipment and storage and packaging containers being an important source of metals. Concern over the contamination of game meat with toxic minerals is mainly related to metals such as cadmium, lead and arsenic. The presence of heavy metals in meat can become a serious problem because of their toxicity, bioaccumulation and biological magnification along the food chains, since when these elements occur above the permissible levels in the animals, they have harmful effects not only in the animals but also on the health of the consumer. It is well known that heavy metals accumulate in animal tissues, mainly liver and kidney, and its concentration in these organs can become very high [72]. The effects of this accumulation on health will depend on the chemical form of the element administered to the animal, its toxicity and the quantity and frequency of the meat consumption.

26.4.1 European Union and international regulatory/advisory aspects

Toxic minerals are routinely monitored in meat both to accumulate background levels for assessing long-term exposure and to enforce regulatory standards. The latter are established by national governments and supranational organizations such as the European Union (EU);

Table 26.3 Maximum levels (mg/kg) for contaminant minerals in meat and meat products.

Commodity	Cd	Pb	Sn
European Union:			
Commission Regulation (EC) No. 1881/2006*			
Commission Regulation (EC) No. 629/2008†			
Meat of bovine animals, sheep, pig and poultry	0.050	0.10	
Horsemeat, excluding offal	0.20		
Offal of bovine animals, sheep, pig and poultry		0.50	
Liver of bovine animals, sheep, pig, poultry and horse	0.50		
Kidney of bovine animals, sheep, pig, poultry and horse	1.0		
Codex Alimentarius Commission:			
Codex Stan 193-1995‡			
Meat of cattle, sheep, pig and poultry		0.1	
Edible offal of cattle, pig and poultry		0.5	
Products in tinplate containers (cooked cured chopped meat, cooked cured ham, cooked cured pork shoulder, corned beef, luncheon beef)			200
Products in containers other than tinplate (cooked cured chopped meat, cooked cured ham, cooked cured pork shoulder, corned beef, luncheon meat)			50

*European Union [74].
†European Union [75].
‡Codex Alimentarius Commission [73].

in addition, international organizations set harmonized standards to facilitate world trade and protect the consumer, and are taken into consideration in the development or adaptation of national food safety legislation. The latter applies to standards developed by the Codex Alimentarius Commission (CAC) (http://www.codexalimentarius.org/). CAC has established maximum levels of lead and tin [73] which are recommended to be applied to meat commodities moving in international trade (Table 26.3). The Codex maximum level (ML) for a contaminant is the maximum concentration of that substance recommended by the CAC to be legally permitted in that commodity. With the aim of reducing the presence of contaminants in foodstuffs, the EU has also established MLs for the heavy metals cadmium and lead in meat commodities in Commission Regulation (EC) No. 1881/2006 [74], amended by Commission Regulation (EC) No. 629/2008 (Table 26.3) [75]. Despite the well-known toxic effects of arsenic, current European legislation has no MLs for total arsenic in foodstuffs. One reason is that the different arsenic species exhibit very different toxicity, the inorganic forms being the most toxic species in which the metal can appear. In addition, seafood, which is the main foodstuff contributing to dietary arsenic exposure, contains mainly organic arsenic compounds.

Toxic minerals are assessed for safety by comparing dietary intake estimates with recommended safe levels. For the latter, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa) has established the provisional tolerable weekly intake (PTWI) and provisional tolerable monthly intake (PTMI) for toxic minerals from food. PTWI and PTMI are end points used for food contaminants such as heavy metals with cumulative properties and their values represent permissible human weekly and monthly exposure to those contaminants unavoidably associated with the consumption of otherwise wholesome and nutritious foods, respectively. The PTWI for inorganic arsenic is 0.015 mg/kg body weight, equivalent to 125 μg/day for a 60-kg person [76]. Nevertheless, recently the Panel on Contaminants in the Food Chain (CONTAM Panel) of the European Food Safety Authority (EFSA; http://www.efsa.europa.eu/) has reviewed this PTWI value [77] and concluded that it is no longer appropriate, since more recent data showing effects at lower doses of inorganic arsenic should be considered. For cadmium, a PTWI of 7 μg/kg body weight was established in 1988 [78]. In 2010, the previous evaluation on cadmium was reviewed and a PTMI of 25 μg/kg body weight was established, which corresponds to 5.8 μg/kg body weight as weekly intake.

The CONTAM Panel recommended in 2011 [79] the reduction of the PTWI to a tolerable weekly intake (TWI) of 2.5 μg/kg body weight in order to ensure a high level of protection of consumers. With respect to lead, in 1986 a PTWI of 25 μg/kg body weight was established by JECFA [80] and endorsed by the Scientific Committee for Food (SCF) of the European Commission in 1990. In 2010 the CONTAM Panel concluded that the establishment of a PTWI was not appropriate, since there was no evidence for a threshold of a number of critical end points including adult nephrotoxicity and developmental neurotoxicity [81]. The PTWIs for inorganic mercury and methylmercury were set by JECFA at 4 [82] and 1.6 μg/kg [83] body weight, respectively. Considering the new developments regarding the toxicity of these mercury species, these values were reviewed by the CONTAM Panel, which established a TWI of 4 μg/kg body weight for inorganic mercury and a TWI of 1.3 μg/kg body weight for methylmercury [84], both expressed as mercury.

26.4.2 Content and distribution

Arsenic

Arsenic occurs naturally in both inorganic and organic forms and also in different valence states (+III, +V, 0, –III). It is well known that inorganic arsenic compounds, arsenite [As(III)] and arsenate [As(V)], are significantly more toxic than organic arsenic compounds such as the methylated species monomethylarsonic acid (MMA) and dimethyarsinic acid (DMA). In turn the trivalent forms of arsenic are more toxic than the pentavalent forms and the latter are considered toxic only after metabolic conversion to the trivalent form of arsenic. Besides, compounds such as arsenobetaine (AB), arsenocholine (AC) and other arsenosugars are considered non-toxic. Arsenic accumulates in the human and animals body and is stored mainly in the liver, kidney, skin and hair [85]. Data on arsenic occurrence in food show that most of the arsenic in the diet comes from fish. In meat, DMA is the main species found in most studies, together with AB and minor amounts of MMA. In poultry meat, the presence of high concentrations of nitarsone, a phenylarsonic acid used in poultry production as a feed additive to increase weight gain, improve feed efficiency and prevent blackhead disease, has also been reported.

In 2011, JECFA reported data provided by several countries on total arsenic contents in food commodities [82]. Table 26.4 shows the range of total arsenic concentrations (minimum to maximum) reported for meat and meat products (data about concentrations of inorganic arsenic were not provided for this food category). Some meat products, together with fish and fish products, were one of the food categories with the highest total arsenic concentrations (0.060–66 mg/kg). However, although arsenic was detected/quantified in all the samples of fish and fish products analysed (n=1818), the percentage of non-detectable/non-quantifiable results in meat and meat products was 74%. On the other hand, dietary exposure estimates for inorganic arsenic (all the food commodities) was less than 1 μg/kg body weight per day for adults and less than 2 μg/kg body weight per day for infants and children, in all the cases below the PTWI [76]. EFSA also issued a call for data on arsenic in foodstuffs in 2008 [77]. Table 26.5 shows the ranges of total arsenic concentrations (mean lower bound to mean upper bound) found in the category 'meat, meat products and offal'. The mean percentage of results below the limit of detection (LOD) or the limit of quantification (LOQ) was 70%. Mean total and inorganic arsenic dietary exposure (all the food commodities) ranged from 0.45 to 4.6 μg/kg body weight per day and from 0.13 to 0.56 μg/kg body weight per day, respectively, concentrations very much lower than the PTWI [76]. Therefore, it can be concluded that meat and meat products do not significantly contribute to the overall total arsenic exposure, and therefore their contribution to inorganic arsenic exposure could be considered as negligible.

Cadmium

Foodstuffs account for most of the human exposure to cadmium for the non-smoking general population, except in the vicinity of cadmium-emitting industries. Although cadmium absorption after dietary exposure in humans is relatively low (3–5%), it is efficiently retained in the kidney and liver in the human body, with a very long biological half-life of 10–30 years [86]. Absorption and tissue accumulation of dietary cadmium are mainly influenced by gender, age, dose and nutritional status as well as by the dietary intake of other mineral elements (e.g. Ca, Fe and Zn) [87].

Data on cadmium contents in meat commodities obtained from JECFA [88] are shown in Table 26.4. As can be seen, the highest cadmium concentrations are found in offal. Meat was found to be among the main

Table 26.4 Levels of arsenic, cadmium and lead in meat and meat products reported by FAO/WHO.

Commodity	*n*	Range of national mean concentrations (mg/kg)
As (total)*		
Meat	4,977	0.004–0.78
Offal	2,074	0.009–0.45
Meat products	50	0.003–3.25
Cd†		
Meat and poultry muscle, not further specified	20,154	0.008–0.04
Meat and poultry offal, not further specified	16,049	0.1
Meat muscle	1,715	0.001–0.003
Meat offal	1,406	0.03–0.5
Poultry muscle	2,500	0.0002–0.01
Poultry offal	1,224	0.006-0.5
Pb†		
Muscle meat, excluding poultry	1,817	0.0001–0.013
Meat not included elsewhere	131	0.22–0.25
Organ meats except kidney	102	0.10–018
Muscle meat and poultry combined	40,313	0.004–0.25
Muscle minced	69	0.001
Kidney	537	0.013–0.14
Muscle poultry	1,589	0.003–0.021
Offal	73	0.006–0.042

n, number of samples.

*Joint FAO/WHO Expert Committee on Food Additives [82].

†Joint FAO/WHO Expert Committee on Food Additives [88].

dietary sources of cadmium in several regions within China. In Europe, animal offal was among the main dietary sources of cadmium. Mean cadmium exposure for adults ranged from 0.07 to 0.4 μg/kg body weight per day. In 2007, EFSA issued a call for information on cadmium concentrations in foodstuffs and data received covered the period 2003–2011 [89]. As can be seen in Table 26.5, the highest cadmium concentrations occurred in horse meat and edible offal. In fact, the highest cadmium concentration in all the foodstuffs analysed in this study was found in horse kidney. Nevertheless, it should be taken into account that many of the reported results for the 'meat and edible offal category' were sampled under Directive 96/23 [90] and might include a high proportion of samples targeting potential problem areas. In addition, although cadmium may be found at high levels in offal and horse meat, these commodities are generally eaten in relatively small amounts and exposure to cadmium from these sources is therefore small for most segments of the population. The cadmium dietary exposure for the European population was estimated at about 2.04 μg/kg body weight per week, which is within the TWI of 2.5 μg/kg body weight recommended by EFSA, the contribution of meat and edible offal being 7.7%. From these data it is obvious that there is a need to reduce exposure to cadmium at the population level because of the limited safety margin.

Levels of cadmium are higher in the kidneys and liver of animals living near industrial areas or other sources of pollution, these organs being used as biomarkers for ecological safety. However, although one may expect that domestic animals are exposed to higher levels of cadmium in the feed than game animals, several authors have reported that the level of cadmium in liver and kidney of big game animals is often so high that their consumption should be severely restricted [33, 91].

Lead

Lead occurs in the environment in inorganic and organic forms, the inorganic species prevailing. Lead can accumulate in the body, primarily in the skeleton, and from the latter is released gradually into the bloodstream [92].

Table 26.5 Levels of arsenic, cadmium, lead and mercury in 'meat, meat products and offal' reported by EFSA.

Commodity	*n*	Range of national mean concentrations (µg/kg)
As (total)*		
Meat, meat product and substitutes		5.0–14.5
Bovine, sheep and goat meat	2,102	3.9–13.7
Pig meat	2,013	3.7–12.8
Poultry meat	2,099	5.0–13.7
Game meat	1,451	7.5–17.4
Other meat	504	7.7–14.1
Processed meat products	1,721	5.1–16.2
Edible offal and offal products		4.9–15.4
Liver bovine, sheep, pig, poultry, horse	4,256	3.6–12.9
Kidney bovine, sheep, pig, poultry, horse	3,964	6.0–17.7
Liver and kidney of game animals	284	8.5–20.6
Other offal products	252	6.1–15.6
Meat-based preparations	378	12.1–18.5
Cd†		
Meat and edible offal		
Livestock meat	15,462	7.60–15.0
Horse meat	1,402	359–366
Poultry	4,821	3.21–12.8
Game mammals	2,479	3.04–5.69
Game birds	680	1.78–7.63
Mixed meat	184	1.23–4.25
Edible offal	18,296	315–319
Preserved meat	416	2.61–12.1
Sausages	2,663	6.19–25.6
Meat specialties	88	2.64–6.72
Pâtés and terrines	310	4.86–17.7
Meat imitates	311	22.2–23.6
Pb‡		
Livestock meat		
Beef	7,432	13–21
Veal	102	2–11
Pork/piglet meat	6,755	7–16
Mutton/lamb meat	1,989	12–21
Goat/kid meat	100	2–12
Horse, asses, mules or hinnies meat	1,074	5–14
Rabbit meat	517	17–24
Poultry		
Chicken meat	2,543	7–17
Turkey meat	894	5–16
Duck meat	595	32–41
Goose meat	274	9–18
Pigeon meat	46	15–21
Guinea fowl meat	23	4–12
Ostrich meat	72	2–10
Game mammals		
Boar meat (wild pig)	966	1140–1146
Venison meat	733	47–49
Elk meat	47	13–17
Reindeer meat	490	55–66
Hare meat	149	149–162

Table 26.5 (Continued)

Commodity	*n*	Range of national mean concentrations (µg/kg)
Game birds		
Pheasant meat	426	342–347
Quail meat	80	5–15
Mixed meat		
Mixed beef and pork meat	23	12–13
Mixed poultry meat	17	1–5
Edible offal, farmed animals		
Cattle liver	4,359	33–42
Calf liver	72	16–30
Pig liver	4,322	14–28
Sheep/lamb liver	915	43–51
Chicken liver	2,089	11–25
Turkey liver	810	6–18
Duck liver	160	18–28
Goose liver	17	19–27
Cattle kidney	1,929	129–136
Calf kidney	13	20–23
Pig kidney	4,355	17–26
Sheep/lamb kidney	172	46–66
Tongue (cattle, calf, sheep, lamb, pig)	2	28
Heart (cattle, calf, sheep, lamb, pig)	12	2–6
Giblets (cattle, calf, sheep, lamb, pig)	76	2–8
Preserved meat		
Ham, pork	49	16–27
Beef, dried	3	10–15
Bacon	63	7–15
Luncheon meat	54	39–40
Sausages		
Fresh and lightly cooked sausage	97	8–16
Uncooked smoked sausage	51	6–14
Cooked sausage	182	7–29
Cooked smoked sausage	164	5–14
Semi-dry sausage	73	27–28
Dry sausage	412	13–24
Meat specialties		
Head cheese (brawn)	26	9–10
Pastes, pâtés and terrines		
Meat paste	247	28–29
Meat imitates		
Textured soy protein	16	21–22
Hg§		
Meat and meat products	10,304	1.9–3.5

n, number of samples.
*European Food Safety Authority [77].
†European Food Safety Authority [89].
‡European Food Safety Authority [93].
§European Food Safety Authority [84].

Food, in which only inorganic compounds of lead have been found, is the major source of human exposure to this metal for the general population. Over the last few decades, lead levels in foodstuffs have decreased significantly owing to control measures taken to reduce emissions, especially from petrol.

Levels of lead in meat and meat products reported by JECFA are shown in Table 26.4 [88]. In this report, meat and especially offal were among the food categories with the highest frequency of detectable lead, with concentrations ranging from 0.1 to 250 μg/kg. The Committee assessed the dietary exposure to lead for the general population and a range of 0.02–3 μg/kg body weight per day was found. The contribution of meat commodities to overall dietary exposure was 8%. EFSA has also been collecting data about the occurrence of lead in food through a specific call [93]. A total of 51,455 data were reported for the 'meat and edible offal' food category, covering the period 2003–2011 (Table 26.5). Mean lead concentrations were 3–1143 μg/kg. The maximum lead concentrations were found in wild boar meat and pheasant (game meat). These results agree with several studies that have reported the retention of ammunition residues in the tissues of hunter-killed animals and, as a consequence, high concentrations of lead in subsistence hunter populations [94]. In these cases, the risk of lead intake can be reduced by accurate trimming of the meat around wounds and bullet pathways [95]. Because of the high lead concentrations in game meat, only a few consumers declared that they ate these commodities. The lead dietary exposure was estimated at 0.68 μg/kg body weight per day in the European population. The contribution of meat and edible offal to the lead dietary exposure ranged from 1.9% (infants) to 6.1% (adolescents).

Mercury

Mercury can be found in several chemical forms: elemental mercury, mercurous ion (Hg_2^{2+}), mercuric ion (Hg^{2+}) and organic mercury (i.e. methylmercury, ethylmercury or phenylmercury). Mercury is present in fish and seafood products largely as methylmercury. Nevertheless, methylmercury and also total mercury levels in terrestrial animals are usually very low, the latter containing mostly inorganic mercury forms. Occasionally, the use of fishmeal as terrestrial animal feed can lead to higher methylmercury levels. Inorganic mercury is poorly absorbed and considerably less toxic than methylmercury.

According to the conclusions of the 72nd meeting of JECFA [96], while total mercury concentrations found in fish and other seafood are up to 11,400 μg/kg, the concentrations observed in other foods are generally low (0.1–50 μg/kg), with about 80% of the samples containing concentrations below the LOQ. No data about inorganic mercury were supplied. On the other hand, in the last report of EFSA about the occurrence of mercury in food [84], a total of 10,304 samples of 'meat and meat products', covering the period 2002–2011, were analysed for total mercury. The mean total mercury concentration was in the range 1.9–3.5 μg/kg (Table 26.5). The percentage of samples in which mercury was not detected or not quantified was below 12% and about 56%, respectively. Although no data about inorganic mercury content were provided, as considered by the CONTAM Panel for food other than 'fish and other seafood', mercury is presumed to be present as inorganic mercury. The mean dietary exposure to inorganic mercury varied from 0.13 to 2.16 μg/kg body weight per week. The contribution of 'meat and meat products' to the dietary exposure to inorganic mercury was estimated to be in the range 2.3% (toddlers) to 13 % (adults).

26.4.3 Methods of analysis

Provisions for methods of sampling and analysis for the official control of lead, cadmium and mercury in foodstuffs are laid down in Commission Regulation (EC) No. 333/2007 [97]. Laboratories will select any validated method of analysis with LOD less than one-tenth of the corresponding ML in Regulation (EC) No. 1881/2006, except when the ML for lead is less than 100 μg/kg (for the latter, LOD will be less than one-fifth of the ML).

Arsenic

As stated, arsenic is present in meat predominantly in organic form. Although most studies have reported only total arsenic concentrations, speciation of arsenic compounds has become increasingly important due to the differential toxicity of arsenic compounds. Most of the studies on arsenic speciation in animal tissues carried out in recent years have been targeted to the characterization of organic arsenic species profiles in foodstuffs and currently there is a need for validated methods for selective extraction and determination of inorganic arsenic species and similarly for certified materials for inorganic species in foods.

Sample treatment for the determination of total arsenic in meat is carried out by dry ashing [98] or wet digestion. Dry ashing is sometimes performed in the presence of a solid oxidant, $MgO/Mg(NO_3)_2$, to avoid arsenic losses by volatilization [100]. Wet digestion is usually performed with HNO_3 or HNO_3/H_2O_2 and microwave-assisted [99, 101]. Other acids also used include $HClO_4/H_2SO_4$ [102] and H_2SO_4/H_2O_2 [103]. Some authors propose a previous pre-digestion step with HNO_3 overnight before wet digestion [102]. For arsenic speciation, the extraction procedure will have to be performed under mild conditions in order to avoid the interconversion of species. Most of the methods reported in the literature for arsenic speciation are focused on tissue from marine animals, and little attention has been paid to other types of samples such as meat. The extraction of arsenic species from meat samples is usually carried out with H_2O, CH_3OH/H_2O, CH_3OH/Cl_3CH or H_3PO_4 [102, 104]. As an example, Pizarro *et al*. [102] proposed a procedure for extraction of As(III), As(V), MMA and DMA from a chicken meat powder candidate reference material using a CH_3OH/H_2O (1 : 1, v/v) mixture. No species transformation was detected during the extraction procedure.

The determination of total arsenic content is mostly performed by HG-AAS [100, 101, 105] and ICP-MS [99, 106]. Recently, two methods based on HG-AAS have been accepted as European standards for the determination of arsenic in foodstuffs [107, 108]. ICP-MS is so sensitive that arsenic can be easily measured without a hydride generation step. The well-known spectral interference of $^{40}Ar^{35}Cl$ on ^{75}As is usually resolved by using a collision cell [106]. Other techniques, such as HG-AFS [102] and GF-AAS [101, 109] have also been used.

For arsenic speciation in meat samples, several chromatographic techniques have been proposed such as HPLC [102], capillary electrophoresis (CE) [110] coupled to ICP-MS and HPLC coupled to HG-AFS [104]. The speciation of inorganic and organic arsenic forms is also possible with non-chromatographic separations, by carefully controlling the experimental conditions of the hydride generation, carrying out off-line/on-line sample pretreatment and using cryogenic trapping of hydrides which show different boiling points [111]. As an example, Schoof *et al*. [112] determined As(III), As(V), DMA and MMA in meat and poultry by HG-AAS. The procedure consisted of a first digestion with HCl, generation of the volatile hydride from As(III) at pH 6 and trapping of the arsine on a cryogenic trap. Since HCl dissolves the arsenic compounds without changing the oxidative states of As(III) and As(V) or decomposing MMA and DMA and because As(III) is the only species that generates a volatile hydride at this pH, only As(III) was determined. Quantification of the total inorganic arsenic [As(III) plus As(V)], MMA and DMA was performed in a similar way to that of As(III), but in this case the three corresponding arsines (AsH_3, CH_3AsH_2 and $(CH_3)_2AsH$) were generated at pH 1. The latter were retained on a cryogenic column and quantified by AAS by gradually increasing the temperature, due to their different column retention times. The concentration of As(V) was finally calculated by difference between total inorganic arsenic and As(III).

Cadmium and lead

Analytical methods for the determination of cadmium and lead in foods are well established. The sample preparation procedure used most frequently for the determination of cadmium and lead in meat and meat products is acid digestion with HNO_3 or HNO_3/H_2O_2, usually microwave-assisted [71, 113, 114]. Other acids frequently used include $HNO_3/HClO_4$ [115, 116] and, to a lesser extent, H_2SO_4/H_2O_2 [103]. Dry ashing has also been used [35, 117]. The most frequently used technique in the analysis of both metals in meat and meat products is GF-AAS [113, 118, 119], although many applications based on ICP-MS [39, 120, 121] and AAS [35, 115–117] have also been reported. The use of GF-AAS generally provides lower detection limits (up to 10-fold) than AAS. In the last decade, many technical improvements have been introduced into GF-AAS, such as the design of the atomizer, background correction systems and improvement in the light source and detector, allowing the determination of cadmium and lead in food at the low microgram per kilogram level. Matrix-modifiers have to be used in order to overcome matrix interferences. Usually $Mg(NO_3)_2$, $NH_4H_2PO_4$ or $Mg(NO_3)_2/NH_4H_2PO_4$ for cadmium and H_3PO_4, $Pd(NO_3)_2$ or $PdCl_2$ for lead are used. ICP-MS also provides lower detection limits than AAS but, in addition, it is able to quantify simultaneously cadmium and lead. Sensitivity of ICP-MS can be improved by the use of a sector field (SF-ICP-MS), which shows high sensitivity, large dynamic range and high sample throughput. Forte and Bocca [120] quantified cadmium and lead in offal of calf and pig by SF-ICP-MS, reporting detection limits as low as 3.5 ng/g for cadmium and 2.0 ng/g for lead.

Mercury

Extraction of the mercury species requires aggressive treatment, such as acid digestion, distillation or alkaline extraction, with the option of applying ultrasonic or microwave energy to assist in the procedure. According to some authors, methylmercury may be decomposed in some food matrices with repeated freezing and unfreezing, but relatively little is known about the effect of storage on the stability of methylmercury in food samples [122].

Following acidic digestion of samples with HNO_3/H_2O_2, cold vapour AAS (CV-AAS) is the most widely used technique for determination of total mercury in meat and meat products, because it is sensitive, well established and the required instrumentation is common to analytical laboratories worldwide [98, 123, 124]. The coupling of CV with AFS (CV-AFS) allows enhancement of sensitivity and selectivity and has been widely applied to analysis of total mercury [125, 126]. Both techniques share the same advantage, namely that mercury is separated from the potentially interfering sample matrix. Nevertheless, it is necessary to take special precautions to avoid the interference caused by the nitrous vapours generated when nitric acid is used for digestion of the sample. This interference is usually overcome by resting the solution for 12 hours.

Another approach, which allows measurement of total mercury directly on animal tissues, has recently become available in automated commercial instrumentation. These systems, such as the Advanced Mercury Analyzer AMA-254, integrate combustion of the sample in a stream of oxygen, pre-concentration of mercury by amalgamation on gold, and AAS determination. The whole analysis is performed automatically without the need for preliminary dissolution and/or decomposition of the sample, making it much more rapid than conventional techniques. Furthermore, because the use of liquid reagents is eliminated, these systems reduce the potential for contamination and eliminate reagent waste [127]. As an example, the AMA-254 Analyzer has been applied to the analysis of mercury in muscle, kidney and liver tissues of pigs in the Czech Republic, achieving a sensitivity for mercury of 1 μg/kg and reproducibility of 1.5% [128], and to the analysis of mercury in the organs of red deer [129]. Recently, the Direct Mercury Analyzer DMA-80 has also proven to be appropriate for the direct analysis of total mercury in meat and offal, achieving a detection limit of 0.3 ng/g dry weight [130].

ICP-MS achieves better sensitivity than CV-AAS and CV-AFS in the analysis of total mercury, but it requires a more expensive and sophisticated instrumentation; in addition, it presents some problems such as the adhesion of mercury to the walls of the sample introduction system [10, 131]. Consequently, it has been applied in a minor way to the analysis of mercury in meat. Other alternative techniques for the analysis of mercury are CV-ICP-AES [132] and CV-ICP-MS [133].

Determination of the speciation of mercury in meat samples is not usual and it is generally assumed that all the mercury present in the samples is inorganic mercury. Therefore, there is a current need for fully validated, standardized methods for determination of methylmercury and inorganic mercury in meat. One of the few methods reported for mercury speciation in meat was proposed by Cheng *et al*. [130]. These authors analysed methylmercury in meat and offal of beef and pork with a method based on ethylation, purge-and-trap collection and gas chromatography separation with CV-AFS detection. The samples were previously subjected to alkaline digestion with a 25% KOH/CH_3OH solution. The extraction of methylmercury in KOH/CH_3OH, first reported by Bloom [133], is the most efficient method for extraction of this mercury species from biological samples since it appears to be more stable than in acid media [122].

Abbreviations

AAS: Atomic absorption spectrometry
AB: Arsenobetaine
AC: Arsenocholine
CAC: Codex Alimentarius Commission
CE: Capillary electrophoresis
CONTAM Panel: Contaminants in the Food Chain Panel
CV: Cold vapour
CV-AAS: Cold vapour atomic absorption spectrometry
CV-AFS: Cold vapour atomic fluorescence spectrometry
CV-ICP-OES: Cold vapour inductively coupled plasma optical emission spectrometry
CV-ICP-MS: Cold vapour inductively coupled plasma mass spectrometry
DAN: Diaminonaphthalene
DMA: Dimethyarsinic acid
DRI: Daily recommended intake
EFSA: European Food Safety Authority

FAAS: Flame atomic absorption spectrometry
GF-AAS: Graphite furnace atomic absorption spectrometry
HG-AAS: Hydride generation atomic absorption spectrometry
HG-AFS: Hydride generation atomic fluorescence spectrometry
HPLC: High-performance liquid chromatography
IAM: Iodoacetamide
ICP-MS: Inductively coupled plasma mass spectrometry
ICP-OES: Inductively coupled plasma optical emission spectrometry
JECFA: Joint (FAO/WHO) Expert Committee on Food Additives
LOD: Limit of detection
LOQ: Limit of quantification
ML: Maximum level
MMA: Monomethylarsonic acid
MREs: Multiple regression equations
NAA: Neutron activation analysis
NIR: Near-infrared spectroscopy
PTMI: Provisional tolerable monthly intake
PTWI: Provisional tolerable weekly intake
SCF: Scientific Committee for Food
SEC: Size-exclusion liquid chromatography
SeCys: Selenocysteine
SeMet: Selenomethionine
SF-ICP-MS: Sector field inductively coupled plasma mass spectrometry
SPDA: *N*,*N*′-Bis(salicylidene)-1,3-propanediamine
TMAH: Tetramethylammonium hydroxide
TPTZ: 2,4,6-Tri(2′-pyridyl)-1,3,5-triazine
TWI: Tolerable weekly intake

References

1 European Union. Regulation (EC) No. 853/2004 of 29 April 2004 laying down specific hygiene rules for food of animal origin. Available at http://www.dardni.gov.uk/reg-ec-853-as-amended-consolidated-1-7-12.pdf.
2 Heinz G, Hautzinger P. *Meat Processing Technology for Small- to Medium-scale Producers*. FAO, Regional Office for Asia and the Pacific (RAP), Bangkok, 2007.
3 Nohr D, Biesalski HK. 'Mealthy' food: meat as a healthy and valuable source of micronutrients. *Animal* 2007;**1**: 309–316.
4 Corpet DE. Red meat and colon cancer: should we become vegetarians, or can we make meat safer? *Meat Sci* 2011;**89**: 310–316.
5 World Cancer Research Fund and American Institute for Cancer Research. *Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective*. World Cancer Research Fund International, Washington, DC, 2007.
6 Tajik H, Rezaei SA, Alamouti MRP, Moradi M, Dalir-Naghadeh B. Mineral contents of muscle (longissimus dorsi thoracis) and liver in river buffalo (*Bubalus bubalis*). *J Muscle Foods* 2010;**21**:459–473.
7 Florek M, Litwińczuk Z, Skałecki P, Kędzierska-Matysek M, Grodzicki T. Chemical composition and inherent properties of offal from calves maintained under two production systems. *Meat Sci* 2012;**90**:402–409.
8 Williams P. Nutritional composition of red meat. *Nutr Diet* 2007;**64**:S113–S119.
9 Reykdal O, Rabieh S, Steingrimsdottir L, Gunnlaugsdottir H. Minerals and trace elements in Icelandic dairy products and meat. *J Food Compos Anal* 2011;**24**:980–986.
10 Sales J. The rhea, a ratite native to South America. *Avian Poult Biol Rev* 2006;**17**:105–124.
11 US Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 26. Available at http://www.ars.usda.gov/ba/bhnrc/ndl (accessed 12 September 2013).
12 De Moreno LA, Vidal A, Huerta-Sánchez D, Navas Y, Uzcategui-Bracho S, Huerta-Leiden N. Análisis comparativo proximal y de minerales entre carnes de iguana, pollo y res. *Arch Latinoam Nutr* 2000;**50**:409–415.
13 Hermida M, Gonzalez M, Miranda M, Rodríguez-Otero JL. Mineral analysis in rabbit meat from Galicia (NW Spain). *Meat Sci* 2006;**73**:635–639.
14 Sales J, Hayes JP. Proximate, amino acid and mineral composition of ostrich meat. *Food Chem* 1996;**56**:167–170.
15 Majewska D, Jakubowska M, Ligocki M *et al*. Physicochemical characteristics, proximate analysis and mineral composition of ostrich meat as influenced by muscle. *Food Chem* 2009; **117**:207–211.
16 Ramos A, Cabrera MC, del Puerto M, Saadoun A. Minerals, haem and non-haem iron contents of rhea meat. *Meat Sci* 2009;**81**:116–119.
17 Pegg RB, Amarowicz R, Code WE. Nutritional characteristics of emu (*Dromaius novaehollandiae*) meat and its value-added products. *Food Chem* 2006;**97**:193–202.
18 Polidori P, Antonini M, Torres D, Beghelli D, Renieri C. Tenderness evaluation and mineral levels of llama (*Lama glama*) and alpaca (*Lama pacos*) meat. *Meat Sci* 2007;**77**: 599–601.
19 Salvá BK, Zumalacárregui JM, Figueira AC, Osorio MT, Mateo J. Nutrient composition and technological quality of meat from alpacas reared in Peru. *Meat Sci* 2009;**82**: 450–455.
20 Giuffrida-Mendoza M, Arenas De Moreno L, Uzcátegui-Bracho S, Rincón-Villalobos G, Huerta-Leidenz N. Mineral content of longissimus dorsi thoracis from water buffalo and Zebu-influenced cattle at four comparative ages. *Meat Sci* 2007;**75**:487–493.

21 Littledike ET, Wittum TE, Jenkins TG. Effect of breed, intake, and carcass composition on the status of several macro and trace minerals of adult beef cattle. *J Anim Sci* 1995;**73**:2113–2119.
22 Kadim IT, Mahgoub O, Al-Marzooqi W, Al-Zadjali S, Annamalai K, Mansour MH. Effects of age on composition and quality of muscle longissimus thoracis of the Omani Arabian camel (*Camelus dromedarius*). *Meat Sci* 2006;**73**: 619–625.
23 Gerber N, Scheeder MRL, Wenk C. The influence of cooking and fat trimming on the actual nutrient intake from meat. *Meat Sci* 2009;**81**:148–154.
24 Zanardi E, Ghidini S, Conter M, Ianieri A. Mineral composition of Italian salami and effect of NaCl partial replacement on compositional, physico-chemical and sensory parameters. *Meat Sci* 2011;**86**:742–747.
25 Jiménez-Colmenero F, Pintado T, Cofrades S, Ruiz-Capillas C, Bastida S. Production variations of nutritional composition of commercial meat products. *Food Res Int* 2010;**43**: 2378–2384.
26 Demirbaş A. Proximate and heavy metal composition in chicken meat and tissues. *Food Chem* 1999;**67**:27–31.
27 Polidori P, Vincenzetti S, Cavallucci C, Beghelli D. Quality of donkey meat and carcass characteristics. *Meat Sci* 2008;**80**: 1222–1224.
28 Da Silva CS, Nunes AM, Oreste EQ, Acunha TS, Vieira MA, Ribeiro AS. Evaluation of sample preparation methods based on alkaline and acid solubilization for the determination of Na and K in meat samples by atomic spectrometric techniques. *J Braz Chem Soc* 2012;**23**:1623–1629.
29 Sulieman AME, Abdelhai MH, Babiker ERB. Some chemical and microbiological characteristics of Agashi meat product. *Food Nutr Sci* 2012;**3**:851–856.
30 Association of Official Analytical Chemists. *Official Methods of Analysis of AOAC International*, 16th edn. AOAC International, Arlington, VA, 1995.
31 Hassan AA, Sandanger TM, Brustad M. Selected vitamins and essential elements in meat from semi-domesticated reindeer (*Rangifer tarandus tarandus L.*) in mid- and northern Norway: geographical variations and effect of animal population density. *Nutrients* 2012;**4**:724–739.
32 Husáková L, Urbanová I, Šrámková J *et al*. Analytical capabilities of inductively coupled plasma orthogonal acceleration time-of-flight mass spectrometry (ICP-oa-TOF-MS) for multi-element analysis of food and beverages. *Food Chem* 2011;**129**: 1287–1296.
33 Avegliano RP, Maihara VK, da Silva FF. A Brazilian Total Diet Study: evaluation of essential elements. *J Food Compos Anal* 2011;**24**:1009–1016.
34 Onianwa PC, Adeyemo AO, Idowu OE, Ogabiela EE. Copper and zinc contents of Nigerian foods and estimates of the adult dietary intakes. *Food Chem* 2001;**72**:89–95.
35 Falandysz J. Some toxic and essential trace metals in cattle from the northern Poland. *Sci Total Environ* 1993;**136**:177–191.
36 Barclay MNI, MacPherson A, Dixon J. Selenium content of a range of UK foods. *J Food Compos Anal* 1995;**8**:307–318.
37 Tinggi U, Reilly C, Patterson CM. Determination of selenium in foodstuffs using spectrofluorimetry and hydride generation atomic absorption spectrophotometry. *J Food Compos Anal* 1992;**5**:269–280.
38 Lombardi-Boccia G, Lanzi S, Aguzzi A. Aspects of meat quality: trace elements and B vitamins in raw and cooked meats. *J Food Compos Anal* 2005;**18**:39–46.
39 López Alonso M, Miranda M, Castillo C, Hernández J, García-Vaquero M, Benedito JL. Toxic and essential metals in liver, kidney and muscle of pigs at slaughter in Galicia, north-west Spain. *Food Addit Contam* 2007;**24**:943–954.
40 Falandysz J. Some toxic and essential trace metals in swine from Northern Poland. *Sci Total Environ* 1993;**136**:193–204.
41 Falandysz J, Kotecka W, Kannan K. Mercury, lead, cadmium, manganese, copper, iron and zinc concentrations in poultry, rabbit and sheep from the northern part of Poland. *Sci Total Environ* 1994;**141**:51–57.
42 Murphy J, Cashman KD. Selenium content of a range of Irish foods. *Food Chem* 2001;**74**:493–498.
43 Holland B, Welch AA, Unwin ID, Buss DH, Paul AA, *Southgate DAT (eds) McCance and Widdowson's The Composition of Food*, 5th edn. Royal Society of Chemistry, Cambridge, 1991.
44 Geissler C, Singh M. Iron, meat and health. *Nutrients* 2011;**3**:283–316.
45 Lombardi-Boccia G, Martinez-Dominguez B, Aguzzi A. Total heme and non-heme iron in raw and cooked meats. *J Food Sci* 2002;**67**:1738–1741.
46 Kadim IT, Al-Karousi A, Mahgoub O *et al*. Chemical composition, quality and histochemical characteristics of individual dromedary camel (*Camelus dromedarius*) muscles. *Meat Sci* 2013;**93**:564–571.
47 Cabrera MC, Ramos A, Saadoun A, Brito G. Selenium, copper, zinc, iron and manganese content of seven meat cuts from Hereford and Braford steers fed pasture in Uruguay. *Meat Sci* 2010;**84**:518–528.
48 Valenzuela C, López de Romaña D, Olivares M, Morales MS, Pizarro F. Total iron, heme iron, zinc, and copper content in rabbit meat and viscera. *Biol Trace Elem Res* 2011;**143**: 1489–1496.
49 Bügel S, Sandström B, Skibsted LH. Pork meat: a good source of selenium? *J Trace Elem Med Biol* 2004;**17**:307–311.
50 Franke, B.M, Gremaud, G, Hadorn, R, Kreuzer, M. Geographic origin of meat-elements of an analytical approach to its authentication. *Eur Food Res Technol* 2005; **221**: 493–503.
51 Mareta, W, Sandstead, H.H. Zinc requirements and the risks and benefits of zinc supplementation. *J Trace Elem Med Biol* 2006; **20**: 3–18.
52 Devatkal S, Mendiratta SK, Kondaiah N, Sharma MC, Anjaneyulu ASR. Physicochemical, functional and microbiological quality of buffalo liver. *Meat Sci* 2004;**68**:79–86.

53 Korsrud GD, Meldrum JB, Salisbury CD, Houlahan BJ, Aschenbrecker PW, Tittiger F. Trace element levels in liver and kidney from cattle, swine and poultry slaughtered in Canada. *Can J Comp Med* 1985;**49**:159–163.

54 Tomović VM, Petrović LS, Tomović MS, Kevrešan ŽS, Džinić NR. Determination of mineral contents of semimembranosus muscle and liver from pure and crossbred pigs in Vojvodina (northern Serbia). *Food Chem* 2011;**124**:342–348.

55 Demirezen D, Uruc K. Comparative study of trace elements in certain fish, meat and meat products. *Meat Sci* 2006;**74**: 255–260.

56 Nunes AM, Acunha TS, Oreste EQ *et al*. Determination of Ca, Cu, Fe and Mg in fresh and processed meat treated with tetramethylammonium hydroxide by atomic absorption spectrometry. *J Braz Chem Soc* 2011;**22**:1850–1857.

57 Dufailly V, Nöel L, Guérin T. Determination of chromium, iron and selenium in foodstuffs of animal origin by collision cell technology, inductively coupled plasma mass spectrometry (ICP-MS), after closed vessel microwave digestion. *Anal Chim Acta* 2006;**565**:214–221.

58 Abdel-Azeem SM, Bader NR, Kuss HM, El-Shahat MF. Determination of total iron in food samples after flow injection preconcentration on polyurethane foam functionalized with N,N-bis(salicylidene)-1,3-propanediamine. *Food Chem* 2013;**138**:1641–1647.

59 Hornsey HC. (1956) The colour of cooked cured pork. I. Estimation of the nitric oxide–haem pigments. *J Sci Food Agric* 1956;**7**:534–540.

60 Lombardi-Boccia G, Martínez-Domínguez B, Aguzzi A, Rincón-León F. Optimization of heme iron analysis in raw and cooked meat. *Food Chem* 2002;**78**:505–510.

61 Ramos A, Cabrera MC, Saadoun A. Bioaccessibility of Se, Cu, Zn, Mn and Fe, and heme iron content in unaged and aged meat of Hereford and Braford steers fed pasture. *Meat Sci* 2012;**91**:116–124.

62 Rebouche CJ, Wilcox CL, Widness JA. Microanalysis of non-heme iron in animal tissues. *J Biochem Biophys Method* 2004;**58**:239–251.

63 Hong JH, Yasumoto K. Near-infrared spectroscopic analysis of heme and nonheme iron in raw meats. *J Food Compos Anal* 1996;**9**:127–134.

64 Sirichakwal PP, Puwastien P, Polngam J, Kongkachuichai R. Selenium content of Thai foods. *J Food Compos Anal* 2005; **18**:47–59.

65 Pappa EC, Pappas AC, Surai PF. Selenium content in selected foods from the Greek market and estimation of the daily intake. *Sci Total Environ* 2006;**372**:100–108.

66 Díaz-Alarcón JP, Navarro-Alarcón M, López-García de la Serrana H, López-Martínez MC. Determination of selenium in meat products by hydride generation atomic absorption spectrometry: selenium levels in meat, organ meats, and sausages in Spain. *J Agric Food Chem* 1996;**44**:1494–1497.

67 Vieira MA, Ribeiro AS, Dias LF, Curtius AJ. Determination of Hg and Se in biological materials by chemical vapor generation electrothermal vaporization inductively coupled plasma mass spectrometry using isotope dilution calibration after microwave-assisted digestion with aqua regia. *J Braz Chem Soc* 2006;**17**:923–928.

68 Bierla K, Dernovics M, Vacchina V, Szpunar J, Bertin G, Lobinski R. Determination of selenocysteine and selenomethionine in edible animal tissues by 2D size-exclusion reversed-phase HPLC-ICP MS following carbamidomethylation and proteolytic extraction. *Anal Bioanal Chem* 2008;**390**: 1789–1798.

69 Daun C, Lundh T, Önning G, Åkesson B. Separation of soluble selenium compounds in muscle from seven animal species using size exclusion chromatography and inductively coupled plasma mass spectrometry. *J Anal Atom Spectrom* 2003;**19**:129–134.

70 Matos WO, Menezes EA, Gonzalez MH, Costa LM, Trevizan LC, Nogueira ARA. Partial microwave-assisted digestion of animal tissue using a baby-bottle sterilizer for analyte determination by inductively coupled plasma optical emission spectrometry. *Spectrochim Acta B* 2009;**64**:615–618.

71 Yabe J, Nakayama SMM, Ikenaka Y, Muzandu K, Ishizuka M, Umemura T. Uptake of lead, cadmium, and other metals in the liver and kidneys of cattle near a lead-zinc mine in Kabwe, Zambia. *Environ Toxicol Chem* 2011;**30**:1892–1897.

72 Miranda M, López-Alonso M, Castillo C, Hernández J, Prieto F, Benedito JL. Some toxic elements in liver, kidney and meat from calves slaughtered in Asturias (Northern Spain). *Eur Food Res Technol* 2003;**216**:284–289.

73 Codex Alimentarius Commission (CAC). Codex general standard for contaminants and toxins in food and feed, Codex Stan 193-1995, Rev. 2009, amended 2012. Available at http://www.codexalimentarius.org/standards/en/ (accessed 12 September 2013).

74 European Union. Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Available at https://www.fsai.ie/uploadedFiles/Consol_Reg1881_2006.pdf.

75 European Union. Regulation (EC) No. 629/2008 of 2 July 2008 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. Available at http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32008R0629.

76 Joint FAO/WHO Expert Committee on Food Additives. *Toxicological Evaluation of Certain Food Additives and Contaminants*. WHO Food Additives Series No. 24. FAO/WHO, Geneva, 1989.

77 European Food Safety Authority. Scientific Opinion of the Panel on Contaminants in the Food Chain on arsenic in food. *EFSA J* 2009;**7**(10):1351.

78 Joint FAO/WHO Expert Committee on Food Additives. *Evaluation of Certain Food Additives and Contaminants. Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives*. WHO Technical Report Series No. 776. WHO, Geneva, 1989.

79 European Food Safety Authority. Scientific Opinion of the Panel on Contaminants in the Food Chain on the statement on tolerable weekly intake for cadmium. *EFSA J* 2011; **9**(2):1975.

80 Joint FAO/WHO Expert Committee on Food Additives. *Toxicological Evaluation of Certain Food Additives and Contaminants. Thirtieth report of the Joint FAO/WHO Expert Committee on Food Additives*. WHO Food Additives Series No. 21. WHO, Geneva, 1987.

81 European Food Safety Authority. Scientific Opinion of the Panel on Contaminants in the Food Chain on lead in food. *EFSA J* 2010;**8**(4):1570.

82 Joint FAO/WHO Expert Committee on Food Additives. *Evaluation of Certain Food Additives and Contaminants. Seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives*. WHO Technical Report Series No. 959. WHO, Geneva, 2011.

83 Joint FAO/WHO Expert Committee on Food Additives. *Evaluation of Certain Food Additives and Contaminants Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives*. WHO Technical Report Series No. 922. WHO, Geneva, 2004.

84 European Food Safety Authority. Scientific Opinion of the Panel on Contaminants on the risk for public health related to the presence of mercury and methylmercury. *EFSA J* 2012;**10**(12):2985.

85 Vreman K, Van der Veen NG, Van der Molen EJ, de Ruig WG. Transfer of cadmium, lead, mercury and arsenic from feed into milk and various tissues: chemical and pathological data. *Neth J Agric Sci* 1986;**34**:129–144.

86 European Food Safety Authority. Scientific Opinion of the Panel on Contaminants in the Food Chain on cadmium in food. *EFSA J* 2009;**980**:1–139.

87 Elsenhans B, Strugala GJ, Schäfer SG. Small-intestinal absorption of cadmium and the significance of mucosal metallothionein. *Hum Exp Toxicol* 1997;**16**:429–434.

88 Joint FAO/WHO Expert Committee on Food Additives. *Evaluation of Certain Food Additives and Contaminants. Seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives*. WHO Technical Report Series No. 960. WHO, Geneva, 2011.

89 European Food Safety Authority. Scientific Report on cadmium dietary exposure in the European population. *EFSA J* 2012;**10**(1):2551.

90 European Union. Council Directive 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC. Available at http://ec.europa.eu/food/food/chemicalsafety/residues/council_directive_96_23ec.pdf.

91 Falandysz J. Some toxic and trace metals in big game hunted in the northern part of Poland in 1987–1991. *Sci Total Environ* 1994;**141**;59–73.

92 Rabinowitz MB. Toxicokinetics of bone lead. *Environ Health Perspect* 1991;**91**:33–37.

93 European Food Safety Authority. Scientific Opinion of the Panel on Contaminants in the Food Chain on lead dietary exposure in the European population. *EFSA J* 2012;**10**(7):2831.

94 Tsuji LJS, Wainman BC, Jayasinghe RK, Van Spronsen EP, Liberda EN. Determining tissue-lead levels in large game mammals harvested with lead bullets: human health concerns. *Bull Environ Contam Toxicol* 2009;**82**:435–439.

95 Dobrowolska A, Melosik M. Bullet-derived lead in tissues of the wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*). *Eur J Wildlife Res* 2008;**54**:231–235.

96 Joint FAO/WHO Expert Committee on Food Additives. *Safety Evaluation of Certain Food Additives and Contaminants. Seventy-second meeting of the Joint FAO/WHO Expert Committee on Food Additives*. WHO Food Additives Series No. 63. WHO, Geneva, 2011.

97 European Union. Regulation (EC) No. 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a) pyrene in foodstuffs. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32007R0333.

98 Vos G, Lammers H, van Delft W. Arsenic, cadmium, lead and mercury in meat, livers and kidneys of sheep slaughtered in the Netherlands. *Z Lebensm Unters Forsch* 1988;**187**:1–7.

99 Waegeneers N, Pizzolon J, Hoenig M, De Temmerman L. Accumulation of trace elements in cattle from rural and industrial areas in Belgium. *Food Addit Contam* 2008;**26**: 326–332.

100 Sapunar-Postružnik J, Bažulić D, Kubala H. Estimation of dietary intake of arsenic in the general population of the Republic of Croatia. *Sci Total Environ* 1996;**191**:119–123.

101 Shah AQ, Kazi TG, Arain MB *et al*. Comparison of electrothermal and hydride generation atomic absorption spectrometry for the determination of total arsenic in broiler chicken. *Food Chem* 2009;**113**:1351–1355.

102 Pizarro I, Gómez M, Cámara C, Palacios MA. Arsenic speciation in environmental and biological samples. Extraction and stability studies. *Anal Chim Acta* 2003;**495**:85–98.

103 Mariam I, Iqbal S, Nagra SA. Distribution of some trace and macrominerals in beef, mutton and poultry. *Int J Agric Biol* 2004;**6**:816–820.

104 Sánchez-Rodas D, Gómez-Ariza JL, Oliveira V. Development of a rapid extraction procedure for speciation of arsenic in chicken meat. *Anal Bioanal Chem* 2006;**385**:1172–1177.

105 Salisbury CDC, Chan W, Saschenbrecker PW. Multielement concentrations in liver and kidney tissues from five species of Canadian slaughter animals. *J AOAC Int* 1991;**74**:587–591.

106 Nriagu J, Bughanen M, Linder A *et al*. Levels of As, Cd, Pb, Cu, Se, and Zn in bovine kidneys and livers in Jamaica. *Ecotoxicol Environ Saf* 2009;**72**:564–571.

107 European Committee for Standardization (CEN). EN 14546:2005. Foodstuffs. Determination of trace elements.

Determination of total arsenic by hydride generation atomic absorption spectrometry (HG-AAS) after dry ashing.
108 European Committee for Standardization (CEN). EN 14627:2006. Foodstuffs. Determination of trace elements. Determination of total arsenic and selenium by atomic absorption spectrometry (AAS) hydride technique after pressure digestion.
109 Dabeka RW, McKenzie AD, Lacroix GM *et al*. Survey of arsenic in total diet food composites and estimation of the dietary intake of arsenic by Canadian adults and children. *J AOAC Int* 1993;**76**:14–25.
110 Liu L, Yun Z, Sun J, Jiang G. Speciation analysis of arsenic compounds by capillary electrophoresis on-line coupled with inductively coupled plasma mass spectrometry using a novel interface. *J Chromatogr A* 2013;**1304**:227–233.
111 Braman RS, Foreback CC. Methylated forms of arsenic in the environment. *Science* 1973;**182**:1247–1249.
112 Schoof RA, Yost LJ, Eickhoff J *et al*. A market basket survey of inorganic arsenic in food. *Food Chem Toxicol* 1999;**37**: 839–846.
113 Karavoltsos S, Sakellari A, Dassenakis M, Scoullos M. Cadmium and lead in organically produced foodstuffs from the Green market. *Food Chem* 2008;**106**:843–851.
114 Danieli PP, Serrani F, Primi R, Ponzetta MP, Ronchi B, Amici A. Cadmium, lead, and chromium in large game: a local-scale exposure assessment for hunters consuming meat and liver of wild boar. *Arch Environ Contam Toxicol* 2012;**63**:612–627.
115 Oforka NC, Osuji LC, Onwuachu UI. Asessment of heavy metal pollution in muscles and internal organs of chickens raised in Rivers State, Nigeria. *J Emerg Trends Eng Appl Sci* 2012;**3**:406–411.
116 Ihedioha JN, Okoye COB. Dietary intake and health risk assessment of lead and cadmium via consumption of cow meat for a population in Enugu State, Nigeria. *Ecotoxicol Environ Saf* 2013;**93**:101–106.
117 Abou-Arab AAK. Heavy metal contents in Egyptian meat and the role of detergent washing on their levels. *Food Chem Toxicol* 2001;**39**:593–599.
118 Rahimi E, Rokni N. Measurement of cadmium residues in muscle, liver and kidney of cattle slaughtered in Isfahan abattoir using graphite furnace atomic absorption spectrometry (GFAAS): a preliminary study. *Iran J Vet Res* 2008;**9**:174–177.
119 Abedi A, Ferdousi R, Eskandari S, Seyyedahmadian F, Khaksar R. Determination of lead and cadmium content in sausages from Iran. *Food Addit Contam* 2011;**4**:254–258.
120 Forte G, Bocca B. Quantification of cadmium and lead in offal by SF-ICP-MS: method development and uncertainty estimate. *Food Chem* 2007;**105**:1591–1598.
121 Abduljaleel SA, Shuhaimi-othman M, Babji A. Assessment of trace metals contents in chicken (*Gallus gallus domesticus*) and quail (*Coturnix coturnix japonica*) tissues from Selangor (Malaysia). *J Environ Sci Technol* 2012;**5**:441–451.
122 Leermakers M, Baeyens W, Quevauviller P, Horvat M. Mercury in environmental samples: speciation, artifacts and validation. *Trends Anal Chem* 2005;**24**:383–393.
123 López Alonso M, Benedito JL, Miranda M, Castillo C, Hernández J, Shore RF. Mercury concentrations in cattle from NW Spain. *Sci Total Environ* 2003;**302**:93–100.
124 Rubio C, Gutiérrez A, Burgos A, Hardisson A. Total dietary intake of mercury in the Canary Islands, Spain. *Food Addit Contam* 2008;**25**:946–952.
125 Ullrich SM, Ilyushchenko MA, Tanton TW, Uskov GA. Mercury contamination in the vicinity of a derelict chlor-alkali plant. Part II: Contamination of the aquatic and terrestrial food chain and potential risks to the local population. *Sci Total Environ* 2007;**381**:290–306.
126 Laird BD, Chan HM. Bioaccessibility of metals in fish, shellfish, wild game, and seaweed harvested in British Columbia, Canada. *Food Chem Toxicol* 2013;**58**:381–387.
127 Száková J, Kolihová D, Miholová D, Mader P. Single-purpose atomic absorption spectrometer AMA-254 for mercury determination and its performance in analysis of agricultural and environmental materials. *Chem Pap* 2004;**58**:311–315.
128 Ulrich R, Raszyk J, Nápravník A. Variation in contamination by mercury, cadmium and lead on swine farms in the district of Hodonín in 1994 to 1999. *Vet Med Czech* 2001;**46**: 132–139.
129 Lazarus M, Orct T, Blanuša M, Vicković I, Šoštarić B. Toxic and essential metal concentrations in four tissues of red deer (*Cervus elaphus*) from Baranja, Croatia. *Food Addit Contam* 2008;**25**:270–283.
130 Cheng Z, Wang HS, Du J *et al*. Dietary exposure and risk assessment of mercury via total diet study in Cambodia. *Chemosphere* 2013;**92**:143–149.
131 Batista BL, Grotto D, Hornos Carneiro MF, Barbosa F Jr. Evaluation of the concentration of nonessential and essential elements in chicken, pork, and beef samples produced in Brazil. *J Toxicol Environ Health A* 2012;**75**:1269–1279.
132 Chibunda RT, Janssen CR. Mercury residues in free-grazing cattle and domestic fowl from the artisanal gold mining area of Geita district, Tanzania. *Food Addit Contam* 2009;**26**: 1482–1487.
133 Bloom N. Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. *Can J Fish Aquat Sci* 1989;**46**:1131–1140.

CHAPTER 27
Fish and seafood

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Abstract: This chapter includes a detailed review on the content, distribution and accumulative processes of metals, metalloids and alkaline/alkaline earth metals within fish and seafood. The inclusion of minerals in the human diet, as well as their role in metabolism, growth and health, are discussed. Moreover, the main analytical methods as usually applied to determine the contents of these elements in fish and seafood are fully considered, including alternative processes of mineralization, sample treatments, and the diversity of analytical instrumentation. The exposure of human populations to potentially toxic metals through fish and seafood consumption is also reviewed, and the most significant case studies analysed: mercury in fish and shellfish species; cadmium in squids; copper and zinc in bivalves, molluscs and crustaceans; arsenic and selenium in seafood; calcium, magnesium, sodium and potassium in fish and shellfish, with analysis of the corresponding trends in accumulation, detoxification processes, toxicity levels, and usual exposure levels within different environments. Finally, the major risks and diseases that can occur in human populations as a consequence of exposure to the toxic metal content of fish and seafood are discussed. The main historical events have been considered within this framework, and the most important effects of these elements on health are included.

Keywords: heavy metals, seafood, related diseases, case studies

27.1 Introduction

The study of essential and toxic trace element content in foodstuffs, as well as study of metalloids, alkaline and alkaline earth metals, has developed extensively in recent decades, particularly with regard to the potential health benefits and risks of seafood consumption. Evaluation of the risks and benefits of the consumption of fish and other seafood items has been particularly controversial. Numerous nutritionists have asserted that these products are an important source of high-quality proteins, minerals and essential fatty acids such as omega-3. Conversely, a number of toxicologists tend to regard seafood as a major vector for toxic substances such as metal trace elements and persistent organic pollutants. The scientific viewpoint is more complex and reconciliation of these two viewpoints requires an integrated consideration of both nutritional properties and toxic substance residues within food products as well as the corresponding consumer behaviour in relation to these products.

The inclusion of minerals in the diet is essential for the development of biological processes; minerals play a vital role in metabolic functions, normal growth and transitional stages of the organism. Physiologically, the most important macrominerals are calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K). The main roles of these elements include maintenance of pH, osmotic pressure, nerve conduction, muscle contraction, energy production and almost all other aspects of biological life [1, 2]. Consequently, inadequate dietary intakes leading to deficiency or excess of these elements leads inevitably to health problems. The importance of optimal intakes of essential mineral elements to maintain peak health is therefore widely recognized [3].

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Moreover, the trace elements (metals and metalloids) play different roles and have different activities within the biological organism. Some of them (i.e. iron, zinc, copper, selenium, molybdenum and chromium) can cause deleterious effects not only in deficiency but also in excess, and are usually called 'essentials'. However, others like manganese have a number of biological functions (e.g. as an enzyme cofactor); however, both low and high intakes do not cause substantial problems and are called 'beneficial'. Finally, there is a group of trace elements (i.e. cadmium, lead, mercury, arsenic) that produce significant biological disorders when present in increased concentrations, and are called 'non- essential' or 'detrimental' [4].

As a safeguard for human health, numerous guidelines and regulations have been issued that stipulate maximum permissible levels of toxic substances (i.e. trace metals) in fish and seafood, including the dietary exposure limit for consumers. Thus different international institutions with responsibility for environmental affairs and human health have proposed limits for dietary exposure to toxic elements related to aquatic and/or marine foodstuffs [5–8]. These estimations of dietary exposure are crucial for risk assessment, as well as for determining the relationship between adverse effects on human health and dietary exposure to particular substances. Dietary exposure to elements such as cadmium (Cd), mercury (Hg), arsenic (As) and lead (Pb) has been clearly associated with toxic and adverse health effects [9]. Unfortunately, conclusions are not so straightforward in the case of elements such as lithium (Li), chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), selenium (Se) and molybdenum (Mo), which are essential micronutrients that need to be consumed in adequate amounts to maintain normal physiological functions [10] but which can produce toxicity at high concentrations [11, 12]. All trace elements, including the essential ones, may be toxic when taken in excessive amounts, or may lead to deficiencies when taken in insufficient amounts.

The World Health Organization (WHO) has suggested the application of three complementary approaches for assessing a population's dietary exposure to food contaminants [13]: (i) the study of individual food items, (ii) the duplicate diet method, and (iii) the total diet study (TDS). It is important to note that the TDS, which has been widely used in many countries, has the advantage of yielding more realistic exposure data compared with other dietary exposure assessment methods, since it basically consists of the analysis of a representative 'market basket' of foods commonly prepared for normal consumption [14].

Both essential and non-essential metals can originate from natural sources (volcanism, geochemical structure, mineral composition) or anthropogenic sources (industrial wastes, agriculture, urban sewage, mining of metals) and their high concentrations have produced pollution processes that have been identified as a concern in coastal environments [15–17]. Heavy metals can be accumulated by aquatic organisms through a variety of pathways, including respiration, adsorption and ingestion and often enter the human body by ingestion [18]. With regard to this framework, numerous scientific papers have reported different items of seafood as potential sources of trace metals to top predators in their ecosystems, including humans. Several studies can be mentioned among the numerous published: As bioaccumulation in organs of the catfish *Clarias batrachus* (L.) from Varanasi, India [19]; mercury in Pacific bluefin tuna *Thunnus orientalis* from different regions of Japan [20]; accumulation of Fe, Mn, Cu, Zn, As, Se, Cd, Hg and Pb in two hagfish species, *Paramyxine nelsoni* and *Myxine formosana*, from Taiwan waters [21]; metal accumulation (Al, Cd, Co, Cu, Cr, Fe, Hg, Ni, Pb, Zn) in tissues of the blue crab *Callinectes danae* S. from São Vicente Channel, São Paulo State, Brazil [22]; levels of metals (Li, Cr, Mn, Co, Ni, Cu, Zn, Se and Mo) in items on the French TDS [23]; metal concentration (Cd, Cr, Cu, Pb, Zn, Fe and Ni) in mussels *Mytilus galloprovincialis* from the Dardanelles, Canakkale Strait, Turkey [24]; the content of Ni, Cd, Cu and Pb in seven seaweed species, including one green alga (*Ulva intestinalis*), three brown algae (*Padina pavonica*, *Cystoseira myrica*, *Sargassum angustifolium*) and three red algae (*Acanthophora spicifera*, *Gracilaria corticata*, *Hypnea cervicornis*) from the Persian Gulf, Iran [25]; and concentrations of organotin compounds (DBT, TBT, ΣBT) in 33 fish species, six cephalopod species and six oyster species usually included in Korean seafood [26].

Consequently, the aim of this chapter is to review the information on trace metals, metalloids and alkaline/alkaline earth elements within seafood (fish, shellfish, seaweeds, etc.) and the role of these items as a significant source of the mentioned pollutants to human populations.

27.2 Methods for determining metals in fish and seafood

Contamination of food products by heavy metals is becoming an unavoidable problem. Air, soil and water pollution are contributing to the presence of harmful elements such as Cd, Pb, Hg and As in foodstuffs [13]. The accumulation of heavy metals in fish and other marine organisms not only has a detrimental influence on the animals and plants themselves, but also affects human health through the food chain. In addition, some heavy metals are found in fishmeals that are used as raw materials for aquaculture feeds.

Different edible tissues of fish and seafood are extracted to determine the corresponding heavy metal content. The first step in the determination of heavy metal concentrations in fish and seafood is the sampling stage, where the generation of cross-contamination must be avoided. Strategies for sampling will depend on the selected species, but must also consider the following important prerequisites: (i) the sample must be representative of the real population and (ii) it must be catchable in sufficient numbers to obtain enough homogenized tissue/organs/specimen in dry or wet weight for subsequent chemical analysis. Moreover, the applied analytical method depends on the range of heavy metal concentrations within the samples, the selected metal, and the characteristics of the selected organism. The shorter the time between collection of a sample and its analysis, the more reliable the analytical results [27].

Immediately fish and seafood samples are obtained, they must be transported in a cooler to the laboratory for dissection; if this is not possible the samples can be stored in plastic bags at –20 °C [28]. During dissection, edible tissues (e.g. axial muscle tissue for scale fish; tail meat for lobsters; foot muscle for abalone) are extracted for trace metal analysis [29]. In order to determine the quality or health status of fish, it is also desirable to assess metals in tissues such as liver, gill and gonad.

27.2.1 Sample mineralization in the laboratory

The first step in the laboratory determination of trace metals in fish and seafood is transformation of the solid matrix (i.e. sample of the fish or foodstuff as obtained) into a solution prior to determination. Wet and dry oxidation procedures have been extensively applied; these involve strong reaction conditions such as use of strong mineral acids, oxidizing agents and both high temperature and pressure conditions [30–32]. In all cases it is strongly recommended that the samples are analysed three times for each metal. The standard addition method can be used to correct for matrix effects [28].

Each tissue sample is homogenized manually or using a mechanical stomacher [29]. The samples are then digested using different methods. First, a weighed sample of edible tissue of fish or seafood (5–7 g) is placed into a glass tube (50 mL borosilicate) [29]. Then acid digestion can be carried out with concentrated analytical quality nitric acid (10 mL), or with different combinations of strong mineral acids (nitric and perchloric, hydrochloric, hydrofluoric or sulfuric acids) to produce complete digestion [27, 29]. Type of reaction mixture, its volume, heating time and final volume of obtained extract vary between authors. For example, one method uses a mixture of concentrated strong acid (3–5 mL of nitric acid 64% plus 1 mL perchloric acid) in an open system, heating to 120 ± 5 °C on a hot plate until solution is colourless [33], or is evaporated to the lowest possible volume before precipitation [27] and making up final volume of 10 mL with nitric acid 0.7% [33]. Others suggest heating the acidified sample at 40 °C until the tissue has dissolved, and then at 90 °C for 3 hours. Nitric acid addition and heating will continue until a clear solution is obtained. During these operations organic matter is completely eliminated [29].

A similar protocol has been proposed by Dural *et al.* [28], which includes tissue sample digestion with a mixture of strong acids (concentrated nitric acid and perchloric acid, 2 : 1 v/v) at 60 °C on a hot plate for 3 days, and then all samples are diluted (to 50 mL in volumetric flask) with double distilled water or with a solution of 0.7% nitric acid.

The Association of Official Analysis Chemists (AOAC) proposes fish sample preparation in two different ways. One is AOAC Method 999.10, the official technique applicable to the determination of metals in a variety of food items by flame atomic absorption spectrometry (FAAS) (i.e. Zn, Cu, and Fe) or graphite furnace atomic absorption spectrometry (GFAAS) (i.e. Cd and Pb) after microwave digestion. The principle of the method involves the digestion of selected products (fresh, maximum 2 g; dry, 0.2–0.5 g) with 5 mL HNO_3 plus 2 mL 30% H_2O_2 under pressure in a closed vessel heated by microwave [35]. The second method is AOAC 999.11, applicable to the determination of the above-mentioned

metals, with preparation of fish samples by conventional dry ashing using a muffle furnace. The samples are dried and then ashed at 450 °C, applying a gradual increase in temperature (≤50 °C/hour). Then, 5 mL 6 M HCl (1 + 1) is added and the solution is evaporated to dryness. The obtained residue is dissolved in 0.1 M HNO_3 and the analytes are determined by FAAS and/or GFAAS [36].

In addition, ultrasound-assisted extraction of trace elements from a variety of solid matrices using both bath and probe ultrasonic processors has emerged as an efficient approach for sample preparation in trace element analysis, the advantage of the method being that it avoids the intensive and destructive sample treatments of dry ashing or acid digestion [37]. It has already been shown to be a very promising technique for the extraction of mercury species in biological tissues [38], especially as there is no risk that the extraction solution evaporates to dryness [39]. Ultrasonic probes provide some advantages over ultrasonic baths, namely short extraction times, extraction efficiency approaching 100%, and feasibility of using low acidic conditions for extraction as a result of the enhanced ultrasonic power directly focused onto the sample [37]. However, although both baths and probes are useful for ultrasound-assisted acid digestion (UAD), the use of ultrasonic bath in laboratories is preferred over that of a probe, bearing in mind that they are cheaper and easily available [39].

Bashir *et al.* [34] analysed Cd, Cu, Mn and Zn in homogenized dried samples of muscle and gills (0.5 g) as well as of liver (0.1 g) from two marine fish from coastal waters of Kapar and Mersing, Malaysia. They used HNO_3 (65% v/v) + H_2O_2 (30% v/v) in Teflon vessels within a microwave oven digestive system. The residues were filtered using 0.45-μm Whatman filter paper and then were transferred to a 50-mL volumetric flask, and diluted with deionized water.

Kazi *et al.* [39] proposed the development of a UAD method for fast and reproducible recovery of some heavy metals (Cd, Cr, Ni and Pb) in both environmental (i.e. sediment, sewage sludge, soil) and biological (i.e. vegetables, fish muscle) samples in order to set up an alternative to classical and non-classical digestion techniques. About 100 mg of dry sample mass was weighed in 25-mL flasks, and 1.0 mL of the acid mixture (HNO_3/H_2O_2, 2 : 1) was added. The optimal parameters influencing UAD for biological samples are (i) pre-sonication time: 8 min; (ii) sonication time: 6 min for Cd, 8 min for Ni, 10 min for Pb and Cr; (iii) solvent system: mixture of HNO_3/H_2O_2 (2 : 1) shows higher recoveries than exclusively HNO_3; and (iv) temperature of ultrasound bath: 60 °C showed to be optimal for Cd, while maximum recoveries of Cr, Ni and Pb were obtained at 70 °C.

Organic matter also has an important role in controlling the release of metals from the sample. The oxidation of biological samples with high organic matter is usually incomplete using only HNO_3 and, traditionally, the use of a strongly oxidizing acid such as $HClO_4$ is necessary. Considering that during heating H_2O_2 dissociates to hydroxyl radicals (OH^-) which could attack proteins, carbohydrates and polyunsaturated fatty acids in the biological samples (i.e. fish muscle), the combined use of H_2O_2 and HNO_3 has been proposed, yielding clear final solutions and improved recovery [39].

As previously stated, a proposed method for pretreatment of fish and shellfish samples is ultrasound-assisted extraction (fast, accurate, low cost and simple), and this has been used for As, Se, Ni and V determination in combination with electrothermal atomic absorption spectrometry (ET-AAS). Moreover, high-intensity ultrasonic probes employ 10 mg of solid freeze-dried matrix, ultrasound amplitude of 50%, low acidic extractant (1.5 mL HNO_3, 3 or 5% v/v) and a short extraction time (maximum 3 min) [37]. Metal contents in seafood samples depend on the analysed species, for example As concentrations can range from 12.6 μg/g for mussel to 190 μg/g for prawn [37].

Extraction of mercury species from a complex sample such as biological tissues is recognized as one of the most crucial steps in their determination. One of the most popular approaches for Hg extraction based on the use of ultrasonic probe sonication is acid leaching (acid hydrolysis). López *et al.* [38] found that the optimal conditions for quantitative extraction of mercury were 7 mol/L HCl (2 mL extraction solution) and 5 min of sonication at 40% ultrasonic amplitude. One important advantage of ultrasonic probe sonication is the ability to handle small amounts of sample (5–50 mg) in volumes as low as microlitres. Another validated method is enzymatic extraction in order to improve quantification of Hg by ICP-MS. An increased efficiency in total Hg recovery was demonstrated with the application of 2-mercaptoethanol 2.5% (v/v) as extractant, due to the affinity of Hg for sulfhydryl groups. This method is simple and rapid (sample treatment time ~10 min), and transformations between mercury species were not detected. It is suitable for mercury extraction in tissue samples of fish and mussel, and is also applicable to

analysis of microsamples (5–50 mg) [38]. The novelty of this procedure is its ability to be applied to small size samples, which is particularly relevant for those cases where large amounts of samples are not available or where they are highly valuable [38].

27.2.2 Analytical methods

Several analytical techniques are available for trace element determination in seafood samples following acid digestion or pre-concentration methods. The most important are atomic absorption spectrometry with air–acetylene flame (FAAS) [28] and graphite furnace atomic absorption spectrometry (GF-AAS), which is more convenient when determining Cd and Pb. Other metals can be analysed by inductively coupled plasma optical emission spectrometry (ICP-OES) [40, 41], inductively coupled plasma mass spectrometry (ICP-MS) [30, 38, 34], high-performance liquid chromatography inductively coupled plasma mass spectrometry (HPLC-ICP-MS) (e.g. Hg) [38], hydride generation atomic absorption spectrometry (HG-AAS) (e.g. As) [42], cold vapour atomic absorption spectrometry (CV-AAS) (e.g. Hg) [42], hydride generation atomic fluorescence spectrometry (HG-AFS) (e.g. As) [43] and ET-AAS [39]. Determination of As in fish by ET-AAS requires Zeeman correction to compensate for the presence of a structured background [37].

A brief consideration of the analytical method applied to mercury determination may be instructive. Mercury speciation in the extracts resulting from enzymatic hydrolysis [38] with 2-mercaptoethanol can be analysed by HPLC-ICP-MS. Volume injected is 100 µL. The chromatographic column is a reverse-phase column (Symmetry Shield RP18, 150 × 3.9 mm, 5 µm) and the mobile phase contains 0.1% (v/v) formic acid, 0.1% (v/v) HFBA, 0.02% (w/v) l-cysteine (to prevent retention of Hg in the mentioned column) and 2% (v/v) methanol. The separation is very fast (about 7 min) and the chromatogram can show a single peak corresponding to methylmercury depending on the type of biological sample being analysed [38].

27.3 Human populations exposed to potentially toxic metals through aquatic food

The effects of marine pollution, produced as a consequence of contamination of the margins of the oceans by humans, can be best described by its different manifestations, such as damage to individual animals, decrease in abundance of local populations, or other damage which may affect entire ecosystems [44]. It is easy to identify specific abnormalities in animals from the most impacted areas: deformed fish embryos and larvae can be observed in plankton collections, juvenile fish with skin ulcerations and fin erosion, and adult fish with cancerous or precancerous liver lesions [45, 46]. Effects at a population level are more difficult to detect, except by observing gradual disappearances of less resistant species from areas of heaviest contamination or by cataloguing the reduced reproductive success of vulnerable resident species [47, 48]. At the ecosystem level, evidence for disturbance may be recorded as mass mortalities of fish or shellfish, extensive blooms of toxic algae, or the sudden intrusion and temporary dominance of noxious non-native organisms [49, 50].

27.4 Case studies

27.4.1 Mercury in fish and shellfish species

Mercury has been regarded as one of the most hazardous aquatic pollutants due to its toxicity and bioaccumulative and non-biodegradable properties [51, 52]. Mercury contamination is an issue of global concern because of its global atmospheric dispersion [53] and subsequent deposition through both wet and dry deposition mechanisms [54]. Hg enters the aquatic environment from a number of sources, including natural causes (volcanic activity, rock weathering and oceanic emission) [53] and anthropogenic pathways (e.g. coal combustion facilities, manufacture of temperature and pressure devices, chlorine and caustic soda production, paint industries, pharmaceutical manufacture of drugs, disinfectants and antiseptic dressings, in formulation of a large number of fungicides) [51, 54]. Industrialization and artisanal mining of precious metals have contributed to the increment in Hg levels, affecting mainly coastal areas [55]. Some physicochemical processes may interfere in Hg bioavailability, such as adsorption by clays, sulfides or organic matter, precipitation, sedimentation, changes in pH, salinity or dissolved oxygen. These factors will determine which species of mercury will be available within the ecosystem [56].

In addition to the elemental form, Hg can be found in two essential varieties in aquatic systems: inorganic mercury (mercurous, Hg_2^{2+}, and mercuric, Hg^{2+}) and organic mercury (methylmercury, $HgCH_3$) [57]. This latter form

(MeHg) is by far the most common form of organic mercury, and is bioaccumulated and passed up the food chain [13]. It has carcinogenic characteristics, hence it is considered one of the most toxic and prevalent metals by the WHO [58, 59]. MeHg is the result of biomethylation reactions in biological systems, primarily performed by anaerobic sulfur-reducing bacteria [54], where the methyl-B_{12} coenzyme transforms inorganic mercury salts into MeHg and dimethylmercury [55]. Within the aquatic environment the methylation process occurs preferentially in anaerobic and organic surface sediments. Meanwhile under aerobic environmental conditions, mercury associated with sulfur may be oxidized, forming soluble Hg(II), which is assimilated by bacteria and soon methylated [58]. The rate of methylation is influenced by the microbial activity and depositional loadings (suspended sediment load, dissolved organic carbon, nutrient content, pH, temperature) [54]. Within estuaries, Hg methylation is facilitated by the presence of wetlands, which act as sources of dissolved organic matter, reducing oxygen levels and thereby increasing the activity of sulfate-reducing bacteria [60].

Mercury is a non-essential element [55] and its compounds have no recognized biological function. Therefore, if this element is present in any living organism, it may cause both cytochemical and histopathological effects [51]. Aquatic organisms can absorb mercury through biotic and abiotic processes, concentrating these substances in their tissues and organs (bioaccumulation) [55, 60]. This bioaccumulation occurs mainly in fish, especially large long-lived predators like sharks, whose muscular tissues can exhibit high mercury levels [55]. Both ionic mercury and MeHg have a great ability to block the activity of enzymes involved in the control of metabolic reactions, resulting in structural and functional impairment of proteins [51, 55].

Within this framework several study cases may be mentioned. Muscles of four shark species (smooth hammerhead shark *Sphyrna zygaena*, blue shark *Prionace glauca*, mako shark *Isurus oxyrinchus* and silky shark *Carcharhinus falciformis*), as well as their main prey, were analysed to determine Hg contents in Baja California peninsula, Mexico [55]. Each manifested different concentrations of Hg, showing differences between species (mean concentrations 0.98, 1.96, 1.05 and 3.40 μg/g, respectively) as well as between individuals of the same species. Some causes of these differences may be that larger individuals have more efficient predation mechanisms (and consequently accumulate more), different trophic habits (feeding on pelagic organisms, piscivorous), that some species (i.e. blue shark) have more efficient Hg elimination mechanisms (higher synthesis of metallothionein, a detoxification protein), and ontogenic trophic changes. The same study showed a tendency for increasing Hg concentration at larger sizes, showing that younger sharks have been exposed for less time to the metal than adults [55].

Newman *et al*. [53] measured Hg concentrations in three deepwater chondrichthyans (*Etmopterus princeps*, *Centroscymnus coelolepis*, and *Hydrolagus affinis*) collected from three North Atlantic Ocean locations. Mean concentrations were 1.76, 4.92 and 2.38 μg/g wet weight, respectively, and all axial muscle concentrations exceeded the current US human health screening value of 0.3 μg/g wet weight for fish.

Mercury content varies widely among different fish species and is highest in predatory fish [57]. It has been shown that Hg bioaccumulation factor in carnivorous fish species is about 10 times higher than in non-carnivorous fish [56]. Mercury is transferred through the trophic web, and its concentration increases at each level (biomagnification) [60]. The relative contribution of Hg in fish tissues depends on the trophic position of finfish species, as well as on the presence or absence of migratory behaviour [53]. Most of the Hg found in the edible portions (muscle tissue) of trophic level 4 fish (TL4, top carnivores, e.g. *Morone chrysops*, hybrid striped bass) is reported to be in the methylated form [54]. The mackerel *Scomber japonicus* (0.57 ± 0.02 μg/g), lantern fish *Symbolophorus evermanni* (0.34 ± 0.13 μg/g), pelagic red crab *Pleuroncodes planipes* (0.13 ± 0.01 μg/g) and giant squid *Dosidicus gigas* (0.12 ± 0.05 μg/g) are all shark prey and exhibited Hg concentrations lower than different shark species. Therefore they can bioaccumulate Hg and then transfer it to their predators. An increase in Hg concentration between trophic level is thus produced [55]. Mercury biomagnification by piscivorous fish in temperate systems is highly dependent on food chain length [60].

Bioaccumulation and biomagnification of Hg has been verified in the muscle of four fish species from Bahía Blanca estuary, Argentina (*Mustelus schmitti*, *Micropogonias furnieri*, *Cynoscion striatus*, *Basilichthys bonaeriensis*), with mean values of 890, 370, 390 and 220 ng/g wet weight, respectively. Variations over time have also been shown: in the case of *M. schmitti*, the concentration

reported corresponds to the period 1982–1988, but this decreased to 90 ng/g during the period 2001–2003 [58].

A study of Hg in tissues by Rodrígues *et al.* [56] selected fish species with different characteristics, namely well-distributed species feeding on benthic organisms (*Genidens genidens* and *Aspistor luniscutis*), a carnivorous species feeding on benthic invertebrates and small fish (*Haemulon steindachneri*) and a carnivorous species feeding on zooplankton, with high commercial value and migratory as adults (*Micropogonias furnieri*). All species showed total Hg levels in muscle below 200 ng/g, except *H. steindachneri* with a mean concentration of 310 ng/g, which also showed the highest mercury concentrations (maximum 817.0 ng/g).

Mercury has been detected in one of the longest-living fish in the oceans, orange roughy (*Hoplostethus atlanticus*). The Hg concentrations in fillets of this fish species (high-level carnivore) varied between 0.06 and 1.1 μg/g wet weight, showing a good positive correlation with age (some of analysed individuals were 136 to 139 years old) [61]. Yadav and Trivedi [52] noted that in killifish (*Fundulus heteroclitus*) inorganic mercury had a teratogenic effect and that MeHg induced chromosomal aberrations and micronuclei.

The main exposure pathway of Hg to humans is through the consumption of fish, where Hg is present mainly in the methylated form [51, 54]. MeHg can be absorbed through the lung and by ingestion, but there is almost no absorption through skin [51]. Mercuric chloride is a highly potent poison that after absorption may be dispersed in all tissues [51, 57]. Exposure to elevated concentrations of Hg, particularly MeHg, causes a number of adverse effects, such as developmental neurotoxicity, damage to the nervous system, immune system, kidney and liver, and other deleterious human health effects, mostly associated with the nervous system [52, 54]. Mercury poisoning in the adult brain is characterized by damage to discrete visual cortex areas and neuronal loss in the cerebellum granule layer [62]. One of the most glaring examples of Hg poisoning as a consequence of water pollution by industry is Minamata disease. This biological disaster took place at Minamata City, Japan in the 1950s, and it was attributed to the intake of fish and zoobenthos contaminated with MeHg [13, 51].

According to the criterion of the US Environmental Protection Agency (EPA), Hg concentrations in fish tissue cannot exceed 0.3 μg/g if used as human food [54]. The tolerable weekly intake (TWI), which is the amount of a given substance that can be consumed every week over the course of a lifetime without causing any appreciable health effects for consumers, has been established as 1.3 μg/kg body weight for MeHg, and 4.4 μg/kg body weight for inorganic mercury [57]. Consequently, the type of fish and seafood, the frequency of consumption, and meal size are essential issues in the balance of benefits and risks of a regular consumption of fish [63].

Marine shellfish have become commercially very important species, with an annual production growth rate of about 8%, as a consequence of increasing global seafood consumption in recent years. Shellfish farming is widespread in European maritime states [64]. Shellfish farming is an important source of pollution to coastal environments. Intensive shrimp farming has recently occupied extensive coastal areas worldwide, and it depends on large inputs of artificial feed like fishmeal, fertilizers and other chemical additives. The ubiquitous presence of Hg in these components produces an increase of this metal in aquaculture effluents. Hg is also of great significance by its toxicity toward phytoplankton and the shrimps. In north-east Brazil, shrimp farming has increased exponentially during the last decade, from an annual production of about 7000 t to over 90,000 t in 2003. Since Hg may be present as an impurity in the feed and chemicals used in aquaculture, the rapid increase in intensive shrimp farming in north-east Brazil may have also increased Hg emission to local estuaries, with concentrations of Hg in shrimp biomass of 16.2 ± 1.2 ng/g wet weight. This value is far below the maximum permissible in seafood for human consumption adopted by Brazilian legislation [65]. A recent study on the Adriatic coast of Croatia [64] showed various Hg concentrations in whole soft tissues of four shellfish species (mussels, oysters, variegated scallops and warty venus) from 19 sites, values ranging from 0.005 to 0.680 ng/g wet weight. The Hg concentration in wild mussels from the central Adriatic (site 14) exceeded the regulatory limits. The highest Hg concentrations were recorded in wild mussel (0.680 ng/g wet weight) and variegated scallop (0.512 ng/g wet weight) collected from wild sites. However, investigation of Hg in mussels collected from the natural beds and mussel farms in the Slovenian part of the northern Adriatic Sea revealed a higher Hg concentration in farmed mussels.

Eating fish is recommended because of its therapeutic and nutritional benefits, due to the content of high-quality protein, high content of two types of omega-3

polyunsaturated fatty acids and other nutrients. All these prevent coronary heart diseases [62]. The American Heart Association (AHA) suggests eating a variety of fish (preferably fatty fish) at least twice per week [62, 63]. However, several authors have shown that a potential risk of dietary fish is its content of heavy metals, with evidence that a high content of Hg in its organic form in some fish may counteract the cardioprotective effect of fish intake [62]. A study in British Columbia salmon compared the flesh quality of farmed ($n = 110$) and wild ($n = 91$) sources with respect to concentrations of total mercury (THg) and MeHg and selected fatty acids. The results showed that THg and MeHg levels in all samples were well below the Health Canada guideline (0.5 ng/g wet weight) and the US Food and Drug Administration (FDA) action level (1.0 ng/g wet weight). On average, THg in farmed salmon was similar to or lower than that in wild salmon. All this would indicate that the benefits outweigh the risks [63].

27.4.2 Cadmium in squid

Cadmium in its inorganic form is a natural element in the environment; anthropogenic sources have further contributed to background levels of Cd in soil, water and living organisms. Food is one of the most important Cd sources to the human populations [66, 67]. It can cause kidney failure and has been statistically associated with an increased risk of cancer. The Joint FAO/WHO Expert Committee on Food Additives [68] established a provisional tolerable monthly intake of 25 μg/kg body weight, whereas the EFSA Panel on Contaminants in the Food Chain [69] nominated a TWI of 2.5 μg/kg body weight to ensure sufficient protection of all consumers.

Trace elements in cephalopods have received increasing attention in recent decades as they are important components of marine food webs, fisheries and an increasingly valuable direct human resource [70]. Squid accumulate traces of heavy metals, including Cd [71–73], from water and food sources and hence constitute an important source of Cd for cephalopod predators and act as vectors through biomagnification, increasing the contaminant load in higher trophic levels, including humans [74–77]. The commercial significance of cephalopods, especially squid, to world fisheries is relatively recent but growing in importance [78, 79]. In the 1990s alone, there was a 40% increase in squid catches worldwide [80].

Most commercially exploited squid species have a lifespan of approximately 1 year, at the end of which there is a single spawning event followed by death. As with other cephalopods, they are characterized by a high growth rate, high mortality rate and high fecundity. Several studies have demonstrated their ability to rapidly accumulate high concentrations of trace elements in their tissues, making them good bioindicators of environmental levels but also a potential source of human intoxication [77, 81].

Many studies highlight that the digestive gland in squid has a central role in the bioaccumulation of trace elements, particularly toxic metals such as Cd [82–84]. Reported Cd concentrations in the digestive gland of squids are higher than in other tissues, independent of habitat and geographical origin, and are extremely variable between species and individuals [74, 85]. Cd levels between 4.77 μg/g dry weight in *Loligo forbesi* [88] and 826.5 μg/g dry weight in *Ommastrephes bartrami* have been detected [87]. On the other hand, the mantle of squid, which is consumed by humans, is among the tissues with the lowest heavy metal concentration, with observed values between 0.08 μg/g dry weight in *Illex argentinus* [88] and 1 μg/g dry weight in *Onychoteuthis borealijaponica* [89]. Concentrations of Cd in squid tissues from various recent studies has been summarized in Table 27.1.

Considering body size, in some squid species a correlation has been observed between size (mantle length) and trace element concentrations in its tissues [86, 93]. Such an observation is common across a large spectrum of marine species. Frequently, given the bioaccumulating properties of trace elements, their concentrations tend to increase with size (and presumably age) of the individual. Conversely, studies in other squid species have shown high Cd concentrations in the digestive gland and muscle of small individuals [88, 90]. A plausible explanation for this negative correlation could be attributed to the marked modification of the squid diet during its life cycle. It is common for squid to experience a drastic change in diet during ontogeny [90, 94, 95]. Another explanation could be the existence of a 'dilution' effect linked to the rapid increase in tissue mass due to growth with regard to proportionally lower intake of trace elements [90]. On the other hand, the absence of significant differences in Cd concentrations between the sexes has been demonstrated [73, 86, 90].

Some authors have established that squid species from sub-polar areas show higher levels of Cd than those living in temperate regions [74], while exposure

Table 27.1 Cadmium concentrations (mean ± SD, *n* in parentheses, μg/g dry weight, except as noted) in squid from different areas.

Species	Area	Tissue	Cd concentration	References
Alloteuthis sp.	UK waters	Digestive gland	9.48 ± 2.30 (5)	[90]
Ommastrephes bartrami	North Pacific	Digestive gland	211.0*	[91]
		Digestive gland	826.5 ± 369.1 (10)	[87]
Loligo forbesi	Scotland (west coast)	Digestive gland	4.77 (17)	[86]
	UK waters	Digestive gland	12.2 ± 9.92 (105)	[90]
		Mantle muscle	0.093 ± 0.146 (101)	
Illex argentinus	Argentina	Digestive gland	144.8 ± 65.0	[87]
	Argentina (Buenos Aires province)	Digestive gland (m)	194.30 ± 63.53 (7)	[88]
		Digestive gland (f)	163.96 ± 21.83 (5)	
		Mantle muscle (m)	0.16 ± 0.02 (7)	
		Mantle muscle (f)	0.08 ± 0.02 (5)	
	Argentina (northern Patagonia)	Digestive gland (m)	339.00 ± 129.10 (3)	
		Digestive gland (f)	485.01 ± 118.74 (3)	
		Mantle muscle(m)	0.80 ± 0.17 (3)	
		Mantle muscle (f)	0.74 ± 0.40 (3)	
	Brazilian waters	Digestive gland	1002.9 ± 566 (15)*	[73]
Onychoteuthis borealijaponica	North Pacific	Digestive gland	154.0 ± 37.0	[89]
		Mantle muscle	1.0 ± 0	
Todarodes sagittatus	UK waters	Digestive gland	65.3 ± 61.1 (11)	[90]
		Mantle muscle	0.304 ± 0.292 (12)	
Todaroces filippovae	South Pacific Ocean (Tasmania)	Digestive gland	98.5 ± 67.2 (30)	[86]
		Mantle muscle	0.20 ± 0.14 (32)	
	Indian Ocean (Amsterdam)	Digestive gland	246 ± 187 (24)	
		Mantle muscle	0.93 ± 0.792 (20)	
Todaropsis eblanae	UK waters	Digestive gland	25.1 ± 25.9 (23)	[90]
		Mantle muscle	1.55 ± 2.15 (23)	
Architeuthis dux	North-east Atlantic (Bay of Biscay)	Digestive gland	60.8 ± 46.2 (5)	[92]
	Mediterranean	Digestive gland	90.7	
Sthenoteuthis oualaniensis	Northwest coast of India	Mantle muscle	1.44 ± 0.29 (30)*	[93]
		Digestive gland	435.22 ± 61.27 (30)*	

f, female; m, male.
*μg/g wet weight.

to soluble metals in natural sea water is extremely low in polar regions [96, 97]. As a general rule, Cd concentrations in various phyla increase 'naturally' from the temperate zone to sub-polar and polar waters [98–100], and thus the bioaccumulation effect is more evident at high latitudes. On the other hand, the higher Cd concentrations in the digestive gland of oceanic squid compared with those in squid from coastal regions may be because the surface layer of the ocean contains low levels of Cd compared with deeper regions [101].

Finally, cephalopods may constitute a significant source of Cd intake for humans [78, 102]. In most European countries, viscera are removed before consumption but in southern Europe the consumption of complete bodies of small squid may also contribute significantly to Cd intake, since their digestive glands account for about 5% of total body mass [90]. Moreover, in Japan the digestive gland is considered to be a delicacy, called *kimo*, and is essential for making a widely consumed food named *shiokara*. Thus, regardless of the squid's origin, digestive glands should be avoided since in most cases Cd concentrations exceed the authorized limits. As mentioned, heavy metal contamination of squid has potential implications for human health and the conservation of marine mammals [103], so it should be monitored on a regular basis.

27.4.3 Copper and zinc in bivalve molluscs and crustaceans

Aquatic organisms can either regulate concentrations of trace essential elements like copper and zinc in their bodies or accumulate them, as many invertebrates usually do. For instance, marine bivalves have contrasting abilities to assimilate Zn. Molluscs like oysters concentrate up to 10 times higher amounts of Zn than mussels or clams. As for Cu, bioaccumulation in these organisms has been known since the discovery of 'green-sick' oysters 120 years ago by Lankester [104], where the green colour was attributed to the Cu contained in the flesh. Specific information regarding differences in heavy metal accumulation has been reviewed elsewhere [105].

Even though marine organisms are nutritious food [106, 107], edible seafood can accumulate substantial concentrations of toxic elements according to their habitat and feeding behaviours [106, 108]. Since these foods have a high consumption, trace metals in the soft tissue of crustaceans and bivalve molluscs should be evaluated with respect to human health. Several case studies have been published in view of the potential risk for human populations. One of the first places evaluated was Restronguet Creek, which is a classic contaminated site with large amounts of heavy metals, including Zn, as a result of mining activities at the beginning of the twentieth century [109].

Water courses in Taiwan are popular areas under study. A series of published articles analysed the impact of metal pollution on seafood. Back in 1994 in the Erhjin Chi estuary, the first reports appeared of the inherent health threat from consuming seafood from Cu-contaminated coastal environments [110]. The oyster *Crassostrea gigas* showed the highest concentration (4.401 ± 79 μg/g dry weight), this average being 14 times higher than international limits. Further results showed that the area around the estuary was affected by discharges of heavy metals from acid cleaning of metal scrap on the riverbanks. Moreover, studies done on the Hsianghsan coastal area concluded that the ranges of Cu and Zn concentrations in oysters differed significantly from other seafood evaluated (shrimps and clams) as follows: Cu, 0.860–909 μg/g; Zn, 17.4–1293 μg/g (dry weight). Oysters exhibited the ability to concentrate these two metals, increasing the risks of consumption [111]. Likewise, researchers reported high Cu concentrations in green oysters from the Sien-San coastal area [112] and after a high-tech industrial park, a garbage dump and an incinerator facility were established in the vicinity of the area. All specimens showed a minimum Cu concentration of 506 μg/g dry weight. Zn also showed a similar pattern, with the highest concentration of 5829 μg/g dry weight. These high concentration observed in the Sien-San area are some of the highest in the available literature.

On the French Atlantic coast, where oyster farming is the primary aquaculture practice, analyses were carried out [113]. The study provided a realistic estimate of trace metal bioavailability in oysters (*C. gigas*). For Cu, the intake amounts were at the lower end of the recommended level. However, since ingestion of 10–15 mg of Cu at one time has been reported toxic to humans, the consumption of two dozen oysters from the French coast under analysis could be dangerous. Bioavailable quantities of Zn were also above the recommended intake (122–344%). Other investigations included commercial shellfish species: green mussels (*Perna viridis*), scallops (*Chlamys nobilis*), clams (*Marcia hiantina*), oysters (*Saccostrea cucullata*, *Crassostrea gigas*, *Ostrea edulis*) and neogastropod molluscs (*Buccinum undatum*) from western Europe (France, UK) and Asia (Hong Kong) [107]. For Cu, oysters showed mean concentrations higher than the Australian maximum permitted levels specifically established for molluscs. For Zn, mean concentrations were higher in all the studied oyster species as well as in *B. undatum* and scallops.

Research in the Jiulong River estuary, China reported a case study analysing green oysters (*Crassostrea angulata*) and blue oysters (*Crassostrea hongkongensis*). Concentrations of heavy metals were elevated, with Zn levels as high as 24,000 μg/g dry weight (equivalent to 2.4% of their tissue weight). Cu levels up to 14,000 μg/g dry weight were also found in the oysters (1.4% of their tissue weight). In fact, these individuals were blue. Another publication in China focused on determining trace elements in these marine invertebrates [114]. Total Cu concentrations were 0.62–190 μg/g dry weight and usually exceeded the limit levels (50 μg/g dry weight) established by China. Despite the habitat, Cu concentrations in oysters were much higher than those in other invertebrates [109].

Another study on the Romanian Black Sea coast analysed tissues from the mussel *Mytilus galloprovincialis* [106]. High Zn and Cu concentrations (Zn >50 μg/g; Cu 5.88 μg/g and 9.87 μg/g dry weight) in all tested samples were above recommended limits (Zn, 50 mg/kg dry weight; Cu, 5 mg/kg dry weight).

In Mexico [115], the mangrove oyster *Crassostrea corteziensis* was selected for investigation because it is used for human consumption. Even though certain areas required great quantities of Cu and Zn-based products for crop protection, maximum values of the elements were found in regions near a thermoelectric power plant as well as industrial and municipal effluents. This was probably because of the Cu-based antifouling paints and the wastewaters from industries.

In other studies, results of Zn and Cu concentrations in edible molluscs and crustaceans did not show levels above the limits of consumption, indicating that there is no risk to humans [22, 116–118]. Allowable concentrations of Cu and Zn from different areas of the world are reviewed elsewhere [119].

27.4.4 Arsenic and selenium in seafood

Arsenic (As) is a metalloid that occurs in different inorganic and organic forms, and is found in the environment naturally as well as a result of anthropogenic activity [19]. The inorganic forms of As are more toxic than the organic forms. Seafood like fish, crustaceans and molluscs contain As at exceedingly high concentrations and these high levels are present as organically bound species. Arsenobetaine is the most widespread and abundant of the organoarsenic compounds found in marine fish and most other seafood [120]. It is a harmless compound which, after ingestion by humans, is rapidly excreted unchanged in the urine. The dominance of this form of As in marine animals is likely related to the similar chemistry between As and nitrogen. Some species of As show structural similarities to certain biologically important forms of nitrogen and phosphorus, which probably facilitates arsenic's uptake and retention in marine organisms [121]. Arsenobetaine is structurally very similar to glycine betaine, which contains nitrogen and serves as an osmolyte protecting osmoconforming organisms from changes in the salinity of ambient water [122]. It thus seems possible that the cell is unable to distinguish between these ions and takes up arsenobetaine instead of glycine betaine. Another possibility is that organoarsenic compounds are found in marine organisms at high concentrations because they are used by the organism for a particular role and their biosynthesis is directed by biochemical requirements of the organism [120]. Over 50 additional As species have been identified in marine organisms, but the most important are arsenosugars, because they occur at high levels; arsenate, because it has toxic properties; and dimethylarsinate, because it has important metabolites. However, for most of these 50 compounds, human toxicology and their metabolism remains to be investigated [120]. In humans, the uptake of As occurs mainly via ingestion of seafood, with As found at concentrations ranging from 1 to 200 μg/g dry weight [123]. Additionally, the levels of inorganic As are typically below 0.2 μg/g dry weight [124]. However, not all seafood shows low concentrations of inorganic As; for example, edible seaweed (*Hizikia fusiformis*) exhibits levels of 60 μg/g dry weight [125].

Table 27.2 shows As concentrations in different seafoods consumed by humans worldwide. Uneyama *et al.* [126] produced a meta-analysis of the published data on As concentrations found in food. This showed that fish and seafood, including seaweed, are the major worldwide food sources of total As.

The International Agency for Research on Cancer (IARC) classifies inorganic As in group 1 as carcinogenic when people are exposed to As in the environment or through their diet. This was based on the induction of primary skin, lung and urinary bladder cancer. Arsenic is also more acutely toxic than other metallic compounds and continual low-level exposure to As is associated with skin, vascular and nervous system disorders. On the other hand, long-term ingestion of inorganic As has been associated with skin lesions, neurotoxicity, cardiovascular diseases and diabetes. There is also evidence of negative impacts on fetal and infant development [125]. The European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM Panel) has assessed the risks to human health related to the presence of As in food.

The inorganic As exposure from food and water in European countries has been estimated to range from 0.13 to 0.56 μg/kg body weight per day for average consumers, and from 0.37 to 1.22 μg/kg body weight per day for 95th percentile consumers. In this report the CONTAM Panel recommended:

1. that inorganic As through dietary exposure be reduced;
2. the need to produce speciation data for different food commodities to support dietary exposure assessment and dose–response data for possible health effects;
3. the need for improved understanding of the human metabolism of organoarsenicals in foods (arsenosugars, arsenolipids, etc.) and the human health implications;

Table 27.2 Arsenic and selenium concentrations in different fish and seafood consumed by humans.

Fish and seafood	*n*	Arsenic (μg/g d.w.)		Selenium (μg/g d.w.)		Reference
		Mean	Max.	Mean	Max.	
Bivalve molluscs	664	3.4	150	—	—	[125]
Cephalopods	189	3.9	66.80	—	—	
Crustaceans	344	5.69	100.40	—	—	
Seafood and seafood products	1347	5.01	150	—	—	
Fish and fish products	3503	1.45	195	—	—	
Fish-based preparations	233	1.15	20.17	—	—	
Cephalopod (*Todarodes filippovae*)						[87] Tasmania and
Branchial heart	32	<DL	<DL	—	—	Indian Ocean
Digestive gland	30	17.1	41.3	—	—	
Gills	32	21	32.3	—	—	
Muscle	32	15.3	22.6	—		
Fish						[127] Lake
Acanthopagrus australis	10	6.2	—	5.7	—	Macquarie Estuary,
Gerres subfasciatus	10	6	—	9.2	—	Australia
Monacanthus chinensus	9	11	—		—	
Anadara trapezia	10	—	—	5.2	—	
Platycephalus fuscus	7	—	—	9.3	—	
Pelagic fish type						[128] Central North
Thunnus alalunga	50	—	—	1.25	—	Pacific
Coryphaena hippurus	30	—	—	0.53	—	
Katsuwonus pelamis	10	—	—	1.57	—	
Thunnus obesus	50	—	—	0.99	—	
Xiphias gladius	50	—	—	0.39	—	
Isurus oxyrinchus	10	—	—	0.32	—	
Thunnus alalunga	20	—	—	0.88	—	
Food item				(μg Se per 100 g w.w.)		[129] Bangkok,
Batrachian catfish	10	—	—	47.3	68.1	Thailand
Short-bodied mackerel	10	—	—	88.1	113.7	
Silver pomfret	10	—	—	52.3	71.7	
Serraed mud crab	10	—	—	46.1	59.9	
Green mussel	10	—	—	42.6	51.5	
Pacific oyster	10	—	—	29.3	38.5	
Green tiger, prawn	10	—	—	35.4	58.9	
Fish				(mg/kg w.w.)		[43] Modena, Italy
Lophius piscatorius		—	—	0.173	—	
Thunnus thynnus		—	—	0.734	—	
Sardina pilchardus				0.678	—	
Clam						
Venerupis decusata		—	—	0.330	—	
Crab						
Callinectessapidas		—	—	0.454	—	
Prawn						
Paeneus kerathuru		—	—	0.257	—	

Table 27.2 (Continued)

Fish and seafood	*n*	Arsenic (μg/g d.w.)		Selenium (μg/g d.w.)		Reference
		Mean	Max.	Mean	Max.	
Mussel						
Mytilus edulis		—	—	0.605	—	
Octopus						
Octopus vulgaris		—	—	0.176	—	
Squid						
Loligo vulgaris		—	—	0.444	—	
Fish				(nmol/g)		[130] Río de Janeiro Coasts, Brazil
Centropomus undecimalis	18	—	—	10.11	18.81	
Micropogonias furnier	73	—	—	6.2	13.10	
Paralonchurus brasiliensis	17	—	—	8.85	17.55	
Anchoa filifera	10			2.81	3.24	
Cephalopod						
Loligo sanpaulensis	32	—	—	0.95	1.7	
Bivalve						
Perna perna	153	—	—	2.75	4.48	

4 the need for robust validated analytical methods for determining inorganic As in a range of food items;
5 to incorporate better characterization of exposure to inorganic As including food sources in epidemiological studies [125].

Selenium (Se) is a nutritional element essential for the metabolic activity of several enzymes that play important roles in the brain and endocrine tissues [131]. These enzymes perform numerous antioxidant functions [132] and, together with other Se-containing molecular species, appear to be important in selenium's role in the prevention of cancer [133] and in supporting a healthy immune system. Selenium is present in all foods, but is particularly abundant in ocean fish and seafood, ranging from 0.4 to 1.5 μg/g dry weight [134]. The molecular forms that predominate in foods are selenocysteine and selenomethionine. However, high amounts of Se have toxic effects. This trace element is harmful at dietary intakes in excess of 1 mg/day, but selenium deficiency can also lead to symptomatic disturbances in humans and animals [135]. Dietary intake averaging 19 and 14 μg/day for adult men and women, respectively, protects against several diseases [136]. Additionally, the US recommended daily allowance (RDA) for selenium is 15–20 μg for infants, 20–30 μg for children, 40–55 μg for adults, 60 μg for pregnant women and 70 μg for lactating mothers [134]. Table 27.2 shows studies on Se concentrations in different fish and seafood consumed by humans worldwide. In all cases the levels are within the allowable values for human consumption.

In addition, Se acts as a protection against Hg toxicity [137]. It is believed that the toxicity of MeHg is linked to its interaction with selenium because the target tissues of MeHg toxicity are the neuroendocrine and nervous systems. Selenium readily complexes with mercury because of the high binding affinity between these metals, and recent research suggests that supplemental Se protects against Hg toxicity by offsetting the loss and sequestration of Se by Hg. Thus, the presence of Se in ocean fish and the protective effects of this metal against Hg are important considerations in the seafood safety issue. In this sense, the molar ratio of Hg and Se in the diet appears to be the essential criterion of risk from Hg exposure rather than considering Hg content alone. Se/Hg molar ratios exceeding 1 : 1 are thought to provide Se-dependent health benefits, but not necessarily those with Se/Hg molar ratios approaching 1 : 1 since that set of fish would necessarily include fish with mercury in molar excess of selenium. Foods that supply more Se than Hg protect against mercury toxicity and provide Se-dependent health benefits, whereas those that contain more Hg than Se are associated with mercury health risks. In the case

of marine fish, they generally contain far more Se than Hg, and consuming marine fish has been shown to prevent rather than contribute to Hg toxicity [131]. Although studies in animals showed that selenite reduces inorganic Hg toxicity, there is almost no evidence for protection from MeHg toxicity by the organic forms of selenium found in the human diet. Moreover, there are no human studies which show a protective role for selenium against Hg neurotoxicity [138].

27.4.5 Calcium, magnesium, sodium and potassium in fish and shellfish

Fish consumption has increased at a global level from an average of 10.1 kg per capita per year in 1965 to 16.4 kg per capita per year in 2005 [139]. As a result, in many cases this may reduce the risk of multiple adverse health disorders associated with a imbalance of several important ingested nutrients (i.e. macrominerals). However, the volume of fish consumption varies among different regions of the world. In all regions, fish consumption per capita has increased, except in sub-Saharan Africa and Latin America/Caribbean, where it has stagnated from the early 1970s [140]. On the other hand, these food items represent more than 70% of the diet in Maldives, Sierra Leone and Solomon Islands [140].

Fish and a variety of other organisms from the aquatic environment have been much appreciated as food by humans over the ages. The term 'seafood' includes fish and shellfish (crustacean and molluscs), which have been recognized as part of a healthy and balanced diet, being an important sources of nutrients as proteins, polyunsaturated lipids and phospholipids, as well as of several micronutrients, group B vitamins, vitamins A and D and minerals (phosphorus, potassium, sodium, calcium, magnesium, iron and iodine) [141, 142]. These minerals are present in different proportions depending on the animal group: 10% for fish, 8% for crustaceans and 11% for molluscs [142].

A number of analyses have investigated the nutritional mineral content of seafood and the effects of cooking methods, although it is difficult to generalize and to establish mean mineral values because they depend on several factors such as species, sex, biological cycle and portion of fish analysed, as well as on ecological factors such as season, place of development, nutrient availability, temperature and salinity of the water [143].

The recommended daily intakes (dietary reference intakes or DRIs) for the main four minerals are as follows: sodium, 1500 mg; potassium, 4700 mg for adolescents and adults, 300 mg for children 1–3 years of age, 3800 mg for children 4–8 years of age, and 4500 mg for children aged 9–13 years; calcium, 1000–1300 mg (therefore usual intakes are only 38–50% of DRI); and magnesium, 420 mg for adult men, 300 mg for women [144, 145].

Nutritional changes caused by processing of fish and shellfish is an important issue. Although it destroys certain vitamins and changes mineral concentrations, processing still greatly contributes to extending the period over which seafood can be consumed [139, 146, 147].

A variety of studies have assessed the effect of different methods of cooking (baking, grilling, microwaving and frying) on the mineral content of seafood, using values from raw fillets as a reference (Table 27.3). Although these methods affect the composition of minerals such as calcium, magnesium, potassium and sodium, it has been concluded that baking and grilling are the cooking methods that can be recommended, since increasing values of Ca, Mg, K and Na are generally observed at the end of those processes [146, 147].

Other studies have considered the presence and/or absence of bone in manufactured fish and shellfish. From a nutritional point of view the results demonstrated that products which include bone were good sources of Mg and Ca. Thus addition of bone can be considered an important supplement to the minerals in the diet [142, 143].

Another benefit of including fish and seafood in the human diet is the significant contribution they make to improving bone mineral density in both men and women. Intakes greater than 250 g of fish and seafood per week produce high benefit [149]. Although fish and seafood are significant sources of Na, Ca, K and Mg, those people who suffer from diseases such as hypertension should be careful with sodium intake; however, an improved reduction in pressure is seen when reduction of salt intake is combined with increased intakes of potassium, calcium and magnesium [144].

27.5 Risks and consequences of high exposure to metals: major diseases and syndromes

People may be exposed to potentially harmful chemical, physical and biological agents in air, food, water and soil. However, exposure does not result only from the

Table 27.3 Mean mineral contents of raw fish fillets (mg/kg).

Species	Ca	K	Mg	Na	Country
Clarias gariepinus (African catfish) [151]	40.1 ± 0.08	1817 ± 132.4	184 ± 18.5	308 ± 0.35	Turkey
Oncorhynchus mykiss (rainbow trout) [152]	632 ± 136	3060 ± 56.8	409 ± 13.2	455 ± 24.5	Turkey
Pterois volitians (lionfish) [153]	355 ± 184	3481 ± 425	322 ± 38	700 ± 146	Jamaica

presence of a harmful agent in the environment. The key word in the definition of exposure is 'contact'. There must be contact between the agent and the outer boundary of the human body, such as the airways, the skin or the mouth. Exposure is a function of concentration and time [150]. This is an important point: because of their toxicity, persistence and tendency to accumulate in water and sediment, heavy metals and metalloids become severe poisons for all living organisms when occurring in higher concentrations [151].

Persistent environmental pollutants (PEPs) in seafood have gained special attention from consumers, scientists, and environmental and governmental organizations due to the widespread consumption of seafood. Several studies have determined PEPs in aquatic organisms from both freshwater and marine environments. In addition to the marine ecosystem, products from aquaculture are also associated with increased risk of contamination by these pollutants, as well as antibiotic residues. Within this in mind, it is important to monitor trace element contents in seafood intended for human consumption, not only directly but also indirectly, and much attention has been focused on potential human exposures to mercury, cadmium, lead and arsenic [152].

27.5.1 Mercury

The general population is primarily exposed to Hg via food, and fish is a major source of MeHg exposure [6, 8]. Once this element has been incorporated in the human body, Hg in urine is related to exposure to inorganic compounds, whereas blood Hg indicates exposure to MeHg. Acute Hg exposure may give rise to lung damage. Chronic poisoning is characterized by neurological and psychological symptoms, for example tremor, changes in personality, restlessness, anxiety, sleep disturbance or depression [153]. MeHg poisoning is related to nervous system damage. The earliest symptoms are paraesthesias and numbness in hands and feet. These signs are usually followed by coordination difficulties and concentric constriction of the visual field, as well as auditory symptoms. High doses may lead to death (Minamata disease in the 1950s or Iraq in the 1970s) [154, 155]. In addition, a high dietary intake of Hg from consumption of fish has been hypothesized to increase the risk of coronary heart disease [156, 157].

Another important effect which must be highlighted is that mercury is a potent neurotoxin because the body lacks external barriers or internal detoxification systems for mercury [158]. MeHg passes the blood–brain barrier (BBB) and nuclear membranes to react directly with both nuclear and cellular components. Hg accumulation in the brain, compared with blood and muscle, occurs much less in fish than in mammals. The blood-to-brain Hg ratio has been shown to be 100 times higher in terrestrial mammals than in fish [159]. Neither reproductive performance nor teratological effects are affected by exposure to Hg chloride.

In addition, the effects produced by marine pollution are best described by various manifestations, such as damage to individual animals, decrease in abundance of local populations, or damage to entire ecosystems [160]. It is easy to identify specific abnormalities in animals from the most impacted areas: deformed fish embryos and larvae can be observed in plankton collections, juvenile fish with skin ulcerations and fin erosion, and adult fish with cancerous or precancerous liver lesions [161]. Effects at population level are more difficult to detect, except by observing gradual disappearances of less resistant species from the areas of heaviest contamination or cataloguing the reduced reproductive success of vulnerable resident species [162]. At the ecosystem level, evidence for disturbance may be recorded as mass mortalities of fish or shellfish, extensive blooms of toxic algae, or the sudden intrusion and temporary dominance of noxious non-native organisms [163].

The effects on biota could be expressed by biomarker responses, in laboratory experiments and in field studies. Exposure of fish to different Hg concentrations produces effects during the larval stage, alterations in haematological parameters, decreasing enzymatic activities

(e.g. acetylcholinesterase), histopathological alterations in liver and kidney, problems during gonad development, reduction of egg incubation success, decreased locomotor activity, genotoxic effects, brain lesions and death [56]. A study revealed that mercuric chloride ($HgCl_2$) is a nephrotoxic agent, causing severe damage and altered histomorphology of fish kidney (primary target site), and therefore might affect its function [51].

The genotoxic potential of mercury can be observed in terms of induction of micronuclei in peripherical erythrocytes of fish blood. The micronucleus assay is a significant, useful and potent tool for evaluating genotoxicity and clastogenic effects due to exposure to inorganic/organic Hg in aquatic systems. Fish inhabiting polluted waters have greater frequencies of micronuclei, and this may vary according to the type of pollution involved, season and species of fish. The incidence of micronuclei has showed positive correlations with Hg in fish muscle [52, 56]. Micronuclei are formed by chromosome fragments or whole chromosomes that lag at cell division due to lack of a centromere, damage to the erythrocyte chromosome, or a defect in cytokinesis [56].

Finally, it is important to remember that most of the effects described in different aquatic animal species have been demonstrated to be highly risky for humans, as has unfortunately been demonstrated by Minamata disease or events in Iraq [154, 155].

27.5.2 Cadmium

Food is the most important Cd exposure in the general non-smoking population in most countries [164]. With regard to the incorporation of this metal, it has been reported that gastrointestinal absorption of Cd may be influenced by nutritional factors such as Fe status [165].

Blood Cd levels generally reflect current exposure, while urine Cd levels are proportional to kidney Cd concentration [166]. Cd exposure may cause kidney damage, which manifests as tubular dysfunction (increased excretion of low-molecular-weight proteins or enzymes), decreased glomerular filtration rate, and occurrence of kidney stones possibly related to increased excretion of Ca in urine following tubular damage [167]. End-stage renal disease has been described linked to the association between Cd exposure and chronic renal failure [168].

Long-term high Cd exposure may cause skeletal damage, first reported in Japan where itai-itai disease was discovered in the early 1950s [169]. This condition produces low bone mineral density (osteoporosis) and fractures [170]. Complementary studies have also shown an excess risk of cardiovascular mortality in Cd-exposed persons with signs of kidney tubule damage compared with individuals without kidney damage [171, 172].

There is consistent evidence for Cd as a human carcinogen, particularly after oral exposure. The IARC has classified Cd as 'probably carcinogenic for humans' (IARC Group 2A) [173], which is in accordance with the EC classification of some Cd compounds (Carcinogen Category 2, Annex 1 to the Directive 67/548/EEC) [174, 175].

27.5.3 Lead

The general population is exposed to lead from air and food in roughly equal proportions [150]. Pb in blood is bound to erythrocytes, and elimination is slow and via urine; in addition, Pb is accumulated in the skeleton [176]. Inorganic Pb does not penetrate the BBB, but this barrier is less developed in children [177, 178]. Thus the high gastrointestinal uptake and permeable BBB make children especially susceptible to lead exposure, and subsequent brain damage [179]. Organic Pb compounds penetrate body and cell membranes, as well as skin, and may cross the BBB in adults, producing Pb encephalopathies [177, 178].

The main symptoms of Pb poisoning are headache, irritability, abdominal pain, sleeplessness and restlessness. Behavioural disturbances, learning and concentration difficulties, acute psychosis, confusion and reduced consciousness may also be observed. Long-term low-level Pb exposure in children may lead to diminished intellectual capacity [180, 181].

Finally, it is important to highlight that IARC classify Pb as a 'possible human carcinogen', with lung cancer, stomach cancer and gliomas the most likely candidates [182].

Abbreviations

AHA: American Heart Association
AOAC: Association of Official Analytical Chemists
BBB: Blood–brain barrier
CV-AAS: Cold vapour atomic absorption spectroscopy
DBT: Dibutyltin
DOC: Dissolved organic carbon
DRI: Dietary reference intake
EFSA: European Food Safety Authority
ET-AAS: Electrothermal atomic absorption spectrometry
FAAS: Flame atomic absorption spectrometry

GFAAS: Graphite furnace atomic absorption spectrometry
HG-AAS: Hydride generation atomic absorption spectrometry
HG-AFS: Hydride generation atomic fluorescence spectrometry
HPLC-ICP-MS: High-performance liquid chromatography inductively coupled plasma mass spectrometry
IARC: International Agency for Research into Cancer
ICP-MS: Inductively coupled plasma mass spectrometry
ICP-OES: Inductively coupled plasma optical emission spectrometry
MeHg: Methylmercury
PEP: Persistent environmental pollutant
RDA: Recommended daily allowance
ΣBT: Sum of butyltin compounds
TBT: Tributylyin
TDS: Total diet study
THg: Total mercury
TWI: Tolerable weekly intake
UAD: Ultrasound-assisted acid digestion method
US EPA: United States Environmental Protection Agency
US FDA: United States Food and Drug Administration
WHO: World Health Organization

References

1 Institute of Medicine. *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride*. National Academy Press, Washington, DC, 1997. Available at http://www.nap.edu/openbook.php?record_id=5776&-page=190 (accessed 18 June 2013).

2 Institute of Medicine. *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate*. National Academy Press, Washington, DC, 2005. Available at http://www.nap.edu/openbook.php?record_id=10925 (accessed 18 June 2013).

3 Chekri R, Noël L, Millour S *et al*. Calcium, magnesium, sodium and potassium levels in foodstuffs from the second French Total Diet Study. *J Food Compos Anal* 2012;**25**:97–107.

4 Nordberg GF, Sandström B, Becking G, Goyer RA. Essentiality and toxicity of metals. In: Sarkar B (ed.) *Heavy Metals in the Environment*. Marcel Dekker, New York, 2002, pp. 1–34.

5 Joint FAO/WHO Expert Committee on Food Additives. JECFA 61st Meeting. Rome (Italy), 10–19 June 2003. Available at ftp://ftp.fao.org/es/esn/jecfa/jecfa61sc.pdf.

6 WHO/UNEP. *Guidance for Identifying Populations at Risk from Mercury Exposure*. World Health Organization (WHO)/United Nations Environment Programme (UNEP), Inter-Organization Programme for the Sound Management of Chemicals (IOMC), Geneva, 2008. Available at http://www.who.int/foodsafety/publications/chem/mercuryexposure.pdf.

7 European Food Safety Authority. Scientific opinion on lead in food. EFSA Panel on Contaminants in the food chain (CONTAM). *EFSA J* 2010;**8**:1570.

8 FAO/WHO. Working document for information and use in discussions related to contaminants and toxins in the GSCTFF (General Standard for Contaminants and Toxins in Foods). Joint FAO/WHO Food Standards Programme Codex Committee in Contaminants in Foods, Codex Alimentarius Commission, CF/5-INF/1, March 2011. Available at ftp://ftp.fao.org/codex/meetings/CCCF/cccf5/cf05_INF.pdf.

9 FAO/WHO. Summary of evaluations performed by the joint FAO/WHO expert committee on food additives (JECFA 1956–2007) (first through 68th meetings). FAO/WHO, Washington, DC, 2007.

10 Goldhaber SB. Trace elements risk assessment: essentiality vs. toxicity. *Regul Toxicol Pharmacol* 2003;**38**:232–242.

11 Momot O, Synzynys B. Toxic aluminum and heavy metals in groundwater of Middle Russia: health risk assessment. *Int J Environ Res Public Health* 2005;**2**:214–218.

12 Seixas TG, Kehrig HA, Di Beneditto APM, Souza CMM, Malm O, Moreira I. Trace elements in different species of Cetacean from Rio de Janeiro coast. *J Brazil Chem Soc* 2009;**20**:243–251.

13 Zukowska J, Biziuk M. Methodological evaluation of method for dietary heavy metal intake. *J Food Sci* 2008;**73**:21–27.

14 World Health Organization. Total diet studies: a recipe for safer food. Available at http://www.who.int/foodsafety/chem/TDS_recipe_2005_en.pdf.

15 Nriagu JO. Global inventory of natural and anthropogenic emissions of trace metals to the atmosphere. *Science* 1979;**279**:409–411.

16 Reimann C, de Caritat P. Distinguishing between natural and anthropogenic sources for elements in the environment: regional geochemical surveys versus enrichment factors. *Sci Total Environ* 2005;**337**:91–107.

17 Davis HT, Aelion CM, McDermott S, Lawson AB. Identifying natural and anthropogenic sources of metals in urban and rural soils using GIS-based data, PCA, and spatial interpolation. *Environ Pollut* 2009;**157**:2378–2385.

18 Mendil D, Ünal ÖF, Tüzen M, Soylak M. Determination of trace metals in different fish species and sediments from the River Yeşilirmak in Tokat, Turkey. *Food Chem Toxicol* 2010;**48**:1383–1392.

19 Kumar R, Banerjee TK. Analysis of arsenic bioaccumulation in different organs of the nutritionally important catfish, *Clarias batrachus* (L.) exposed to the trivalent arsenic salt, sodium arsenite. *Bull Environ Contam Toxicol* 2012;**89**:445–449.

20 Hisamichi Y, Haraguchi K, Endo T. Levels of mercury and organohalogen compounds in Pacific bluefin tuna (*Thunnus orientalis*) cultured in different regions of Japan. *Arch Environ Contam Toxicol* 2012;**62**:296–305.

21 Chiu KH, Mok HK. Study on the accumulation of heavy metals in shallow-water and deep-sea hagfishes. *Arch Environ Contam Toxicol* 2011;**60**:643–653.

22 Bordon ICAC, Sarkis JES, Tomás ARG *et al*. A preliminary assessment of metal bioaccumulation in the blue crab,

Callinectes danae S., from the São Vicente Channel, São Paulo State, Brazil. *Bull Environ Contam Toxicol* 2012;**88**:577–581.

23 Noël L, Chekri R, Millour S *et al*. Li, Cr, Mn, Co, Ni, Cu, Zn, Se and Mo levels in foodstuffs from the Second French TDS. *Food Chem* 2012;**132**:1502–1513.

24 Çayır A, Coşkun M, Coşkun M. Evaluation of metal concentrations in mussel *M. galloprovincialis* in the Dardanelles Strait, Turkey, in regard of safe human consumption. *Bull Environ Contam Toxicol* 2012;**89**:91–95.

25 Alahverdi M, Savabieasfahani M. Metal pollution in seaweed and related sediment of the Persian Gulf, Iran. *Bull Environ Contam Toxicol* 2012;**88**:939–945.

26 Choi M, Moon HB, Choi HG. Intake and potential health risk of butyltin compounds from seafood consumption in Korea. *Arch Environ Contam Toxicol* 2012;**62**:333–340.

27 Marcovecchio JE, Botté SE, Domini CE, Freije RH. Heavy metals, major metals, trace elements. In: Nollet LML, De Gelder LSP (eds) *Handbook of Water Analysis*, 3rd edn. CRC Press, Boca Raton, FL, 2014, pp. 385–433.

28 Dural M, Lugal Göksu MZ, Akif Özak A. Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon. *Food Chem* 2007;**102**:415–421.

29 Fabris G, Turoczy NJ, Stagnitti F. Rapid communication: Trace metal concentrations in edible tissue of snapper, flathead, lobster, and abalone from coastal waters of Victoria, Australia. *Ecotoxicol Environ Saf* 2006;**63**:286–292.

30 Grotto D, Lemos Batista B. Hornos Carneiro MF, Barbosa F Jr. Evaluation by ICP-MS of essential, non-essential and toxic elements in Brazilian fish and seafood samples. *Food Nutr Sci* 2012;**3**:1252–1260.

31 Tüzen M. Determination of heavy metals in fish samples of the middle Black Sea (Turkey) by graphite furnace atomic absorption spectrometry. *Food Chem* 2003;**80**:119–123.

32 Tüzen M, Soylak M. Evaluation of trace element contents in canned foods marketed from Turkey. *Food Chem* 2007;**102**:1089–1095.

33 Botté SE, Freije RH, Marcovecchio JE. Distribution of several heavy metals in tidal flats sediments within Bahía Blanca Estuary (Argentina). *Water Air Soil Pollut* 2010;**210**:371–388.

34 Bashir FH, Othman MS, Mazlan AG, Rahim SM, Simon KD. Heavy metal concentration in fishes from the coastal waters of Kapar and Mersing, Malaysia. *Turkish J Fish Aquat Sci* 2013;**13**:375–382.

35 Association of Official Analytical Chemists. *Lead, Cadmium, Zinc, Copper and Iron in Food. Atomic Absorption Spectrophotometry after Microwave Digestion*. AOAC Official Method 999.10. AOAC, Rockville, MD, 2002.

36 Association of Official Analytical Chemists. *Determination of Lead, Cadmium, Zinc, Copper and Iron in Foods. Atomic Absorption Spectrophotometry after Dry Ashing*. AOAC Official Method 999.11. AOAC, Rockville, MD, 2002.

37 Lavilla I, Vilas P, Bendicho C. Fast determination of arsenic, selenium, nickel and vanadium in fish and shellfish by electrothermal atomic absorption spectrometry following ultrasound-assisted extraction. *Food Chem* 2008;**106**:403–409.

38 López I, Cuello S. Cámara C, Madrid Y. Approach for rapid extraction and speciation of mercury using a microtip ultrasonic probe followed by LC–ICP-MS. *Talanta* 2010;**82**:594–599.

39 Kazi TG, Jamali MK, Arain MB *et al*. Evaluation of an ultrasonic acid digestion procedure for total heavy metals determination in environmental and biological samples. *J Hazard Mater* 2009;**161**:1391–1398.

40 Ikem A, Egiebor NO. Assessment of trace elements in canned fishes (mackerel, tuna, salmon, sardines and herrings) marketed in Georgia and Alabama (United States of America). *J Food Compos Anal* 2005;**18**:771–787.

41 Uysal K, Emre Y, Köse E. The determination of heavy metal accumulation ratios in muscle, skin and gills of some migratory fish species by inductively coupled plasma-optical emission spectrometry (ICP-OES) in Beymelek Lagoon (Antalya/Turkey). *Microchem J* 2008;**90**:67–70.

42 Plessi M, Bertelli D, Monzani A. Mercury and selenium content in selected seafood. *J Food Compos Anal* 2001;**14**:461–467.

43 Schaeffer RC, Soeroes I, Ipolyi P, Fodor NS, Thomaidis P. Determination of arsenic species in seafood samples from the Aegean Sea by liquid chromatography–(photo-oxidation)–hydride generation–atomic fluorescence spectrometry. *Anal Chim Acta* 2005;**547**:109–118.

44 Ofiara DD, Seneca JJ. Biological effects and subsequent economic effects and losses from marine pollution and degradations in marine environments: implications from the literature. *Mar Pollut Bull* 2006;**52**:844–864.

45 Muramoto S. Vertebral column damage and decrease of calcium concentration in fish exposed experimentally to cadmium. *Environ Pollut* 1981;**24**:125–133.

46 Maceda-Veiga A, Monroy M, Navarro E, Viscor G, de Sostoa A. Metal concentrations and pathological responses of wild native fish exposed to sewage discharge in a Mediterranean river. *Sci Total Environ* 2013;**449**:9–19.

47 Kim SJ, Rodríguez-Lanetty M, Suh JH, Song JI. Emergent effects of heavy metal pollution at a population level: Littorina brevicula a study case. *Mar Pollut Bull* 2003;**46**:74–80.

48 Ryu JS, Khim JS, Kang SG, Kang D, Lee CH, Koh CH. The impact of heavy metal pollution gradients in sediments on benthic macrofauna at population and community levels. *Environ Pollut* 2011;**159**:2622–2629.

49 Medina MH, Correa JA, Barata C. Micro-evolution due to pollution: possible consequences for ecosystem responses to toxic stress. *Chemosphere* 2007;**67**:2105–2114.

50 Rutgers M. Field effects of pollutants at the community level: experimental challenges and significance of community shifts for ecosystem functioning. *Sci Total Environ* 2008;**406**:469–478.

51 Ghosh D, Mandal DK. Histopathological effects and bioaccumulation of mercury in the kidney of an Indian major

carp, *Labeo rohita* (Hamilton). *Bull Environ Contam Toxicol* 2012;**89**:479–483.
52 Yadav KK, Trivedi SP. Sublethal exposure of heavy metals induces micronuclei in fish, *Channa punctate*. *Chemosphere* 2009;**77**:1495–1500.
53 Newman MC, Xu X, Cotton ChF, Tom KR. High mercury concentrations reflect trophic ecology of three deep-water Chondrichthyans. *Arch Environ Contam Toxicol* 2011;**60**:618–625.
54 Emery EB, Spaeth JP. Mercury concentrations in water and hybrid striped bass (*Morone saxatilis* × *M. chrysops*) muscle tissue samples collected from the Ohio River, USA. *Arch Environ Contam Toxicol* 2011;**60**:486–495.
55 Maz-Courrau A, López-Vera C, Galván-Magaña F, Escobar-Sánchez O, Rosíles-Martínez R, Sanjuán-Muñoz A. Bioaccumulation and biomagnification of total mercury in four exploited shark species in the Baja California Peninsula, Mexico. *Bull Environ Contam Toxicol* 2012;**88**:129–134.
56 Rodrígues APC, Maciel PO, Pereira da Silva LCC *et al*. Biomarkers for mercury exposure in tropical estuarine fish. *J Brazil Soc Ecotoxicol* 2010;**5**:9–18.
57 European Food Safety Authority. Scientific opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA J* 2012;**10**:2985–2993.
58 International Programme on Chemical Safety. *Methylmercury*. Environmental Health Criteria EHC 101. WHO, Geneva, 1990. Available at http://www.inchem.org/documents/ehc/ehc/ehc101.htm.
59 World Health Organization/United Nations Environment Programme. *Guidance for Identifying Populations at Risk from Mercury Exposure*. WHO, Geneva, 2008.
60 De Marco SG, Botté SE, Marcovecchio JE. Mercury distribution in abiotic and biological compartments within several estuarine systems from Argentina: 1980–2005 period. *Chemosphere* 2006;**65**:213–226.
61 Julshamn K, Mage A, Tyssebotn IMB, Sæthre LJ. Concentrations of mercury and other toxic elements in Orange Roughy, *Hoplostethus atlanticus*, from the Mid-Atlantic Ridge. *Bull Environ Contam Toxicol* 2011;**87**:70–73.
62 Al-Busaidi M, Yesudhason P, Al-Mughairi S *et al*. Toxic metals in commercial marine fish in Oman with reference to national and international standards. *Chemosphere* 2011;**85**:67–73.
63 Domingo JL. Omega-3 fatty acids and the benefits of fish consumption: is all that glitters gold? *Environ Int* 2007;**33**:993–998.
64 Bogdanović T, Ujević II, Sedak M *et al*. As, Cd, Hg and Pb in four edible shellfish species from breeding and harvesting areas along the eastern Adriatic Coast, Croatia. *Food Chem* 2014;**146**:197–203.
65 Lacerda LD, Soares TM, Costa BGB, Godoy MDP. Mercury emission factors from intensive shrimp aquaculture and their relative importance to the Jaguaribe River estuary, NE Brazil. *Bull Environ Contam Toxicol* 2011;**87**:657–661.
66 European Food Safety Authority. Cadmium dietary exposure in the European population. *EFSA J* 2012;**10**:2551–2573.
67 United States Environmental Protection Agency. Technology Transfer Network Air Toxics Website. Available from http://www.epa.gov/airtoxics/allabout.html.
68 Joint FAO/WHO Expert Committee on Food Additives. Seventy-third meeting, Geneva, 8–17 June 2010. Summary and Conclusions. Available at http://www.who.int/foodsafety/publications/chem/summary73.pdf.
69 European Food Safety Authority. Statement on tolerable weekly intake for cadmium. *EFSA J* 2011;**9**(2):1975.
70 Piatkowski U, Pierce G, Morais da Cunha M. Impact of cephalopods in the food chain and their interaction with the environment and fisheries: an overview. *Fish Res* 2001;**52**:5–10.
71 Finger JM, Smith JD. Molecular association of Cu, Zn, Cd and 210Po in the digestive gland of the squid *Nototodarus gouldi*. *Mar Biol* 1987;**95**:87–91.
72 Bustamante P, Cosson RP, Gallien I, Caurant F, Miramand P. Cadmium detoxification processes in the digestive gland of cephalopods in relation to accumulated cadmium concentrations. *Mar Environ Res* 2002;**53**:227–241.
73 Dorneles PR, Lailson-Brito J, Aguiar dos Santos R *et al*. Cephalopods and cetaceans as indicators of offshore bioavailability of cadmium off Central South Brazil Bight. *Environ Pollut* 2007;**148**:352–359.
74 Bustamante P, Caurant F, Fowler SW, Miramand P. Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean. *Sci Total Environ* 1998;**220**:71–80.
75 Lahaye V, Bustamante P, Spitz J *et al*. Long-term dietary preferences of common dolphins in the Bay of Biscay using a metallic tracer. *Mar Ecol Progr Ser* 2005;**305**:275–285.
76 Storelli MM, Barone G, Marcotrigiano GO. Cadmium in cephalopod mollusks: implications for public health. *J Food Protect* 2005;**68**:577–580.
77 Storelli MM, Giacominelli-Stuffler R, Storelli A, Marcotrigiano GO. Cadmium and mercury in cephalopod mollusks: estimated weekly intake. *Food Addit Contam B* 2006;**23**:25–30.
78 Boyle PR, Pierce GJ. Fishery biology of northeast Atlantic squid: an overview. *Fish Res* 1994;**21**:1–16.
79 Boyle PR, Rodhouse PG. *Cephalopods: Ecology and Fisheries*. Blackwell Science, Oxford, 2005.
80 FAO Fisheries and Aquaculture Information and Statistics Service. Fisheries commodities production and trade 1976–2005. FISHSTAT Plus, Universal software for fishery statistical time series. Available at http://www.fao.org/fi/statist/FISOFT/FISHPLUS.asp.
81 Storelli MM, Garofalo R, Giungato D, Giacominelli-Stuffler R. Intake of essential and non-essential elements from consumption of octopus cuttlefish and squid. *Food Addit Contam B* 2010;**3**:14–18.
82 Ichihashi H, Kohno H, Kannan K, Tsumura A, Yamasaki SI. Multi-elemental analysis of purple back flying squid using high resolution inductively coupled plasma-mass spectrometry (HRICP-MS). *Environ Sci Technol* 2001;**35**:3103–3108.

83 Koyama J, Nanamori N, Segawa S. Bioaccumulation of waterborne and dietary cadmium by oval squid, *Sepioteuthis lessoniana*, and its distribution among organs. *Mar Pollut Bull* 2000;**40**:961–967.

84 Bustamante P, Teyssié JL, Fowler SW *et al*. Biokinetics of zinc and cadmium accumulation and depuration at different stages in the life cycle of the cuttlefish *Sepia officinalis*. *Mar Ecol Progr Ser* 2002;**231**:167–177.

85 Kojadinovic J, Jackson ChH, Cherel Y, Jackson GD, Bustamante P. Multi-elemental concentrations in the tissues of the oceanic squid *Todarodes filippovae* from Tasmania and the southern Indian Ocean. *Ecotoxicol Environ Saf* 2011;**74**: 1238–1249.

86 Craig S, Overnell J. Metals in squid, *Loligo forbesi*, adults, eggs and hatchlings. No evidence for a role for Cu- or Zn-metallothionein. *Comp Biochem Physiol C* 2003;**134**:311–317.

87 Kurihara H, Togawa H, Hatano M. Concentration of cadmium in livers of several kinds of squids and an approach to its elimination. *Bull Fac Fish Hokkaido Univ* 1993;**44**:32–37.

88 Gerpe MS, de Moreno JEA, Moreno VJ, Patat ML. Cadmium, zinc and copper accumulation in the squid *Illex argentinus* from the Southwest Atlantic Ocean. *Mar Biol* 2000;**136**:1039–1044.

89 Castillo LV, Kawaguchi S, Maita Y. Evidence for the presence of heavy metal binding proteins in the squid *Onychoteuthis borealijaponica*. In: Hiranor R, Hanyu I (eds) *The 2nd Asian Fisheries Forum*. Asian Fisheries Society, Manila, Philippines, 1990, p. 456.

90 Pierce GJ, Stowasser G, Hastie LC, Bustamante P. Geographic, seasonal and ontogenetic variation in cadmium and mercury concentrations in squid (Cephalopoda: Teuthoidea) from UK waters. *Ecotoxicol Environ Saf* 2008;**70**:422–432.

91 Castillo LV, Maita Y. Isolation and partial characterization of cadmium binding proteins from the oceanic squid *Ommastrephes bartrami*. *Bull Fac Fish Hokkaido Univ* 1991;**42**: 26–34.

92 Bustamante P, González AF, Rocha F, Miramand P, Guerra A. Metal and metalloid concentrations in the giant squid *Architeuthis dux* from Iberian Waters. *Mar Environ Res* 2008;**66**:278–287.

93 Murthy LN, Panda SK, Madhu VR *et al*. Cadmium in the purpleback flying squid *Sthenoteuthis oualaniensis* (Lesson, 1830) along northwest coast of India. *J Mar Biol Assoc India* 2008;**50**:191–195.

94 Ivanovic ML, Brunetti NE. Food and feeding of *Illex argentines*. *Antarct Sci* 1994;**6**:185–193.

95 Zuyev G, Nigmatullin C, Chesalin M, Nesis K. Main results of long-term worldwide studies on tropical nektonic oceanic squid genus *Sthenoteuthis*: an overview of the Soviet investigations. *Bull Mar Sci* 2002;**71**:1019–1060.

96 Donat JR, Bruland KW. Trace elements in the oceans. In: Salbu B, Steinnes E (eds) *Trace Elements in Natural Waters*. CRC Press, Boca Raton, FL, 1995, pp. 247–281.

97 Mart L, Rützel H, Klahre P *et al*. Comparative studies on the distribution of heavy metals in the oceans and coastal waters. *Sci Total Environ* 1982;**26**:1–17.

98 Rainbow PS. Copper, cadmium and zinc concentrations in oceanic amphipod and euphausiid crustaceans, as a source of heavy metals to pelagic seabirds. *Mar Biol* 1989;**103**:513–518.

99 Zauke GP, Petri G. Metal concentrations in Antarctic crustacean: the problem of background levels. In: Dallinger R, Rainbow PS (eds) *Ecotoxicology of Metals in Invertebrates*. Lewis Publishers, London, 1993, pp. 73–101.

100 Bustamante P, Bocher P, Cherel Y, Miramand P, Caurant F. Distribution of trace elements in the tissues of benthic and pelagic fish from the Kerguelen Islands. *Sci Total Environ* 2003;**313**:25–39.

101 Bewers JM, Barry PJ, MacGregor DJ. Distribution and cycling of cadmium in the environment. In: Nriagu JO, Sprague JB (eds) *Cadmium in the Aquatic Environment*. John Wiley & Sons, Inc., New York, 1987, pp. 1–18.

102 Storelli MM, Marcotrigiano GO. Cadmium and total mercury in some cephalopods from the South Adriatic Sea (Italy). *Food Addit Contam B* 1999;**16**:261–265.

103 Gallien I, Caurant F, Bordes M *et al*. Cadmium-containing granules in kidney tissue of the Atlantic white-sided dolphin (*Lagenorhyncus acutus*) off the Faroe Islands. *Comp Biochem Physiol C* 2001;**130**:389–395.

104 Pan K, Wang WX. Trace metal contamination in estuarine and coastal environments in China. *Sci Total Environ* 2012; **421–422**:3–16.

105 Rainbow PS. Trace metal concentrations in aquatic invertebrates: why and so what? *Environ Pollut* 2002;**120**: 497–507.

106 Stanciu G, Mititelu M, Gutaga S. Pesticides and heavy metals determination in marine organisms from black sea. *Chem Bull 'Politehnica' Univ (Timişoara)* 2005;**50**(64): 123–126.

107 Amiard JC, Amiard-Triquet C, Charbonnier L, Mesnil A, Rainbow PS, Wang W-X. Bioaccessibility of essential and non-essential metals in commercial shellfish from Western Europe and Asia. *Food Chem Toxicol* 2008;**46**: 2010–2022.

108 Canli M, Atli G. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. *Environ Pollut* 2003;**121**: 129–136.

109 Wang WX, Yang Y, Guo X, He M, Guo F, Ke C. Copper and zinc contamination in oysters: subcellular distribution and detoxification. *Environ Toxicol Chem* 2011;**30**: 1767–1774.

110 Han BC, Jeng WL, Chen RY, Fang GT, Hung TC, Tseng RJ. Estimation of target hazard quotients and potential health risks for metals by consumption of seafood in Taiwan. *Arch Environ Contam Toxicol* 1998;**35**:711–720.

111 Han BC, Jeng WL, Hung TC, Ling YC, Shieh MJ, Chien LC. Estimation of metal and organochlorine pesticide exposures and potential health threat by consumption of oysters in Taiwan. *Environ Pollut* 2000;**109**:147–156.

112 Lin S, Hsieh IJY. Occurrences of green oyster and heavy metals contaminant levels in the Sien-San area, Taiwan. *Mar Pollut Bull* 1999;**38**:960–965.

113 Bragigand V, Berthet B, Amiard JC, Rainbow PS. Estimates of trace metal bioavailability to humans ingesting contaminated oysters. *Food Chem Toxicol* 2004;**42**:1893–1902.

114 He M, Wang WX. Bioaccessibility of 12 trace elements in marine molluscs. *Food Chem Toxicol* 2013;**55**:627–636.

115 Frías-Espericueta MG, Osuna-López I, Bañuelos-Vargas I *et al*. Cadmium, copper, lead and zinc contents of the mangrove oyster, *Crassostrea corteziensis*, of seven coastal lagoons of NW Mexico. *Bull Environ Contam Toxicol* 2009;**83**: 595–599.

116 Chaharlang BH, Bakhtiari AR, Yavari V. Assessment of cadmium, copper, lead and zinc contamination using oysters (*Saccostrea cucullata*) as biomonitors on the coast of the Persian Gulf, Iran. *Bull Environ Contam Toxicol* 2012;**88**: 956–961.

117 Gil MN, Torres A, Harvey M, Esteves JL. Heavy metals in marine organisms from the coastal zone of Continental Argentine Patagonia. *Rev Biol Mar Oceanogr* 2006;**41**: 167–176.

118 Páez-Osuna F, Ruiz-Fernández AC, Botello AV *et al*. Concentrations of selected trace metals (Cu, Pb, Zn), organochlorines (PCBs, HCB) and total PAHs in mangrove oysters from the Pacific Coast of Mexico: an overview. *Mar Pollut Bull* 2002;**44**:1296–1313.

119 Pourang N, Dennis JH. Distribution of trace elements in tissues of two shrimp species from the Persian Gulf and roles of metallothionein in their redistribution. *Environ Int* 2005;**3**:325–341.

120 Francesconi KA. As species in seafood: origin and human health implications. *Pure Appl Chem* 2010;**82**:373–381.

121 Francesconi KA, Edmonds JS. Arsenic and marine organisms. *Adv Inorg Chem* 1997;**44**:147–189.

122 Yancey PH, Clark ME, Hand SC, Bowlus RD, Somero GN. Living with water stress: evolution of osmolyte systems. *Science* 1982;**217**:1214–1222.

123 Ysart G, Miller P, Croasdale M *et al*. UK total diet study: dietary exposures to aluminum, arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, tin and zinc. *Food Addit Contam* 1997;**17**:775–786.

124 Sloth JJ, Larsen EH, Julshamn K. Survey of inorganic As in marine animals and marine certified reference materials by anion exchange high-performance liquid chromatography inductively coupled plasma mass spectrometry. *J Agric Food Chem* 2005;**53**:6011–6018.

125 European Food Safety Authority. Scientific Opinion on arsenic in food. *EFSA J* 2009;**7**(10):1351.

126 Uneyama C, Toda M, Yamamoto M, Morikawa K. Arsenic in various foods: cumulative data. *Food Addit Contam* 2007; **24**:447–534.

127 Barwick M, Maher W. Biotransference and biomagnification of selenium copper, cadmium, zinc, arsenic and lead in a temperate seagrass ecosystem from Lake Macquarie Estuary, NSW, Australia. *Mar Environ Res* 2003;**56**: 471–502.

128 Kaneko JJ, Ralston NVC. Selenium and mercury in pelagic fish in the Central North Pacific near Hawaii. *Biol Trace Elem Res* 2007;**119**:242–254.

129 Sirichakwal PP, Puwastien P, Polngam J, Kongkachuichai R. Selenium content of Thai foods. *J Food Compos Anal* 2005;**18**:47–59.

130 Kehrig HA, Seixas TG, Di Beneditto APM, Malm O. Selenium and mercury in widely consumed seafood from South Atlantic Ocean. *Ecotoxicol Environ Saf* 2013; **93**:156–162.

131 Ralston NVC. Selenium health benefit values as seafood safety criteria. *EcoHealth* 2008;**5**:442–455.

132 Behne D, Pfeifer H, Rothlein D, Kyriakopoulos A. Cellular and subcellular distribution of selenium and selenium-containing proteins in the rat. In: Roussel AM, Favier AE, Anderson RA (eds) *Trace Elements in Man and Animals*. Kluwer Academic, New York, 2000.

133 Clark C, Combs GF, Turnbull BW *et al*. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin: a randomized controlled trial. *JAMA* 1996;**276**:1957–1963.

134 Chien LC, Yeh CY, Huang SY, Shieh MJ, Han BC. Pharmacokinetic model of daily selenium intake from contaminated seafood in Taiwan. *Sci Total Environ* 2003; **311**:57–64.

135 World Health Organization. *Selenium*. Environmental Health Criteria EHC 58. WHO, Geneva, 1987. Available at http://www.inchem.org/documents/ehc/ehc/ehc58.htm.

136 Yang G, Zhu L, Liu S. Human selenium requirements in China. In: Combs GF Jr, Spallholz JE, Levander OA, Oldfield JE (eds) *Selenium in Biology and Medicine,Part B*. AVI Publishing Co., Westport, CT, 1987.

137 United States Environmental Protection Agency. *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Volume 2: Risk Assessment and Fish Consumption Limits*, 3rd edn. EPA, Washington, DC, 1997.

138 Mergler D, Anderson HA, Chan HM, Mahaffey KR, Murray M, Sakamoto M. Methyl mercury exposure and health effects in humans: a worldwide concern. *Ambio* 2007;**36**:3–11.

139 Kawarazuka N, Béné C. The potential role of small fish species in improving micronutrient deficiencies in developing countries: building evidence. *Public Health Nutr* 2011;**14**:1927–1938.

140 Food and Agriculture Organization of the United Nations. FAOSTAT. Calculated from food balance sheets. Available at http://faostat3.fao.org/faostat-gateway/go/to/home/E.
141 Calatayud M, Deves V, Virseda JR, Barberá R, Montoro R, Vélez D. Mercury and selenium in fish and shellfish: occurrence, bioaccessibility and uptake by Caco2 cells. *Food Chem Toxicol* 2012;**50**:2696–2702.
142 Rittenschober D, Nowak V, Charrondiere UR. Review of availability of food composition data for fish and shellfish. *Food Chem* 2013;**141**:4303–4310.
143 Martínez-Valverde I, Periago MJ, Santanella M, Ros G. The content and nutritional significance of minerals on fish flesh in the presence and absence of bone. *Food Chem* 2000;**71**:503–509.
144 Karppanen H, Mervaala E. Sodium intake and hypertension. *Progr Cardiovasc Dis* 2006;**49**:59–75.
145 US Department of Health and Human Services, US Department of Agriculture. Dietary Guidelines for Americans. Available at http://www.health.gov/dietaryguidelines/dga2005/document/default.htm.
146 Ersoy B, Özeren A. The effect of cooking methods on mineral and vitamin contents of African catfish. *Food Chem* 2009;**115**:419–422.
147 Gokoglu N, Yerlikaya P, Cengiz E. Effects of cooking methods on the proximate composition and mineral contents of rainbow trout (*Oncorhynchus mykiss*). *Food Chem* 2004;**84**:19–22.
148 Fung LAH, Antoine JMR, Grant CN, Buddo DSA. Evaluation of dietary exposure to minerals, trace elements and heavy metals from the muscle tissue of the lionfish *Pterois volitans* (Linnaeus 1758). *Food Chem Toxicol* 2013;**60**:205–212.
149 Zalloua PA, Hsua Y, Terwedowa H *et al*. Impact of seafood and fruit consumption on bone mineral density. *Maturitas* 2007;**56**:1–11.
150 Järup L. Hazards of heavy metals contamination. *Br Med Bull* 2003;**68**:167–182.
151 Has-Schön E, Bogut I, Strelec I. Heavy metal profile in five fish species included in human diet, domiciled in the end flow of River Neretva (Croatia). *Arch Environ Contam Toxicol* 2006;**50**:545–551.
152 Özçelik B, Uygun Ü, Bayram B. Seafoods and environmental contaminants. In: Alasalvar C, Shahidi F, Miyashita K, Wanasundara U (eds) *Handbook of Seafood Quality, Safety and Health Applications*. Wiley-Blackwell, Chichester, 2010, pp. 303–316.
153 Weiss B, Clarkson TW, Simon W. Silent latency periods in methylmercury poisoning and in neurodegenerative disease. *Environ Health Perspect* 2002;**110**:851–854.
154 Harada M. Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. *Crit Rev Toxicol* 1995;**25**:1–24.
155 Rustam H, Hamdi T. Methyl mercury poisoning in Iraq. *Brain* 1974;**97**:499–510.
156 Guallar E, Sanz-Gallardo MI, van't Veer P *et al*. Heavy metals and myocardial infarction study group. Mercury, fish oils, and the risk of myocardial infarction. *N Engl J Med* 2002;**347**:1747–1754.
157 Yoshizawa K, Rimm EB, Morris JS *et al*. Mercury and the risk of coronary heart disease in men. *N Engl J Med* 2002;**347**:1755–1760.
158 Klaassen CD, Amdur MO, Doull J. *Toxicology: The Basic Science of Poisons*, 3rd edn. Macmillan, New York, 1986.
159 Gordon G, Skett P. *Introduction to Drug Metabolism*. Chapman & Hall, New York, 1986.
160 Livingstone DR. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Mar Pollut Bull* 2001;**42**:656–666.
161 Ergene S, Çavaş T, Çelik A, Köleli N, Kaya F, Karahan A. Monitoring of nuclear abnormalities in peripheral erythrocytes of three fish species from the Goksu Delta (Turkey): genotoxic damage in relation to water pollution. *Ecotoxicology* 2007;**16**:385–391.
162 Zheng N, Wang Q, Zhang X, Zheng D, Zhang Z, Zhang S. Population health risk due to dietary intake of heavy metals in the industrial area of Huludao city, China. *Sci Total Environ* 2007;**387**:96–104.
163 Lim SR, Schoenung JM. Human health and ecological toxicity potentials due to heavy metal content in waste electronic devices with flat panel displays. *J Hazard Mater* 2010;**177**:251–259.
164 Satarug S, Moore MR. Adverse health effects of chronic exposure to low-levels cadmium in foodstuffs and cigarette smoke. *Environ Health Perspect* 2004;**112**:1099–1103.
165 Leazer TM, Liu Y, Klaassen CD. Cadmium absorption and its relationship to divalent metal transporter-1 in the pregnant rat. *Toxicol Appl Pharmacol* 2002;**185**:18–24.
166 Jarup L, Hellstrom L, Alfven T, Carlsson MD, Grubb A, Persson B. Low level exposure to cadmium and early kidney damage: the OSCAR study. *Occup Environ Med* 2000;**57**:668–672.
167 Buchet JP, Lauwerys R, Roels H *et al*. Renal effects of cadmium body burden of the general population. *Lancet* 1990;**336**:699–702.
168 Hsu CY, Iribarren C, McCulloch CE, Darbinian J, Go AS. Risk factors for end-stage renal disease: 25-year follow-up. *Arch Intern Med* 2009;**169**:342–350.
169 Shiraishi Y. Cytogenetic studies in 12 patients with Itai-Itai disease. *Humangenetik* 1975;**27**:31–44.
170 Kjellström T. Mechanism and epidemiology of bone effects of cadmium. In: Nordberg GF, Herber RFM, Alessio L (eds) *Cadmium in the Human Environment: Toxicity and Carcinogenicity*. IARC Scientific Publication No. 118. IARC, Lyon, 1992, pp. 301–310.
171 Elinder CG, Järup L. Cadmium exposure and health risks: recent findings. *Ambio* 1996;**25**:370–373.
172 Messner B, Knoflach M, Seubert A *et al*. Cadmium is a novel and independent risk factor for early atherosclerosis

mechanisms and *in vivo* relevance. *Arterioscl Thromb Vasc Biol* 2009;**29**:1392–1398.

173 International Agency for Research on Cancer. Cadmium and cadmium compounds. In: *Beryllium, Cadmium, Mercury and Exposure in the Glass Manufacturing Industry*. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans No. 58. IARC, Lyon, 1993, pp. 119–237.

174 Kolonel LN. Association of cadmium with renal cancer. *Cancer* 1976;**37**:1782–1787.

175 Mandel JS, McLaughlin JK, Schlehofer B *et al*. International renal-cell cancer study. IV. Occupation. *Int J Cancer* 1995;**61**:601–605.

176 Elliott P, Arnold R, Barltrop D, Thornton I, House IM, Henry JA. Clinical lead poisoning in England: an analysis of routine sources of data. *Occup Environ Med* 1999;**56**: 820–824.

177 Bechmann I, Galea I, Perry VH. What is the blood–brain barrier (not)? *Trends Immunol* 2007;**28**:5–11.

178 Crivori P, Cruciani G, Carrupt P-A, Testa B. Predicting blood–brain barrier permeation from three-dimensional molecular structure. *J Med Chem* 2000;**43**:2204–2216.

179 Mahaffey KR. Nutritional factors and susceptibility to lead toxicity. *Environ Health Perspect* 1974;**7**:107–112.

180 Chisolm J Jr. Lead poisoning. *Sci Am* 1971;**224**:15–23.

181 Needleman H. Lead poisoning. *Annu Rev Med* 2004;**55**: 209–222.

182 Steenland K, Boffetta P. Lead and cancer in humans: where are we now? *Am J Ind Med* 2000;**38**:295–299.

CHAPTER 28
Milk and dairy products

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Abstract: Milk and dairy products are excellent sources of mineral elements, mainly Ca, P, Mg, Na, K and Zn. The mineral elements are present in the form of inorganic ions and salts or are part of organic molecules, mainly proteins and fats. Distribution of the main elements in milk between the soluble and micellar phases contributes not only to milk's nutritional value but also to the stability of the colloidal dispersion. The balance between Ca and P salts can be affected by the varying features of the technical processes (pH, temperature, pressure, concentration) to which milk can be subjected. If processing is mild, the salt balance barely changes and can revert back to initial conditions. Applying harsher conditions usually brings about irreversible changes resulting in the formation of a new salt system that differs from the one intrinsic to milk. Research and development aimed at achieving milk products with higher levels of mineral elements, desirable from a nutritional standpoint, has produced milks and milk products that have been fortified with minerals and trace elements. Dairy products supply necessary major mineral elements and trace elements, making measurement particularly important. Available methods meet the requirements of both research and routine control procedures.

Keywords: milk, cheese, dairy products, fermented milk, micelle, mineral distribution, fortification

28.1 The importance of milk as a food

Milk is a balanced mixture of lipids, proteins, carbohydrates, vitamins and minerals dispersed in water in the form of an emulsion, colloidal dispersion and solution. It is a basic foodstuff whose intrinsic function is to meet the nutritional requirements of newborns and infants. In addition, it plays an essential role in the diets of adolescents and adults thanks to the high density of nutrients it contains. Its health effects are the outcome of the interactions among all its nutrients and extend beyond the simple sum of individual effects. Certain aspects that make milk nutritionally important deserve mention [1].

- It contains a wide range of nutrients, with only iron being deficient.
- It has a good balance between the major components, fats, proteins and carbohydrates, which are present in equivalent proportions.
- It affords high levels of nutrients proportional to the caloric content. This is particularly meaningful for people trying to lower their intake.

Based on the figures for mean consumption of dairy products as a whole, milk products can be deemed to supply more than one-fifth of the recommended daily allowance (RDA) of proteins, Zn, vitamin B_2 and vitamin A, as well as smaller proportions of the RDAs of many other vitamins. Furthermore, likewise based on mean consumption values, the amount of Ca supplied by milk and milk products, nearly 60% of the RDA, is highly noteworthy [2]. If the share of dairy products in our diet were to drop, it would be hard to meet the RDA for some of the nutrients mentioned above.

The various products derived from milk span a broad range of compositions and hence can cover both varied consumption habits and quite diverse nutritional applications [3]. These dairy products include cream and butter (products with enriched levels of milk fat and low levels of the other nutrients), milks with differing fat contents and different mineral and vitamin levels (fortified milks), evaporated milk, fermented milks retaining most all the nutrients of the milk, and different types of cheese containing concentrated fat, some or all of the

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milk proteins (depending on the manufacturing method) and sizeable proportions of the various minerals. All these products are available in the marketplace.

Apart from milk's known nutritional aspects, there are also data attesting to the health benefits afforded by the various components of milk [3]. With the research tools available today, many biologically active minor compounds have been shown to be present in bovine milk [4] and in the milks of other species [5–7].

Milk proteins are highly digestible, contain all the essential amino acids, and are able to satisfy requirements for these nutrients. They have high biological value (91% of the value of the reference protein) and thus meet the definition of high-quality proteins. Because of their high lysine content, milk proteins are good supplements to the proteins in other foods, such as cereals. Bovine milk contains, on average, 0.5% nitrogen (3–3.5% of the proteins) in various fractions of differing import to dairy product food technology and nutrition: caseins, whey proteins, and non-protein nitrogen compounds. The casein fraction contains, within the structure of the proteins, two-thirds of the Ca and half the P in milk, along with practically all the Mn and Zn. In addition, milk proteins contain bioactive peptides [8, 9] that have potentially beneficial health effects and may decrease the risk of disease and which can be released by the action of gastric enzymes and, in fermented products, by the action of the proteolytic enzymes of the microorganisms in the starter cultures. Furthermore, whey proteins, as a functional food component, have the potential to help regulate body weight by providing satiety signals that can influence both the short-term and long-term regulation of food intake [10].

Lipids are some of the most important milk components both in nutritional terms and on account of the physical and organoleptic properties they impart to milk products. Fatty acids are the basic building blocks of fats, making up 90% of the mass of glycerides. The fatty acids are both saturated and unsaturated (having one to four double bonds). The fat content and fat composition of milk varies depending on a range of genetic and environmental factors [11], chief among these being diet and milk type (whole, semi-skim, skim). Milk lipids are involved in the transport of liposoluble vitamins, and milk is a good source of these vitamins. There is evidence that certain components present in milk fat, for example butyric acid, conjugated linoleic acid its physiological precursor vaccenic acid, and certain phospholipids, could have beneficial effects on certain diseases [12].

Lactose is the sole sugar present in significant amounts in milk, serving mainly as an energy source. Lactose is a valuable nutrient because it could facilitate intestinal absorption of minerals and utilization of vitamin D. Milk is also a major source of hydrosoluble vitamins for children and adults. Drinking 1 L of milk supplies the recommended intake of B_1, B_2, B_{12} as well as a large proportion of those of vitamins A, C and pantothenic acid.

Interest in the nutritional significance of milk minerals and trace elements has increased markedly in recent years. Some 20 minerals in milk are considered nutritionally essential for humans (Na, K, Cl, Ca, Mg, P, Fe, Cu, Zn, Mn, Se, I, Cr, Co, Mb, F, As, Ni, Si and B), and milk and dairy products can make an important contribution to the daily intake of certain of these, Ca and P in particular.

This chapter presents data on the mineral element content of milk and milk products, the distribution of the said mineral elements between the soluble and micellar phases of the milk, and how different technical processing may alter the mineral balance of milk and other dairy products. Additionally, a number of issues relating to analytical methods of determination for these components are discussed.

28.2 Mineral elements in milk

The mineral content of milk is not constant but is instead influenced by a number of factors, such as species, individual animal, lactation stage, nutritional status of the animal, dietary regime, environmental and genetic factors, and seasonal variations. The composition of commercial cows' milk is expected to undergo only minor changes over the course of the year, because milk from cow herds pumped into bulk tanks should vary little by season thanks to year-round breeding. However, the case of ovine and caprine milks, mainly produced by seasonal breeding of ewes and does, is quite different [13, 14]. Ovine milk has around 0.9% total minerals or ash, compared with 0.7% in bovine or caprine milk [5, 15]. The highest ash content is found in reindeer milk (Table 28.1).

The most abundant elements are Ca, P, K, Na and Mg, with Zn, Fe, Cu and Mn being the main trace elements.

Table 28.1 Mineral composition and ash content (per 100 mL) of milk from different species producing milk for human consumption (mean value with ranges in parentheses).

	Cow			Sheep		Goat		Buffalo	Camel	Mare	Yak	Reindeer
	(1)	(2)	(3)	(1)	(2)	(1)	(2)	(3)	(3)	(3)	(3)	(3)
Ash (%)	0.7		0.7	0.9		0.8		0.8 (0.7–0.8)	0.8	0.4 (0.3–0.5)	0.8 (0.4–1.0)	1.5 (1.2–2.7)
Ca (mg)	122	120	113 (112–116)	193	(195–200)	134	126	191 (147–220)	114 (105–120)	95 (76–124)	129 (119–134)	320
P (mg)	119	92	84 (59–95)	158	(124–158)	121	97	185 (102–293)	86 (83–90)	58 (43–83)	106 (77–135)	270
Mg (mg)	12	11	10 (7–12)	18	(18–21)	16	13	12 (2–16)	13 (12–14)	7 (4–12)	10 (8–12)	19
K (mg)	152	150	132 (103–163)	136	(136–140)	181	190	112	151 (124–173)	51 (25–87)	95 (83–107)	156
Na (mg)	58	45	43 (26–58)	44	(44–58)	41	38	47	66 (59–73)	16 (13–20)	29 (21–38)	48 (46–50)
Cl (mg)	100	110		160	(110–112)	150	160	57		19		68
Zn (mg)	0.53	0.38	0.40 (0.3–0.5)	0.57	(0.52–0.74)	0.56	0.34	0.50	0.60 (0.4–0.6)	0.20 (0.2–0.3)	0.9 (0.7–1.1)	1.1
Fe (μg)	80	46	30 (0–70)	80	(72–122)	70	55	170	210 (170–260)	100 (30–150)	570 (150–980)	
Cu (μg)	60	22	30 (0–80)	40	(40–68)	50	30	20	150 (120–170)	50 (20–110)	410	
Mn (μg)	20	6	4 (0–8)	7	(5–9)	32	8		11 (6–18)			
I (μg)	21	7		20	10	22	8	4				
Se (μg)	0.96	3.00	3.70 (0–8.8)	1.00	3.10	1.33	2.00					

Source: data from (1) Park *et al*. [5]; (2) Raynal-Ljutovac *et al*. [167]; (3) Medhammar *et al*. [6].

Table 28.1 also sets out representative mean mineral content values for the milks of different species. Ca, P, Mg, Zn, Fe and Cu levels are higher in ovine milk than in bovine milk, while the opposite appears to be the case for K and Na. On the whole, the mineral content of ovine milk seems to be subject to greater variation than that of bovine milk.

The biochemical roles of these essential minerals and trace elements and the nutritional significance of milk as a source of these micronutrients have been described and discussed in detail elsewhere [16–20]. Of all the mineral elements in milk, Ca is the one of most interest because of its involvement in numerous vital functions and its high bioavailability. Lactose, ascorbate, citrate, phosphopeptides and lactoferrin may have a significant impact on the absorption of mineral elements [17, 21]. Furthermore, milk does not contain phytates or other substances that might inhibit mineral absorption [22]. Milk also contains substantial levels of P, and its Ca/P ratio is ideal for optimal absorption. One litre of milk supplies more than 100% of the RDAs of Ca and P and at least 40% of the RDAs of Mg and Zn, though it is, by contrast, quite low in Fe and Cu (Table 28.2). Both the content and bioavailability of minerals in milk products are affected by the technical processing employed, fractionation, acidification on fermentation, or heat treatment, as described below.

Optimal intake of Ca from milk, supplemented by the vitamin D present, in the first three decades of life has been shown to be critical to being able to generate suitable bone mass in humans and thereby achieve the concomitant benefit of reducing the risk of osteoporosis [23]. Milk's high Ca content and supply of such other elements as K and Mg may also have beneficial effects on cardiovascular risk factors like blood pressure, insulin resistance and platelet aggregation [24, 25]. In addition, evidence has also been compiled that the Ca and such other components of dairy products as vitamin D and whey proteins may aid in controlling weight and lowering the body mass index [26–28].

Besides the scientific evidence collected in the publications cited, the European Food Safety Authority (EFSA) and the European Union [29] have recently approved a series of health claims for different mineral elements. Table 28.3 shows the claims for Ca, P, Mg and Zn. For a food to be regarded as a 'mineral source' in connection with these claims, 100 g must supply at least 15% of the RDA for that mineral, and hence milk products fortified with different mineral elements, primarily Ca, Mg and Zn, have lately been brought onto the market. These are discussed later.

Table 28.2 Recommended dietary allowances for different mineral elements in adult men and women (19–70 years) [168].

Mineral	Males	Females
Calcium (mg/day)	1000	1000–1200
Phosphorus (mg/day)	700	700
Magnesium (mg/day)	400–420	310–320
Zinc (mg/day)	11	8
Iron (mg/day)	8	8–18
Copper (μg/day)	900	900
Iodine (μg/day)	150	150

28.3 Analytical methods

Mineral determination in milk and dairy products is a highly demanding field, though one that has undergone significant progress in recent decades. Analytical procedures to determine the main mineral and trace element contents generally comprise a sample preparation step in which organic matter is destroyed, followed by measurement of the elements of interest by different instrumental techniques, preferably atomic absorption-based. Because the subject is so vast, this chapter deals with specific aspects related to dairy products. More detailed information can be found in a fairly comprehensive review [30] that discusses at some length the different analytical alternatives for determining minerals in dairy products.

28.3.1 Sample preparation

Sample preparation constitutes an important stage in mineral analysis. In milk and dairy products it involves digestion and extraction of the analytes before any determinations using the different methods can be carried out. It tends to be the most time-consuming step and is responsible for part of the total analytical error. The main problem in measuring the mineral content of milk products is the nature of the matrix, which may interfere with the analytical technique employed. Very low detection thresholds can only be achieved when concentrations of dissolved inorganic and organic

Table 28.3 European Union register of nutrition and health claims made on foods for Ca, P, Mg and Zn [29]. Source of mineral or vitamin: 15% RDA per 100 g as per the Annex to European Regulation [138].

Nutrient	Claim	Conditions of use of the claim
Ca	Ca is needed for normal growth and development of bone in children Ca contributes to normal blood clotting, energy-yielding metabolism, muscle function, neurotransmission, function of digestive enzymes Ca has a role in the process of cell division and specialization Ca is needed for the maintenance of normal bones and teeth	The claim may be used only for food which is at least a 'source of Ca'
P	Phosphorus contributes to normal energy-yielding metabolism, function of cell membranes Phosphorus contributes to the maintenance of normal bones and teeth	The claim may be used only for food which is at least a 'source of P'
Mg	Mg contributes to a reduction of tiredness and fatigue, electrolyte balance Mg contributes to normal energy-yielding metabolism, functioning of the nervous system, muscle function, protein synthesis, psychological function Mg contributes to the maintenance of normal bones and teeth Mg has a role in the process of cell division	The claim may be used only for food which is at least a 'source of Mg'
Zn	Zn contributes to DNA synthesis, acid–base metabolism, carbohydrate metabolism, cognitive function, fertility and reproduction, macronutrient metabolism, normal metabolism of fatty acids, protein synthesis Zn contributes to the maintenance of normal bones, hair, nails, skin, testosterone levels in the blood, vision, function of the immune system Zn contributes to the protection of cells from oxidative stress Zn has a role in the process of cell division	The claim may be used only for food which is at least a 'source of Zn'

matrix compounds are kept to a minimum. In particular, the presence of dissolved organic carbon may give rise to severe interference in various different measurement methods.

The main preparation methods for dairy products can be divided into complete oxidation procedures (wet digestion and dry ashing) and slurries. In milk products, dry ashing is a successful way to totally decompose the organic matter, minimize matrix effects in elemental analysis and achieve pre-concentration of trace elements during sample preparation. Thus dry ashing is commonly used as a reference procedure [31]. Conventional acid digestion is typically faster than dry ashing but requires continuous operator attention. Another common procedure to eliminate the organic matter in dairy products involves using closed pressurized devices, either microwave or conventionally heated, in the presence of concentrated acids. The microwave system can be controlled and operated unattended, but the mass of the analytical sample must be carefully selected to prevent excessive pressure during digestion. Another of the most common digestion procedures for determining the major elements in milk (Ca, Mg, Na, K) involves precipitating the milk protein and fat with trichloroacetic acid [32, 33]. However, this technique has not been applied to determine the content of trace elements.

Dry ashing and wet digestion are time-consuming procedures and are extremely harsh on samples. Furthermore, they are unsuitable for speciation studies in milk. The use of slurry sampling is an alternative and offers the advantage of direct solid and liquid sampling for direct analysis by electrothermal atomic absorption spectrometry (ETAAS) [34]. The conventional atomizers and injection systems used for liquid samples can also be used for slurry samples, thus allowing the use of heavier samples than in solid sample analysis. Homogeneous suspensions that remain stable for extended periods of time can readily be prepared from dry and skim milk. Placing milk directly into the graphite tube and diluting samples in water are unsuitable in ETAAS because of poor repeatability attributable to both carbon and fat residues in the autosampler capillary tube. A variety of solvents and stabilizers (HNO_3, ethanol, H_2O_2) as well as ultrasonic homogenization devices [35] can be used in preparing the slurries. Diluting samples with water-soluble tertiary amines has allowed direct analysis of different minerals in bovine [36] and

buffalo [37] milk by ETAAS without digestion. The main advantage of this procedure is its simplicity and speed, since it eliminates the slow sample digestion step. Additionally, the major trace elements and iodine have been determined in the same solution, without precipitation or volatilization losses.

28.3.2 Measurement procedures

Measurement of mineral element content in dairy products currently includes a wide array of techniques. Complexometric methods were first employed, mainly to measure the Ca content of milk. Precipitation titrations have also been widely used to determine Ca, Mg and above all Cl. Cl, in particular, cannot be determined by conventional atomic absorption or inductively coupled plasma (not equipped with deep UV optics: 120–180 nm). AOAC methods for the determination of the Cl content of different dairy products include titration with $AgNO_3$ [38].

Ultraviolet/visible spectrophotometry (UV/Vis) is often simpler and less expensive than atomic spectroscopic methods but is not suitable for multi-element analysis and requires derivatization to improve sensitivity and selectivity. Spectrophotometric determination of P in dairy products, involving the formation of a complex with molybdate or reduction of amidol following microwave digestion, is a simple, fast and inexpensive method if only total P is to be measured, making it a suitable procedure for routine determination [38, 39]. Sequential injection systems have also been used for online digestion and colorimetric determination of the total P in milk [40]. The greatest advantage of this approach is its speed (3.5 min per sample). Other minerals have also been measured by UV/Vis. Afkhami *et al*. [41], for instance, simultaneously determined Zn, Ca and Mg in UHT milks and milk powders by ratio spectra-continuous wavelet transforms without prior separation steps.

^{31}P-NMR provides a means of detecting and separating the signals from the different phosphorylated compounds present in milk, since each phosphate displays a characteristic chemical shift according to the chemical nature and structure of the compound to which it is attached [42]. This technique has enabled direct detection and quantification of the different forms of P in milk, namely the P distributed between the soluble and micellar phases, phosphate esterified to micellar caseins, and a minor fraction of phosphate esterified to other compounds present in the soluble phase such as carbohydrates, glycerides, and diphosphates added as stabilizers with simple sample preparation [43, 44].

Although specific electrodes can be utilized to determine a large number of ionic species in milk, the most popular application of this method, by far, is Ca^{2+} determination. Using an ion-selective membrane electrode for the direct determination of Ca^{2+} in skim milk is clearly preferred to the longer and more laborious determination via ion-exchange resins or complexing reagent methods using murexide [45, 46]. Stable electrical output by the electrode, the basis of accurate and reproducible measurement, is the most important operating feature.

Atomic spectrometry methods are the most widely used options for total element analysis in dairy products. The choice of flame atomic absorption spectroscopy (FAAS), ETAAS or inductively coupled plasma absorption emission spectroscopy (ICP-AES) depends on the concentration of the element sought, the accuracy and precision required, and the number of elements to be determined. FAAS is a well-established method for determining such major elements in milk as Ca, Mg, Na and K, although the last two can also be determined by flame atomic emission spectrometry. ETAAS needs only very small sample volumes, and low detection thresholds allow trace and ultratrace elements (Al, Cd, Cu, Fe, Mn, Ni, Se, Zn, Co, Cr and Pb) to be determined. However, this method is prone to spectral and matrix interference. In terms of detection power, ICP-AES is halfway between FAAS and ETAAS. The main advantages are robustness against matrix effects and the possibility of multi-element analysis. Inductively coupled plasma mass spectrometry (ICP-MS) is currently the most powerful detector system in atomic spectrometry, primarily thanks to its high sensitivity, multi-element analytical capability, and ability to measure isotope ratios. The main constraint on ICP-MS is, again, spectral and matrix interference, which can reduce the true analyte signal for the main elements in milk products.

X-ray fluorescence (XRF) provides simultaneous multi-element analysis for elements having an atomic number between 22 and 55. Though a high-cost technique, it is fast, non-destructive and matrix independent and does not need calibration standards similar to the sample composition. XRF exhibits potential as an analytical tool for use in dairy production lines because it is suitable for direct analysis of solid samples without pretreatment (e.g. powdered milk or infant

formula) [47–49]. Determination of minerals in dairy products based on voltammetric techniques is simple and quick and requires only relatively inexpensive instrumentation while affording good sensitivity capable of determining elements accurately at trace to ultratrace levels and the possibility of multi-element determination [35, 50, 51]. These electroanalytical techniques furnish an alternative to the use of atomic spectroscopic methods for heavy metal determinations and are free of the interference associated with these latter procedures. Ion chromatography (IC) is a routine and versatile method widely employed for multi-element and sensitive analyses of metal ions using either pre- or post-column derivatization [52]. The general applicability of IC to milk and milk products makes it a simple tool for systematic studies. IC has attracted the attention of analysts on account of its high sensitivity, speed, ease of operation, and relatively inexpensive and commonly used instrumentation that can be combined for simultaneous anion and cation determinations [38, 53, 54]. Other methods (neutron activation analysis, spectrofluorimetry) are also available for measuring mineral elements, but their applicability to milk and dairy products is more limited [30]. Most of these instrumental techniques are described in more detail in Part 1 of this book.

28.3.3 Speciation

Speciation analysis of elements in foods is of great importance to human health and is still a challenge to analytical chemistry [55]. In the case of speciation analysis in whole milk, gentle centrifugation or ultracentrifugation is normally applied to separate the different components of the milk (fat, serum and casein micelles). The resulting fractions, namely skim milk or whey milk, are then subjected to analysis, usually by different chromatographic techniques, to determine the distribution of metals between molecules, mostly proteins, having a predefined molecular mass range. Hyphenated techniques, such as liquid chromatography (solid-phase extraction, ion exchange or size exclusion), in association with detectors for the major atomic spectrometric methods (FAAS, ETAAS, ICP-AES and ICP-MS) have been used for speciation of the different elements (Cu, Mo, Mg, I, Ca, Fe, Zn and Mn) in milk (Table 28.4). Issues relating to separation mechanisms, mobile phase, pH, and sample preparation are highly significant and must be carefully considered to avoid changes in species and ensure exact characterization of samples. The main problems affecting mineral speciation are contamination, mineral losses, lack of stability of mineral–protein complexes during the separation process, and inadequate detection limits for the element in the eluate [55].

28.4 Partition of the main minerals in milk phases

Milk minerals exist in an equilibrium system between the diffusible and colloidal phases. The diffusible phase, also called the soluble phase, is the liquid or serum obtained from milk by ultracentrifugation, ultrafiltration or dialysis. A nutrient's chemical form may influence its

Table 28.4 Procedures for speciation analysis of mineral elements of interest in milk.

Element	Sample preparation	Separation	Detection	Reference
Ca, Mg, Mn, Fe, Cu, Zn, I	Ultracentrifugation	SEC	ICP-MS	[66]
Ca, Mg, Fe, Zn	Sample dilution	IEC	FAAS	[169]
Ca, Fe, Zn	Enzymatic digestion	RP-HPLC/IEC	FAAS	[170]
Cu, Mn	Precipitation of caseins	SPE	ICP-AES	[72]
Mo	Sample dilution	IEC/SEC	ETAAS	[171]
I	Centrifugation/ultrafiltration	IEC	ICP-MS	[172]
I	Ultracentrifugation	SEC	ICP-MS	[76]
I	Solvent extraction	SPE	NAA	[77]
I	Solvent extraction	IEC	NAA	[78]

ETAAS, electrothermical atomic absorption spectroscopy; FAAS, flame atomic absorption spectroscopy; ICP-AES, inductively coupled plasma absorption emission spectroscopy; ICP-MS, inductively coupled plasma absorption mass spectrometry; IEC, ion-exchange chromatography; NAA, neutron activation analysis; RP-HPLC, reverse-phase high-performance liquid chromatography; SEC, size exclusion chromatography; SPE, solid-phase extraction.

bioavailability: free forms like soluble ions are absorbed well, whereas bound forms are often poorly absorbed. Na, K and Cl are essentially diffusible, while Ca, inorganic phosphate and Mg are partly bound to casein micelles (Table 28.5). About one-third of the Ca, half of the inorganic phosphate, and two-thirds of the Mg are present in the aqueous phase of bovine milk. No macroelement is bound to the fat globules in significant amounts. Ovine and buffalo milks contain considerably more colloidal Ca than bovine milk, with the concentration in caprine milk being similar to that in bovine milk.

Table 28.5 Distribution of minerals in bovine milk in physiological conditions between the diffusible and micellar phases (%).

Component	Micellar phase	Diffusible phase
Calcium	21% Calcium caseinate (directly linked to casein)	11% Ionic Ca (Ca^{2+})
	46% Calcium phosphate	22% Calcium phosphate and citrate
Phosphorus	22% Organic (esterified with proteins)	1% Organic (phospholipids)
	32% Inorganic calcium phosphate	9% Soluble esters
		36% Inorganic phosphate
Magnesium	33%	67%
Zinc	90–95%	5–10%
Manganese	90–95%	5–10%
Iron	24%	32% Low-molecular-weight ligands
		14% Fat
		29% Whey proteins
Copper	44%	47% Low-molecular-weight ligands
		8% Whey proteins
		2% Fat

In the soluble phase, minerals are not totally free in solution, there being a variety of associations of ions [56]. These associations depend on the affinities between cations and anions and the solubilities of the salts. Ca exists as a stable complex mainly with citrate (Figure 28.1) and to a lesser extent with inorganic phosphate (in combination with HPO_4^{2-} and $H_2PO_4^-$) and with chloride. The small quantity of calcium phosphate in the diffusible fraction is due to the low solubility of these salts. Ionic calcium (Ca^{2+}) accounts for only approximately 10% of the total Ca in milk, though this portion is free to react and change the Ca equilibrium and plays a major role in the stabilization, rennetability, heat stability, and surface and rheological properties of milk [46]. The monovalent cations (Na and K) exist mainly as free ions, but a small portion is associated with citrate, inorganic phosphate, and chloride.

Milk's colloidal phase is formed by highly hydrated (3.5 kg of water per kilogram of protein) spherical particles 20–600 nm in diameter called micelles (Figure 28.1), composed of minerals, mainly high-energy amorphous calcium phosphate and caseins (80–90%), a group of four phosphoproteins (α_{s1}, α_{s2}, β and κ) prone to association due to their high hydrophobicity and distribution. A delicate balance between electrostatic and hydrophobic forces, depending on such experimental conditions as pH, temperature and ionic strength, determines the type and extent of the associations. The cation-binding ability of the casein components decreases in the order $\alpha_{s2} > \alpha_{s1} > \beta > \kappa$, according to their decreasing phosphoser-

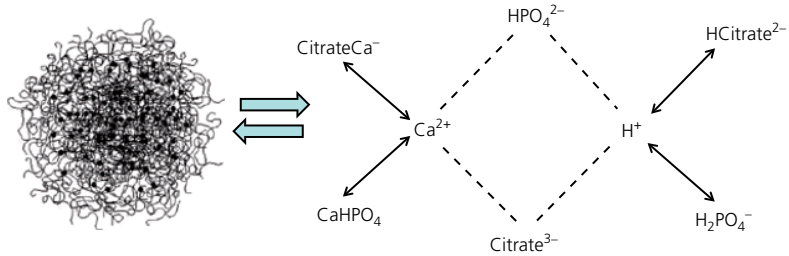


Figure 28.1 Mineral equilibrium between the soluble (right) and micellar (left) phases in milk at pH 6.7. In the micellar phase, Ca is associated with organic phosphate (phosphoseryl residues in casein molecules) and inorganic phosphate to form nanoclusters (black dots inside the micelle). Concentration (in mM) of the different mineral associations in the soluble phase are 0.04 ($HCitrate^{2-}$), 7.50 ($H_2PO_4^-$), 2.65 (HPO_4^{2-}), 0.26 ($Citrate^{3-}$), 2.00 (Ca^{2+}), 6.96 ($CitrateCa^-$) and 0.59 ($CaHPO_4$). Adapted from Gaucheron [19]. Reproduced with permission of the American College of Nutrition.

ine contents. Two types of Ca are present in the micelles (Table 28.5): Ca associated directly with casein molecules in the absence of phosphate (i.e. calcium caseinate) and Ca as an integral part of the micellar calcium phosphate (MCP). Caseins are cross-linked by the ester phosphate groups on the MCP, with at least three phosphate groups needed for cross-linking.

All documented models of casein micelle structure include MCP as an integral component. Based on electron microscopy studies, Schmidt [57] first proposed a subunit structure (submicelles) linked by MCP. More recently, Holt [58] described how small domains of calcium phosphate precipitating from a supersaturated solution could be surrounded and prevented from precipitating by the presence of casein phosphopeptides. The result of this interaction is the formation of calcium phosphate nanoclusters surrounded by casein phosphopeptides and, by extension, the caseins themselves [59]. A wide range of structural methods has established beyond any reasonable doubt that MCP particles are several nanometres in size (≈2.3 nm), that they are formed from an amorphous hydrated calcium phosphate linked to casein phosphate centres, and that they are distributed throughout the protein matrix of the micelles. Accordingly, a typical micelle having a radius of 100 nm and a mass of 10^6 kDa contains approximately 800 calcium phosphate nanoclusters [60]. The nanocluster model can be taken to invert the submicellar model; instead of casein particles linked by calcium phosphate, there are calcium phosphate/casein particles linked by non-covalent bonds.

The most recent electron microscopy results have yielded images that do not appear to support a classical submicellar structure [61]. However, the nanocluster model considers hydrophobic interactions to be important in linking the caseins of the nanoclusters together, even though such interactions would appear to preclude the presence of water in the interior of the micelle. To explain this, Dalgleish [62] postulated that water channels within the micelle can be stabilized by β-casein interacting with the hydrophobic portions of the nanoclusters. Such a model views the casein micelle as a sponge-like structure having a relatively even distribution of nanoclusters but also containing pores stabilized by β-casein. This would account for the hydration of the particles, the presence of β-casein inside the micelle, and the observation of more rigid regions formed by linked nanoclusters (Figure 28.2).

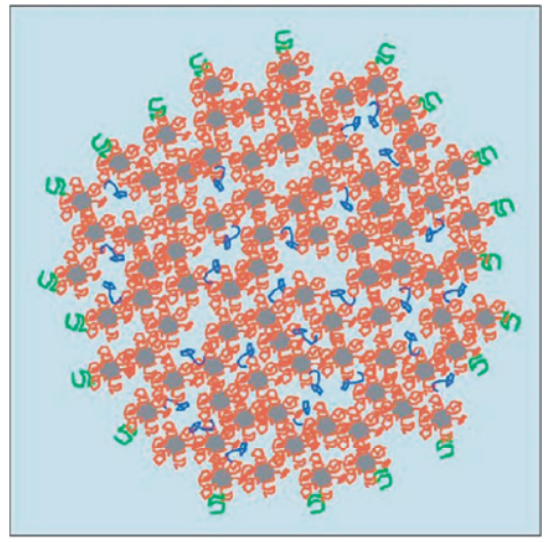


Figure 28.2 Schematic structure of the casein micelle incorporating calcium phosphate nanoclusters (grey) with their attached caseins (red) and κ-casein (green) located on the surface. The 'hydrophobically bound' mobile β-casein is shown in blue, within the water channels inside the micelle. For clarity, the relative sizes of the individual components are not to scale. From Dalgleish [62]. Reproduced with permission of The Royal Society of Chemistry. (See insert for colour representation of the figure.)

It has been widely argued that casein micelle structure is optimized for the nutritional benefit of the young, because the high-energy amorphous state and high surface to volume ratio favour rapid dissolution of the calcium phosphate under the acidic conditions in the stomach. By contrast, the premise that it is intended to control calcification has much to recommend it, since caseins are extremely effective in preventing calcification thanks to their rheomorphic structure and phosphate centres [58]. Milk, like many other biological fluids, is saturated with respect to a number of different calcium phosphate phases, and spontaneous precipitation could be expected to occur; this, if not controlled, could be hazardous to the proper functioning of the mammary gland. Micelles are adapted to fulfil the function of controlling calcium phosphate precipitation in the subcellular organelles where Ca accumulation takes place [63]. According to this postulated role, Ca-sensitive caseins would rapidly bind to calcium phosphate nuclei by their phosphoseryl residues, stabilizing the ion clusters against further growth and preventing maturation to a crystalline state.

28.5 Partitioning of trace elements

Greater awareness of the relationship between dietary trace elements and health has contributed to interest in the distribution of these nutrients in the different fractions of milk (Table 28.5). Zn in ruminant milk is primarily (90–95%) bound to the micelles, and only small amounts are linked to whey proteins and low-molecular-weight ligands in the serum [64–66]. Within the casein micelles, one-third of the Zn is loosely bound to casein, whereas two-thirds is more tightly bound to the MCP. Binding of a large fraction of Zn to casein in ruminant milk may result in entrapment in casein curds formed in the stomach, which may be incompletely digested in the small intestine, thus rendering a significant proportion of this mineral unavailable for absorption [67].

Mn is also associated largely (90–95%) with the micellar phase [65, 68] (Table 28.5). The rest is present in the whey, in the low-molecular-weight fraction [66]. In the case of Fe, 14% of the total Fe content of milk is located in the fat, mostly bound to the outer membrane of fat globules; 24% is bound to the caseins, and the remainder is found in the soluble phase [69]. Looking at the Fe content in the casein fraction, α_s-caseins are an important Fe-binding ligand, more specifically the phosphate groups responsible for the bond. Based on literature data, it is very likely that part of the Fe in the soluble fraction is bound to low-molecular-mass ligands, while another part is bound to whey proteins, especially lactoferrin [69, 70], a multifunctional protein which, among other physiological roles, regulates Fe homeostasis [71].

Available data highlight the importance of caseins [72], mainly β-casein, as Cu-binding ligands (Table 28.5). Cu present in the soluble phase is bound to low-molecular-mass ligands, mainly citrate, and a small percentage (<10%) to whey proteins [69, 73]. Skim bovine milk was found to contain 93% of the total milk Se, mainly associated with the casein fraction [74], with β-casein contributing most to the total milk Se (33%), while 17% was associated with whey proteins. A subsequent study concluded that protein digestibility could influence Se availability in milk to a large extent [75]. Whey obtained by centrifuging fresh milk or in reconstituted milk powders contained more than 90% of the I in milk [76–78], with anionic inorganic I seemingly the principal form. In contrast, lipids and proteins account for only around 5–10% of the total iodine.

28.6 Changes in mineral distribution

The mineral balance, mainly the divalent cations of Ca and Mg and phosphate, is affected by changes in composition and by milk processing (pH, temperature, etc.). Alterations in the salt distribution of milk in turn have a significant effect on the results of processing [61, 79, 80]. Small changes in the mineral balance can produce notable effects on micellar stability. In addition, milk salts, particularly MCP and Ca^{2+}, play an important role in the aggregation stage during rennet coagulation, thus affecting curd structure.

28.6.1 pH-induced changes

Alterations in milk pH, particularly increased acidity, are fundamental to a number of manufacturing processes, and it has been established that substantial changes in the mineral balance and hence in micellar structure can occur as the pH declines [81–83]. Acidification, either by bacterial souring or by the addition of acids, gradually dissolves the MCP and reduces Ca to casein binding. Acidification from pH 6.6 to 5.3 brings about progressive release of Ca, phosphate and Mg from inside the micelles [84, 85]. This loss of MCP nanoclusters has been demonstrated by small angle X-ray scattering [86]. All the inorganic phosphate is soluble at pH 5.2, while complete solubilization of the calcium caseinate takes place only after acidification to pH 3.5 [87, 88]. MCP does not reform when acidified milk is neutralized; instead, other calcium phosphates having different acid–base buffering properties than those in raw milk are formed. Evidence published in the literature suggests that changes become irreversible in the range of pH 5.7–5.8 [58, 88].

Lucey and Fox [89] reported inhibition of rennet coagulation when approximately 30% (equivalent to pH 5.8–6.0) of the MCP had been solubilized as a consequence of the structural changes in the micelles. At the same time, lower pH also leads to increased Ca^{2+} activity. Ca^{2+} is well known to play an important role in reducing repulsion between negatively charged caseins and thus in augmenting the aggregation rate during milk coagulation. Acidification followed by neutralization [81, 83] results in improved renneting properties by raising the level of Ca^{2+} activity.

Pressurized carbon dioxide can be added to milk as an antimicrobial acidifying agent. In addition to its effect of improving the keeping quality of raw milk by reducing the growth of psychrotrophs, attention has also focused

on the subsequent use of CO_2-preserved milks in cheesemaking [90]. Adding CO_2 to milk lowers the pH, removes MCP, increases Ca^{2+} levels and markedly disrupts micellar structure. A comparison of casein micelle distribution in milk acidified with HCl, milk with added CO_2 and untreated milk indicated that diffusible casein levels in the acidified milk were twice those in the untreated milk but only half the levels in the carbonated milk [91]. This finding could be correlated with the greater release of Ca (and Ca^{2+}) into the diffusible phase brought about by adding CO_2 to a pH of 6.1 compared with acidification by adding HCl [92]. Treatment with CO_2 to a pH below 6.0 results in irreversible changes [93–95]. Although diffusible minerals returned to levels similar to those in untreated milk, salt solubilization destabilized the casein micelle structure, and at some point after depressurization the caseins and other forms of calcium phosphate produced by the treatment reassociated to form a new salt system.

28.6.2 Effects of temperature

MCP solubility increases as the temperature decreases, and consequently milk cooling results in dissolution of MCP [96]. This increase in diffusible salt levels could be attributable to solubilization of casein molecules during cooling. Solubilization, in particular of β-casein [97], is a consequence of weakening of the hydrophobic interactions between casein molecules within the micellar structure [82, 84]. The caseins carry the MCP and cross-linked Ca with them to the diffusible phase. Pasteurization or gently warming, re-establishing the hydrophobic interactions between the caseins, is effective in bringing about reabsorption of the β-casein and restoring micellar structure.

The moderate transfer of the Ca, P and Mg from the diffusible to the colloidal phase together with a decrease in Ca^{2+} activity [98] on heat treatment and the partial reversal of these changes on cooling have been well documented in milk [99]. The extent of reversibility is influenced by the intensity of the heating and the cooling temperature. Law [100] showed that the distribution of Ca and P in milk heated at 85 °C for 10 min and cooled at room temperature for 22 hours was similar to that in raw milk. Similarly, on cooling milk heated at 90 °C, reversal of the changes in Ca and P partitioning was likewise found to be largely complete within the first minute or so, followed by a more gradual recovery (24 hours) to levels close to those in raw milk [101].

More severe treatments (>90 °C) give rise to irreversible changes in casein micelles and mineral partitioning. The interaction between the MCP and the casein is weakened, causing disaggregation of the micelles. The content of casein aggregates cross-linked by MCP in the milk decreased from 51.9% to 46.1% on heating at 135–140 °C for 75 s [102]. Another irreversible change is dephosphorylation of the casein. At 130 °C the extent of dephosphorylation is reported to be about two-thirds after 1 hour [103]. Further, changes in the structure of the original MCP, to a more insoluble form, have also been described [104]. This new form of insoluble calcium phosphate does not take part in casein cross-linking because of its different structure, and MCP composition changes, the concentration of the insoluble form increasing at higher treatment intensities. The new phase is not clearly identifiable, though crystalline β-tricalcium phosphate and hydroxyapatite formation has been suggested [80].

28.6.3 Effects of pressure

In addition to inhibiting microbial growth, high pressure has been reported to enhance milk coagulation, increase cheese yields and be an effective method of improving the coagulation characteristics of milk and other important properties in cheesemaking and yoghurt manufacturing [105, 106]. Applying high pressures to milk also breaks down the casein micelles into smaller structures which can subsequently reaggregate under certain conditions. Pressure-induced disruption of casein micelles appears to be driven primarily by the extent of solubilization of the MCP [107]. Hubbard *et al*. [108] estimated solubilization of the MCP using ^{31}P-NMR under pressures of 100–300 MPa. They found that a peak corresponding to free inorganic phosphate increased progressively with pressure, indicative of progressive pressure-induced solubilization of the MCP, in turn leading to disruption of the amorphous calcium phosphate nanoclusters, thereby compromising micellar integrity.

Pressurization markedly increases casein and mineral levels in the diffusible phase but increases the Ca^{2+} concentration only slightly [109, 110]. The absence of correlation between losses of micellar Ca and levels of free Ca^{2+} indicates that pressure-induced dissociation of the micelles is not produced exclusively by broken linkages between the caseins and inorganic constituents. Pressure enhances those reactions associated with decreased volume and thus exerts a disruptive effect on the ionic and hydrophobic interactions, resulting in the loss of micelle fragments together with calcium phosphate from the micelles [111, 112].

The order of casein dissociation ($\kappa > \beta > \alpha_{s1} > \alpha_{s2}$) in the micelles in pressurized milks follows the ester phosphate content, indicating lesser dissociation of the caseins more tightly bound to colloidal phosphate [109]. Various authors [113, 114] have reported that the highest levels of diffusible Ca, Mg and P were reached at 250–300 MPa, a pattern similar to that described for the casein content. Both Ca, Mg and P and casein levels decreased slightly at 400 MPa. However, in contrast to bovine and caprine milk, ovine milk [109, 115] and buffalo milk [116] did not follow this pattern. In the milks of these ruminants, solubilization was highest at about 400 MPa. These interspecies differences in pressure-induced solubilization of minerals appear to be related to the concentrations of colloidal calcium in the milk [106].

Subjecting milk to moderate pressures (150–300 MPa) was conducive to the formation of a large number of small micelles that coexisted with a fraction of larger micelles, while applying higher pressures (>350 MPa) generated smaller casein assemblies at sizes between 30 and 100 nm [110, 117]. Formation of new and bigger colloidal casein particles at low pressures is probably the result of increased intermolecular hydrophobic interactions, possibly aided by additional charge-shielding due to increased concentrations of Ca in solution. Complete solubilization of calcium phosphate nanoclusters, leading to complete disruption of the micellar framework, may prevent casein aggregation at 350 or 400 MPa [107, 118].

28.6.4 Effects of ultrafiltration

Ultrafiltration (UF) is a pressure-driven membrane process in which membranes having a molecular weight cut-off of between 10 and 75 kg/mol [119] are used to separate macromolecules such as proteins, while small molecules like lactose and soluble minerals diffuse through the membranes into the permeate. When applied to milk, UF yields a retentate containing higher proportions of proteins, fats and colloidal minerals than are present in untreated milk. UF allows diffusible components to pass through the membrane, so their concentrations hardly change, while the concentration of colloidal salts increases due to the volume concentration factor [119–121]. Among the major minerals, retention is highest for Ca, Mg and P and lowest for Na and K. The concentration factors of Ca, Mg and P depend on the proportions bound, and amounts relative to casein usually decrease as the factor increases. Renner and Abd El-Salam [122] reported that in concentrates of between 60 and 80%, the densities of Ca and P rose, though they fell back at more elevated concentration factors; however, even in milk protein concentrate containing 80% protein, Ca density was still somewhat higher than in milk.

Because they are associated with casein micelles, enzymes, membranes and fat globules, Mn, Cu, Fe and Zn will have high concentration factors. Increased trace element concentrations proportional to but less than the concentration factors for proteins and fat have been reported, because a fraction of these minerals is not associated with these other components and is lost in the permeate [121, 123, 124].

When milk has been acidified, a portion of the minerals is solubilized and a smaller proportion is retained by the UF membrane, concentrations in the retentate being lower than for non-acidified milk. In contrast, raising the UF temperature reduces the Ca^{2+} and soluble Ca in the permeate, with more Ca being retained in the retentate [98, 125]. Decreases in soluble P were also observed, but results suggest that considerable calcium citrate will precipitate when milk is heated. The effects of temperature, acidification and diafiltration seem be synergistic [126]. Low temperatures and pH shift the mineral balance from the colloidal fraction to the diffusible fraction. During UF, diffusible minerals pass through the UF membrane and accumulate in the permeate. However, low-temperature UF of acidified skim milk leads to dissociation of casein micelles and higher solubility of micellar minerals, causing increased viscosity.

More recent studies [119, 127] have reported that micelles undergo only small changes during concentration by membrane filtration and that the amount of MCP remains constant except at high concentrations. Casein micelles only become irreversibly altered at concentration levels of ×5. Lower insoluble calcium phosphate levels and protein rearrangement within the micelles were the main changes brought about by UF to high concentration levels. These alterations are reflected in the turbidity values and renneting functionality when the retentates are reconstituted back to the conditions of the original milk.

28.7 Mineral elements in dairy products

The proportions of milk components can be altered during milk product manufacture, by separating some or nearly all the fat by means of centrifugation to produce

semi-skim or skim milk; by simply removing the water to produce evaporated or powdered milk; by adding sugar to evaporated milk to produce condensed milk; by precipitating out some of the components to produce different types of cheese; or by chemical or biochemical alteration to produce such other products as yoghurt, fermented milks and the like. The nutritional value of these milk products depends firstly on that of the milk, though also on the technical processes employed. Table 28.6 sets out the contents of Ca, P, Mg and some trace elements in different types of milk and dairy products.

28.7.1 Fermented milks

Many different fermented milk products exist around the globe, though by far the most freely available published material concerns yoghurt. Fermentation has little effect on the mineral content of milk; and, like milk, yoghurt is an excellent source of essential minerals, particularly Ca, P, Mg and Zn (Table 28.6). At the same time, the mineral content of yoghurts has been shown to exhibit significant differences in the levels of the different elements. While some yoghurts have mineral concentrations similar to those generally reported for raw milk, most show higher contents of these nutrients as a consequence of the addition of dairy ingredients during manufacture. This is a common practice during yoghurt manufacture to enhance the product's technical and organoleptic properties. There are numerous factors affecting the mineral composition of yoghurt, chief among these being the method of fortification with different dairy fractions or products used to increase the solids content. Although whey protein concentrate, milk protein concentrate, and caseinate have all been successfully employed, one of the most common approaches in the industry is to use milk with added skim milk powder. The presence of high Ca, Mg, Na and K contents would thus indicate enrichment of the raw milk with a dairy additive, like milk powder. From a nutritional standpoint this may represent an advantage as a source of essential nutrients in the diet compared with other dairy products. Moreover, yoghurt could be an alternative source of minerals for sufferers of lactose intolerance. On the other hand, mean mineral concentrations are not affected by differences in the fat content of yoghurts.

Bacterial production of lactic acid from the lactose in milk is an essential step in yoghurt manufacture, and the physicochemical changes in the casein micelles brought about by this acidification are reflected in changes in the salt balance. As discussed at length above, lowering the pH from 6.7 to 4.5 brings about major alterations in the composition, structure and reactivity of the casein micelles, altering the mineral balance. Yoghurt manufacture results in the redistribution of minerals, mainly those associated with caseins (e.g. Ca, Mg, P, Zn and Mn). The non-sedimentable Ca in yoghurt has ranged from 97 to 99%, whereas levels of P measured in the supernatants have ranged from 63 to 78% [128]. This difference between Ca and P can be explained by how these elements are bound in the micelles. The very small amounts of Ca remaining in the pellet after ultracentrifugation at pH 4.5 is ascribable to Ca bound to casein without being involved in MCP formation. In contrast, the organic phosphate covalently esterified to the phosphoserine groups on the casein molecules (around 20–25% of the total phosphate) is not removed when the pH decreases during yoghurt manufacture and remains in the pellet after ultracentrifugation (Table 28.5). These changes in mineral distribution may have effects on the nutritional properties and may influence mineral bioavailability. Delisle *et al.* [129] postulated that the high Ca^{2+} content in yoghurt during simulated gastrointestinal digestion could be an index of high bioavailability in this dairy product, and Ünal *et al.* [130] concluded, after an *in vitro* determination, that yoghurt had the highest Ca bioavailability compared with other dairy products. However, other trials in rats and humans have found no differences between the use of Ca from yoghurt and that from other milk or mineral sources [22, 131]. Thus, further study is needed to clarify this controversy and its implications for the nutritional properties of yoghurt.

There is rather limited literature on the mineral constituents of other fermented milks. Significant amounts of both major and minor minerals have been reported in kefir produced by the fermentation of milk with a grain-like starter culture. These minerals include Ca, P, K and Mg and such microelements as Zn, Cu, Mn, Fe, Mo and Co [132]. Trace element contents have been observed to be higher in flavoured kefir than in plain kefir, probably due to the use of adjuncts during processing of the milk [133].

28.7.2 Cheese

A large part of milk production worldwide is used to make cheese, which contains a wide range of mineral elements (Table 28.7). The mineral composition of

Table 28.6 Mineral composition of different dairy products (mean values with ranges in parentheses).

Dairy product	Ca	P	Mg	Na	K	Cl	Zn	Fe	Cu	Mn	Se	I	Reference
	Parts per million										μg/100mL		
Skim milk	1,200	950	120	550	1,500	1,000	4	0.5	Tr.	Tr.	1.0	15	[21]
Dried skim milk	12,800	9,700	1,300	5,500	15,900	10,700	40	2.7	Tr.	Tr.	11.0	150	[21]
Evaporated milk	2,900	2,600	290	1,800	3,600	2,500	9	2.6	0.2	Tr.	3.0	11	[21]
Condensed milk	3,300	2,700	330	1,500	4,500	3,000	10	2.3	Tr.	Tr.	3.0	74	[21]
Butter*	150	240	20	7,500	150	11,500	1	2	0.3	Tr.	Tr.	38	[21]
Cream (35–48% fat)	500	500	60	370	650	510	2	2	Tr.	Tr.	Tr.		[21]
Yoghurt	2,000	1,700	190	800	2,800	1,700	7	1	Tr.	Tr.	2.0	63	[21]
Yoghurt (cows' milk)	1,936 (1252–2601)		150 (105–240)				4 (2.6–6.7)				2.8 (0.3–11.4)		[173]
Yoghurt (goat's milk)	1,940 (1556–2601)		178 (146–238)				5 (3.7–6.6)				2.8 (0.8–5.8)		[173]

*Butter to which salt was added.

Tr., trace levels.

Table 28.7 Levels of minerals and trace elements in different cheese varieties (mg/kg).

Cheese	Milk	Type	Ca	P	Mg	Na	K	Cl	Zn	Fe	Cu	Mn	Se	Reference
Brie	Cow	Soft	5,400	3,900	270	7,000	1,000	10,600	22	8	Tr.	Tr.	0.036	[21]
Cream	Cow	Cream	980	1,000	100	3,000	1,600	4,800	5	1	0.4	Tr.	0.010	[21]
Cottage	Cow	Fresh	730	1,600	90	3,800	890	5,500	6	1	0.4	Tr.	0.040	[21]
Edam	Cow	Semi-hard	7,700	5,300	390	10,200	970	15,700	22	4	0.5	Tr.	0.006	[21]
Feta	Cow	Soft	3,600	2,800	200	14,400	950	23,500	9	2	0.7	Tr.	0.050	[21]
Gouda	Cow	Semi-hard	7,400	4,900	380	9,100	910	14,400	18	1	Tr.	Tr.	0.080	[21]
Parmigiano	Cow	Hard	12,000	8,100	450	10,900	1,100	18,200	53	11	3.3	1	0.110	[21]
Stilton	Cow	Blue	3,200	3,100	200	9,300	1,300	14,100	25	3	1.8	Tr.	0.110	[21]
Emmental	Cow	Semi-hard	9,700	5,900	350	450	890		44	3				[174]
Cheddar	Cow	Hard	7,200	4,900	250	6,700	770		23	3				[174]
Camembert	Cow	Soft	3,500	3,100	210	6,500	1,000		27	2				[174]
Roquefort	Ewe	Blue	5,300	4,000	330	16,700	910		16	4				[174]
Cheddar	Goat	Hard	5,990	5,260	420	3,610	110		7	8	5	0.9		[175]
Idiazabal	Ewe	Hard	9,846	6,259	476	5,530	1,099		31	6	1.2	0.4		[135]
Cabrales	Blend	Blue	7,846	5,429	284	18,871	1,113		34	4	0.9	0.2		[135]
Burgos	Cow	Fresh	6,220	3,850	210	2,220	930		24	6	0.8			[176]
Mahón	Cow	Hard	5,590	4,780	210	12,740	1,430		31	4	0.7			[176]
Manchego	Ewe	Semi-hard	6,850	5,440	590	6,700	800		24	5	1			[176]

Tr., trace levels.

cheeses is mainly associated with the raw source milk, which, as already mentioned, is in turn influenced by a variety of factors (e.g. animal species). Cheeses made from ewe's milk have the highest mineral and trace element content, which is to be expected because the mineral content of ovine milk is also highest compared with the milks of other ruminant species. Furthermore, only about 20% of ewe's milk Ca is in the soluble phase [65], less than in bovine milk (**Table** 28.5), and hence higher levels of this element can be incorporated into the curds.

Na, K and Cl in milk are almost entirely soluble and therefore fully available in the whey during cheesemaking. The Na content, depending on cheese variety, increases appreciably during cheesemaking as a result of the NaCl used in salting or brining. However, low-salt cheeses are also sold commercially. In contrast to the monovalent cations, Ca, Mg and P in milk are associated with the colloidal suspension of casein micelles in differing proportions and consequently are partly retained in the curd during manufacture. Thus, the Ca and P content of cheese is much higher than that of milk, four to five times higher in mould cheeses (e.g. Blue-type cheeses), seven to eight times higher in semi-hard cheeses (e.g. Manchego) and up to 10 times higher in hard cheeses (e.g. Parmesan, Cheddar). The lowest levels are found in cream and cottage-type cheeses, in which they are generally no greater than in the source milk [131].

By contrast, the acidification taking place during coagulation and wheying results in solubilization and substantial losses of the minerals, for the most part those linked to the casein micelles (Ca, Mg, P, Zn). This gives place to a lower contents of these minerals in the curds [134]. The use of lactic cultures can also bring about a significant decrease in the pH, thereby augmenting mineral losses during cheese processing and reducing mineral and trace element concentrations in the finished cheeses [135]. Lastly, the mineral and trace element contents of ripened cheeses is considerably higher than in fresh cheeses, because the ripening process increases concentrations of these elements by decreasing the amount of water [135, 136].

28.7.3 Dairy products fortified with minerals

Mineral fortification of milk products is currently receiving a great deal of attention because of the roles of certain elements in disease prevention (see Table 28.3). Milks today are being marketed as enriched in various minerals, chiefly Ca. Milks fortified with Mg and Zn and other dairy products supplemented with Fe, Cu, I and K can also be found in the marketplace, though on a smaller scale. According to European regulation [137, 138], 100 g or 100 mL of these foods should contain at least 15% or 30% of a mineral's RDA to be labelled as a 'source of' or 'high in', respectively, that mineral. Claims of being 'enriched' through the addition of minerals are subject to the same condition for labelling as for labelling as a 'source of'.

Although studies of Ca fortification in cheese [139] and yoghurt [140, 141] exist in the literature, most reports have dealt with liquid milk [44, 142, 143]. Ca-fortified milks account for more than 20% of total milk sales in some countries. Most of these products declare a Ca content of about 1600 mg/L, an increase of about 30% in this element compared with unfortified milk, and therefore under EU regulations [138] they can be labelled as a 'source of Ca'.

Ca enrichment of milk can be achieved by adding dairy ingredients. Adding milk fractions (permeates, concentrated milk, ultrafiltered milk, or the like) or milk powder could bring further benefits, since it would increase the protein content of the final product. Salts, mainly lactates, phosphates and calcium chloride, can also be used. Calcium lactate, for instance, has been used successfully for Ca fortification of heated milks in pilot-scale experiments [142]. However, supplementation of this kind can alter the salt equilibrium of the milk and modify the mineral distribution between the soluble and micellar phases. Adding phosphate salts increases the P content, which has been associated with the failure of Ca salts to stay in solution. Ca enrichment of milk in the form of calcium chloride seems to lead to a reorganization of the micellar caseins, bringing about an increase in micellar density [143]. Mg-enriched milk can also be found in the marketplace in products whose content in this element is around threefold that commonly reported for unfortified milk [44]. In this case magnesium lactate could be used for supplementation [142].

Results obtained when applying *in vitro* methods with Caco-2 cells show that Ca fortification increases not only the Ca content of the milk but Ca bioavailability as well [144]. Also, metabolic studies on mice revealed that Ca bioavailability in bovine [145] and buffalo [146] milks enriched with different Ca salts was higher than in unfortified milks. Consistent with the previous findings

of Goldscher and Edelstein [147], Ranjan *et al*. [146] also inferred that Ca absorption/retention was much higher for organic than for inorganic salts of Ca. López-Huertas *et al*. [148] also determined Ca absorption in humans from a range of fortified milks. They found that Ca-enriched milks were a good source of absorbable Ca and that absorption of Ca added as milk solids and tricalcium phosphate was higher than the absorption of Ca from unfortified milk.

Fe fortification has been the most extensively researched form of microelement enrichment in the past 25 years (Table 28.8). Because milk is very low in Fe, fortification could help meet this nutritional need for people in risk groups, who typically consume more dairy products. There have been many reports of the manufacture of Fe-fortified milk [149–151], yoghurt [152–154] and cheese [155–158]. However, enriching dairy products with Fe is no easy task, because it can produce a metallic taste and unacceptable flavour as a result of fat oxidation or rancidity, undesirable colour changes from interactions with different compounds, and degradation of vitamins and other minerals. Additionally, Fe supplementation can lower the pH, alter the salt balance, change the structure of the casein micelles, and affect factors regulating rennet coagulation. Fairly comprehensive reviews listing different aspects of these concerns in detail can be found in the literature [159, 160]. Each of the alterations mentioned depends on a different factor, mainly the physicochemistry of the added Fe (Fe valence, solubility, degree of chelation, complex formation).

Proper selection of an appropriate source of Fe is important for dairy product quality. Fe can be added in the form of a salt, such as sulfate, chloride, gluconate, fumarate, lactate [155, 157, 161]. These salts have two oxidation states (Fe^{2+} and Fe^{3+}) and dissolve completely in milk. However, there may be a disadvantage, in that they may freely interact with the constitutive elements of the milk and thus may alter the milk's sensory properties [159] and bring about changes in the mineral components of the casein micelles, partially attributable to the formation of a tertiary complex, inorganic phosphate–Fe–organic phosphate [161]. Fe can also be supplemented in a form chelated to proteins or phosphopeptides [152, 155, 157, 162]. The binding sites are mainly phosphoserine. Protein-chelated Fe is generally not exchanged and does not react with milk components. Then again, Fe salts have been microencapsulated using lecithin or lipid microcapsules to fortify milk [149, 150], cheese [156, 157] and yoghurt [153]. Microencapsulation keeps the Fe from coming into contact with the food vehicle, averting the undesirable interactions that may take place when conventional Fe salts are used. A recent study [151] reported the fatty acid esters technique to be the most efficient, inexpensive, simple, rapid and practical method of microencapsulating

Table 28.8 Mineral contents (mg/kg) in different dairy products fortified with minerals.

Mineral	Dairy product	Content		Source of fortification	Reference
		Unfortified	Fortified		
Calcium	Yoghurt	1570	2130–2310	Ca lactate and gluconate	[140]
Calcium	Commercial UHT milks		1371–1793	Various	[44]
Calcium	Buffalo milk	1820	2293–2346	Ca chloride, lactate and gluconate	[146]
Calcium	Cow milk	1262–1275	1741–1772	Ca chloride, lactate and gluconate	[145]
Calcium	Yoghurt	1317	1793	Ca lactate	[141]
Magnesium	Commercial UHT milk		470	Mg citrate	[44]
Zinc	Turkish white cheese	14	72–140	Zn sulfate dihydrate	[164]
Zinc	Italian cheeses	30–43	136–151	Zn aspartate, gluconate and sulfate	[165]
Zinc	Cheddar cheese	44	228	Zn sulfate	[166]
Selenium	Turkish white cheese	0.04	0.39–0.43	Water-soluble Se chelate	[164]
Iron	Cheddar cheese	0.002	46–124	Fe salts/Fe chelated to proteins	[155]
Iron	Yoghurt		11–39	Fe salts/Fe chelated to proteins	[152]
Iron	Mozzarella cheese	8	20–51	Ferric chloride/Fe chelated to proteins	[157]
Iron	Yoghurt	0.7	8–27	Ferrous lactate	[154]

Fe and fortifying pasteurized milk. However, further study will be needed to gain a detailed understanding of the changes in the different compounds of dairy products brought about by Fe fortification.

In contrast to Fe, there have been only a few studies on Zn fortification of dairy products, namely yoghurt [163] and cheese [164–166]. In all cases, fortification was accomplished without adversely affecting product characteristics. In cheeses, more than 90% of the Zn added to the milk used in cheesemaking as sulfate was recovered from the Zn-enriched cheeses [164]. Different varieties of Zn-fortified cheeses have been reported to have four [165] and five [166] times more Zn than control cheeses (Table 28.8). Se fortification of some dairy products based on a water-soluble Se chelate has also been tested. Fortification did not affect the quality of yoghurts [163], while achieving a sevenfold increase in Se levels in cheeses after 60 days of ripening [164].

28.8 Final considerations

Milk and dairy products are excellent sources of mineral elements, chiefly Ca, P, Mg, K and Zn, though Fe levels are low. The natural Na content is not high, but it can increase substantially in cheeses, since salt is ordinarily added during manufacture. The mineral elements are present in the form of inorganic ions and salts or are part of organic molecules, mainly proteins and fats. The nutritional value of these components has been backed by EFSA and the European Union, which has approved a lengthy series of health-related claims concerning Ca, P, Mg and Zn.

Distribution of the main elements in milk between the soluble and micellar phases contributes not only to milk's nutritional value but also to the stability of the colloidal dispersion. The balance between Ca and P salts can be affected by the varying features of the technical processes (pH, temperature, pressure, concentration) to which milk can be subjected. If processing is mild, the salt balance barely changes and can revert to initial conditions. Applying harsher conditions usually brings about irreversible changes to the structure of the micelles, resulting in the formation of a new salt system that differs from the one intrinsic to milk. Research and development aimed at achieving milk products with higher levels of mineral elements, desirable from a nutritional standpoint, has produced milks and milk products like yoghurt and cheese that have been fortified with minerals and trace elements.

Dairy products supply necessary major mineral elements and trace elements, making measurement particularly important. Advances have been made in adapting analytical methods, especially with regard to sample preparation and final determinations, to product characteristics and the specific levels of the individual elements. Available methods meet the requirements of both research and routine control procedures.

Abbreviations

AOAC: Association of Official Analytical Chemists
EFSA: European Food Safety Authority
ETAAS: Electrothermal atomic absorption spectrometry
EU: European Union
FAAS: Flame atomic absorption spectroscopy
IC: Ion chromatography
ICP-AES: Inductively coupled plasma absorption emission spectroscopy
ICP-MS: Inductively coupled plasma mass spectrometry
MCP: Micellar calcium phosphate
NMR: Nuclear magnetic resonance
RDA: Recommended daily allowance
UF: Ultrafiltration
UV/Vis: Ultraviolet/visible spectrophotometry
XRF: X-ray fluorescence

References

1 Gurr MI. Milk products: contribution to nutrition and health. *J Soc Dairy Technol* 1992;**45**:61–67.
2 Heaney RP. Calcium, dairy products and osteoporosis. *J Am Coll Nutr* 2000;**19**:83S–99S.
3 Nagpal R, Behare PV, Kumar M *et al*. Milk, milk products, and disease free health: an updated overview. *Crit Rev Food Sci Nutr* 2012;**52**:321–333.
4 Korhonen HJ. Bioactive components in bovine milk. In: Park YW (ed.) *Bioactive Components in Milk and Dairy Products*. Wiley-Blackwell, Ames, IA, 2009, pp. 15–42.
5 Park YW, Juárez M, Ramos M, Haenlein GW. Physico-chemical characteristics of goat and sheep milk. *Small Ruminant Res* 2007;**68**:88–113.
6 Medhammar E, Wijesinha-Bettoni R, Stadlmayr B, Nilsson E, Charrondiere UT, Burlingame B. Composition of milk from minor dairy animals and buffalo breeds: a biodiversity perspective. *J Sci Food Agric* 2012;**92**:445–474.
7 De la Fuente MA, Ramos M, Recio I, Juárez M. Sheep milk. In: Park YW, Haenlein GFW (eds) *Milk and Dairy Products in Human Nutrition*. John Wiley & Sons Ltd, Chichester, 2013, pp. 554–577.

8 Abd El-Salam MH, El-Shibiny S. Bioactive peptides of buffalo, camel, goat, sheep, mare, and yak milks and milk products. *Food Rev Int* 2012;**29**:1–23.

9 Choi J, Sabikhi L, Hassan A, Anand S. Bioactive peptides in dairy products. *Int J Dairy Technol* 2012;**65**:1–12.

10 Luhovyy BL, Akhavan T, Anderson GH. Whey proteins in the regulation of food intake and satiety. *J Am Coll Nutr* 2007;**26**:704S–712S.

11 Jensen RG. The composition of bovine milk lipids: January 1995 to December 2000. *J Dairy Sci* 2002;**85**:295–350.

12 Parodi PW. Nutritional significance of milk lipids. In: Fox PF, McSweeney PLH (eds) *Advanced Dairy Chemistry*, 3rd edn. Springer, New York, 2006, Vol. **2**, pp. 601–633.

13 Haenlein GFW. Production of goat milk. In: Park YW, Haenlein GFW (eds) *Handbook of Milk of Non Bovine Mammals*. Blackwell Publishing Professional, Oxford, 2006, pp. 11–33.

14 Haenlein GFW, Wendorff WL. Sheep milk. In: Park YW, Haenlein GFW (eds) *Handbook of Milk of Non Bovine Mammals*. Blackwell Publishing Professional, Oxford, 2006, pp. 137–194.

15 Mayer K, Fiechter G. Physical and chemical characteristics of sheep and goat milk in Austria. *Int Dairy J* 2012;**24**:57–63.

16 Flynn A, Cashman K. Nutritional aspects of minerals in bovine and human milks. In: Fox PF (ed.) *Advanced Dairy Chemistry, Vol. 3. Lactose, Water, Salts and Vitamins*. Chapman & Hall, London, 1997, pp. 257–302.

17 Cashman KD. Macroelements, nutritional significance. In: Roginski H, Fuquay JW, Fox PF (eds) *Encyclopedia of Dairy Sciences*. Academic Press, London, 2002, Vol. **3**, pp. 2051–2058.

18 Cashman KD. Trace elements, nutritional significance. In: Roginski H, Fuquay JW, Fox PF (eds) *Encyclopedia of Dairy Sciences*. Academic Press, London, 2002, Vol. **3**, pp. 2059–2065.

19 Gaucheron F. Milk and dairy products: a unique micronutrient combination. *J Am Coll Nutr* 2011;**30**:400S–409S.

20 Gaucheron F. Milk minerals, trace elements, and macroelements. In: Park YW, Haenlein GFW (eds) *Milk and Dairy Products in Human Nutrition*. John Wiley & Sons Ltd, Chichester, 2013, pp. 172–199.

21 Zamberlin S, Antunac N, Havranek J. Mineral elements in milk and dairy products. *Mljekarstvo* 2012;**62**:111–125.

22 Guéguen L, Pointillart A. The bioavailability of dietary calcium. *J Am Coll Nutr* 2000;**19**:119S–136S.

23 Cashman KD. Milk minerals (including trace elements) and bone health. *Int Dairy J* 2006;**16**:1389–1398.

24 Hirschler V, Oestreicher K, Beccaria M, Hidalgo M, Maccallini G. Inverse association between insulin resistance and frequency of milk consumption in low-income Argentinean school children. *J Pediatr* 2009;**154**:101–105.

25 Malik VS, Sun Q, Van Dam RM *et al*. Adolescent dairy product consumption and risk of type 2 diabetes in middle-aged women. *Am J Clin Nutr* 2011;**94**:854–861.

26 Shahar DR, Schwarzfuchs D, Fraser D *et al*. Dairy calcium intake, serum vitamin D and successful weight loss. *Am J Clin Nutr* 2010;**92**:1017–1022.

27 Astrup A, Chaput JP, Gilbert JA, Lorenzen JK. Dairy beverages and energy balance. *Physiol Behav* 2010;**100**:67–75.

28 Abargouei AS, Janghorbani M, Salehi-Marzijarani M, Esmaillzadeh A. Effect of dairy consumption on weight and body composition in adults: a systematic review and meta-analysis of randomized controlled clinical trials. *Int. J. Obes.,* 2012;**36**:1485–1493.

29 European Union. Commission Regulation (EU) No. 432/2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health of 16 May 2012. *Official J Eur Union* **55**(L136):1–40. Available at http://eur-lex.europa.eu/LexUriServ/%20LexUriServ.do?uri=OJ:L:2012:136:0001:0040:EN:PDF.

30 Alegría A, Barberá R, Lagarda MJ, Farré R. Minerals and trace elements. In: Nollet LML, Toldrá F (eds) *Handbook of Dairy Foods Analysis*. CRC Press, Boca Ratón, FL, 2010, pp. 543–577.

31 Gaines TP, West JW, McCallister JF. Determination of calcium and phosphorus in milk. *J Sci Food Agric* 1990;**51**:207–213.

32 International Dairy Federation. Determination of the calcium content of milk. International Standard, International Dairy Federation, FIL-IDF 36, 1966.

33 Zucchetti S, Contarini G. AAS determination of calcium, sodium, and potassium in dairy-products using TCA for extraction. *Atom Spectrosc* 1993;**14**:60–64.

34 Cal-Prieto MJ, Felipe-Sotelo M, Carlosena A *et al*. Slurry sampling for direct analysis of solid materials by electrothermal atomic absorption spectrometry (ETAAS). A literature review from 1990 to 2000. *Talanta* 2002;**56**:1–51.

35 Karadjova I, Girousi S, Iliadou E, Stratis I. Determination of Cd, Co, Cr, Cu, Fe, Ni and Pb in milk, cheese and chocolate. *Microchim Acta* 2000;**134**:185–191.

36 Aleixo PC, Nóbrega JA. Direct determination of iron and selenium in bovine milk by graphite furnace atomic absorption spectrometry. *Food Chem* 2003;**83**:457–462.

37 Pereira JB, Fernandes KG. Muller RCS, Nóbrega JA, Palheta DC. Direct determination of Ca, Mg, Mn and Zn in buffalo milk of the Marajo island by FAAS. *Quim Nova* 2009;**32**:2333–2335.

38 Horowitz W (ed.) *Official Methods of Analysis of AOAC International*, 17th edn, Vols 1 and II. Association of Official Analytical Chemists, Gaithersberg, MD, 2002.

39 De la Fuente MA, Juárez M. Determination of phosphorus in dairy products by sample wet digestion in a microwave oven. *Anal Chim Acta* 1995;**309**:355–359.

40 Reis Lima MJ, Fernandes SMV, Rangel AOSS. A sequential injection system for the in-line digestion and colorimetric determination of phosphorus in milk. *J Food Sci* 2002;**67**:3280–3283.

41 Afkhami A, Madrakian T, Abbasi-Tarighat M. Simultaneous determination of calcium, magnesium and zinc in different foodstuffs and pharmaceutical samples with continuous wavelet transforms. *Food Chem* 2008;**109**:660–669.

42 Wahlgren NM, Drakenberg T. Milk. In: Webb GA, Belton PS, McCarthy MJ (eds) *Annual Reports on NMR Spectroscopy Vol. 31*. Academic Press, London, 1995, pp. 275–312.

43 Belloque J, De la Fuente MA, Ramos M. Qualitative and quantitative analysis of phosphorylated compounds in milk by means of ^{31}P-FT-NMR. *J Dairy Res* 2000;**67**:529–539.

44 De la Fuente MA, Belloque J, Juárez M. Mineral contents and distribution between the soluble and the micellar phases in calcium-enriched UHT milks. *J Sci Food Agric* 2004;**84**:1708–1714.

45 Geerts JP, Bekhof JJ, Scherjon JW. Determination of calcium ion activities in milk with an ion-selective electrode. A linear relationship between the logarithm of time and the recovery of the calcium ion activity after heat treatment. *Neth Milk Dairy J* 1983;**37**:197–211.

46 Lewis MJ. The measurement and significance of ionic calcium in milk. *Int J Dairy Technol* 2011;**64**;1–13.

47 Perring L, Andrey D. ED-XRF as a tool for rapid minerals control in milk-based products. *J Agric Food Chem* 2003;**51**: 4207–4212.

48 Perring L, Blanc J. Faster measurement of minerals in milk powders: comparison of a high power wavelength dispersive XRF system with ICP-AES and potentiometry reference methods. *Food Anal Method* 2008;**1**:205–213.

49 Pashkova GV. X-ray fluorescence determination of element contents in milk and dairy products. *Food Anal Method* 2009;**2**:303–310.

50 Muñoz E, Palmero S. Determination of heavy metals in milk by potentiometric analysis using a home-made flow cell. *Food Control* 2004;**15**:635–641.

51 Tokusoglu O, Aycan S, Akalin S, Kocak S, Ersoy N. Simultaneous differential pulse polarographic determination of cadmium, lead, and copper in milk and dairy products. *J Agric Food Chem* 2004;**52**:1795–1799.

52 Buldini PL, Cavalli S, Trifiro A. State of the art ion chromatographic determination of inorganic ions in food. *J Chromatogr A* 1997;**789**:529–548.

53 Buldini PL, Cavalli S, Sharma JL. Matrix removal for the ion chromatographic determination of some trace elements in milk. *Microchem J* 2002;**72**:277–284.

54 Cataldi TRI., Angelotti M, D'Erchia L, Altieri G, Di Renzo GC. Ion-exchange chromatographic analysis of soluble cations, anions and sugars in milk whey. *Eur Food Res Technol* 2003;**216**:75–82.

55 Ruzik L. Speciation of challenging elements in food by atomic spectrometry. *Talanta* 2012;**93**:18–31.

56 Holt C, Dalglish DG, Jenness R. Calculation of the ion equilibria in milk diffusate and comparison with experiment. *Anal Biochem* 1981;**113**:154–163.

57 Schmidt DG. Association of caseins and casein micelle structure. In: Fox PF (ed.) *Developments in Dairy Chemistry*. Elsevier, London, 1982, pp. 61–86.

58 Holt C. An equilibrium thermodynamic model of the sequestration of calcium phosphate by casein micelles and its application to the calculation of the partition of salts in milk. *Eur Biophys J* 2004;**33**:431–434.

59 Holt C, Timmins PA, Errington N, Leaver J. A core-shell model of calcium phosphate nanoclusters stabilized by beta-casein phosphopeptides, derived from sedimentation equilibrium and small-angle X-ray and neutron-scattering measurements. *Eur J Biochem* 1998;**252**:73–78.

60 Smyth E, Clegg RA, Holt C. A biological perspective on the structure and function of caseins and casein micelles. *Int J Dairy Technol* 2004;**57**:121–126.

61 Dalgleish DG, Corredig M. The structure of the casein micelle of milk and its changes during processing. *Annu Rev Food Sci Technol* 2012;**3**:449–467.

62 Dalgleish DG. On the structural models of casein micelles: review and possible improvements. *Soft Matter* 2011;**7**: 2265–2272.

63 Holt C, Carver JA. Darwinian transformation of a 'scarcely nutritious fluid' into milk. *J Evolution Biol* 2012;**25**: 1253–1263.

64 Singh H, Flynn A, Fox PF. Zinc binding in bovine milk. *J Dairy Res* 1989;**56**:249–263.

65 De la Fuente MA, Olano A, Juárez M. Distribution of calcium, magnesium, phosphorus, zinc, manganese, copper and iron between the soluble and colloidal phases of ewe's and goat's milk. *Lait* 1997;**77**:515–520.

66 Rivero Martino FA, Fernández Sánchez ML, Sanz Medel A. Multi-elemental fractionation in milk whey by size exclusion chromatography coupled on line to ICP-MS. *J Anal Atom Spectrom* 2002;**17**:1271–1277.

67 Pabón M, Lönnerdal B. Bioavailability of zinc and its binding to casein in milks and formulas. *J Trace Elem Med Biol* 2000;**14**:146–153.

68 Lönnerdal B, Keen CL, Hurley LS. Manganese binding proteins in human and cow's milk. *Am J Clin Nutr* 1985; **41**:550–559.

69 Fransson GB, Lönnerdal B. Distribution of trace elements and minerals in human and cow's milk. *Pediatr Res* 1983; **17**:912–915.

70 International Dairy Federation. *Trace Elements in Milk and Dairy Products*. Bulletin of the International Dairy Federation No. 278/1992. International Dairy Federation, Brussels, Belgium, 1992.

71 Ebringer L, Ferenčik M, Krajčovič J. Beneficial health effects of milk and fermented dairy products: review. *Folia Microbiol* 2008;**53**:378–394.

72 Abollino O, Aceto M, Bruzzoniti MC, Mentasti E, Sarzanini C. Speciation of copper and manganese in milk by solid-phase extraction/inductively coupled plasma-atomic emission spectrometry. *Anal Chim Acta* 1998;**375**:299–306.

73 Brulé G, Fauquant J. Interactions des protéines du lait et des oligoéléments. *Lait* 1982 ;**62**:323–331.

74 Van Dael P, Vlaemynck G, Van Renterghem R, Deelstra H. Selenium content of cow's milk and its distribution in protein fraction. *Z. Lebensm Unters Forsch* 1991;**192**:422–426.

75 Shen L, Van Dael P, Deelstra H. Evaluation of an *in-vitro* method for the estimation of the selenium availability from cow's milk. *Z Lebensm Unters Forsch* 1993;**197**: 342–345.

76 Fernández Sánchez L, Szpunar J. Speciation analysis for iodine in milk by size-exclusion chromatography with inductively coupled plasma mass spectrometric detection (SEC-ICP MS). *J Anal Atom Spectrom* 1999;**14**:1697–1702.

77 Isaac-Olive K, Acharya R, Chatt A. Fractionation analysis of iodine in bovine milk by preconcentration neutron activation analysis. *Talanta* 2008;**77**:827–832.

78 Isaac-Olive K, Chatt A. Studies of total, organic and inorganic iodine in Canadian bovine milk samples with varying milk fat content using ion-exchange chromatography and neutron activation analysis. *J Radioanal Nucl Chem* 2012;**294**:479–486.

79 De la Fuente MA. Changes in the mineral balance in milk submitted to technological treatments. *Trends Food Sci Technol* 1998;**9**:281–288.

80 Gaucheron F. The minerals of milk. *Reprod Nutr Dev* 2005;**45**:473–483.

81 Lucey JA, Gorry C, O'Kennedy B, Kalab M, Tan-Kinita R, Fox PF. Effect of acidification and neutralization of milk on some physico-chemical properties of casein micelles. *Int Dairy J* 1996;**6**:257–272.

82 Law AJR, Leaver J. Effects of acidification and storage of milk on dissociation of bovine casein micelles. *J Agric Food Chem* 1998;**46**:5008–5016.

83 Ezeh VN, Lewis MJ. Milk reversibility following reduction and restoration of pH. *Int J Dairy Technol* 2011;**64**: 179–187.

84 Dalgleish DG, Law AJR. pH-induced dissociation of bovine casein micelles. II. Mineral solubilization and its relation to casein release. *J Dairy Res* 1989;**56**:727–735.

85 Le Graët Y, Gaucheron F. pH-induced solubilization of minerals from casein micelles: influence of casein concentration and ionic strength. *J Dairy Res* 1999;**66**:215–224.

86 Marchin S, Putaux JL, Pignon F, Leonil J. Effects of the environmental factors on the casein micelle structure studied by cryo transmission electron microscopy and small-angle x-ray scattering/ultrasmall-angle x-ray scattering. *J Chem Phys* 2007;**126**:045101.

87 Le Graët Y, Brulé G. Les équilibres minéraux du lait: influence du pH et de la force ionique. *Lait* 1993;**73**:51–60.

88 Gastaldi E, Lagaude A, Tarodo de la Fuente B. Micellar transition state in casein between pH 5.5 and 5.0. *J Food Sci* 1996;**61**:59–64, 68.

89 Lucey JA, Fox PF. Importance of calcium and phosphate in cheese manufacture: a review. *J Dairy Sci* 1992;**76**: 1714–1724.

90 Singh P, Wani AA, Karim AA, Langowsky HC. The use of carbon dioxide in the processing and packaging of milk and dairy products: a review. *Int J Dairy Technol* 2012;**65**: 161–177.

91 Chang MK, Zhang H. Carbonated milk: proteins. *J Food Sci* 1992;**57**:880–882.

92 De la Fuente MA, Olano A, Requena T, Juárez M. Salt balance and rennet clotting properties of cow's, ewe's and goat's milks preserved with carbon dioxide. *J Food Protect* 1998;**61**:66–72.

93 Gevaudan S, Lagaude A, Tarodo de la Fuente B, Cuq JL. Effect of treatment by gaseous carbon dioxide on the colloidal phase of skim milk. *J Dairy Sci* 1996;**79**:1713–1721.

94 Guillaume C, Gastaldi E, Cuq JL, Marchesseau S. Effect of pH on rennet clotting properties of CO_2–acidified skim milk. *Int Dairy J* 2004 ;**14**:437–443.

95 Raouche S, Dobenesque M, Bot A, Cuq JL, Marchesseau S. Stability of casein micelles subjected to CO_2 reversible acidification: impact of carbonation temperature and chilled storage time. *Int Dairy J* 2008;**18**:221–227.

96 Raynal K, Remeuf F. Effect of storage at 4 °C on the physicochemical and renneting properties of milk: a comparison of caprine, ovine and bovine milks. *J Dairy Res* 2000;**67**: 199–207.

97 Aoki T, Yamada N, Kako Y. Relation between colloidal calcium phosphate cross-linkage and release of β-casein from bovine casein micelles on cooling. *Agric Biol Chem* 1990;**54**:2287–2292.

98 On-Nom N, Grandison AS, Lewis MJ. Measurement of ionic calcium, pH, and soluble divalent cations in milk at high temperature. *J Dairy Sci* 2010;**93**:515–523.

99 Holt C. The milk salts: their secretion, concentrations and physical chemistry. In: Fox PF (ed.) *Developments in Dairy Chemistry 3*. Elsevier Applied Science, London, 1985, pp. 143–181.

100 Law AJR. Effects of heat treatment and acidification on the dissociation of bovine casein micelles. *J Dairy Res* 1996; **63**:35–48.

101 Pouliot Y, Boulet M, Paquin P. Observations on the heat induced salt balance changes in milk. II. Reversibility on cooling. *J Dairy Res* 1989;**56**:193–199.

102 Aoki T, Umeda T, Kako Y. Cleavage of the linkage between colloidal calcium phosphate and casein on heating milk at high temperature. *J Dairy Res* 1990;**57**:349–354.

103 Dalgleish DG, Pouliot Y, Paquin P. Studies on the heat stability of milk. I. Behaviour of divalent cations and phosphate in milks heated in a stainless steel system. *J Dairy Res* 1987;**54**:29–37.

104 Holt C. Effect of heating and cooling on the milk salts and their interaction with casein. In: Fox PF (ed.) *Heat-induced Changes in Milk*. International Dairy Federation, Brussels, 1995, pp. 105–133.

105 Huppertz T, Kelly AL, Fox PF. Effects of high pressure on constituents and properties of milk. *Int Dairy J* 2002;**12**: 561–572.

106 López-Fandiño R. High pressure-induced changes in milk proteins and possible applications in dairy technology. *Int Dairy J* 2006;**16**:1119–1131.

107 Huppertz T, De Kruif CG. Disruption and reassociation of casein micelles under high pressure: influence of milk serum composition and casein micelle concentration. *J Agric Food Chem* 2006;**54**:5903–5909.

108 Hubbard CD, Caswell D, Ludemann LD, Arnold M. Characterization of pressure-treated skimmed milk powder dispersions: application of NMR spectroscopy. *J Sci Food Agric* 2002;**82**:1107–1114.

109 López-Fandiño R, De la Fuente MA, Ramos M, Olano A. Distribution of minerals and proteins between the soluble and colloidal phases of pressurized milks from different species. *J Dairy Res* 1998;**65**:69–78.

110 Knudsen JC, Skibsted LH. High pressure effects on the structure of casein micelles in milk as studied by cryo-transmission electron microscopy. *Food Chem* 2010;**119**: 202–208.

111 Johnston DE, Austin BA, Murphy BJ. Effects of high hydrostatic pressure on milk. *Milchwissenschaft* 1992;**47**: 760–763.

112 Lee SK, Anema SG, Schrader K, Buchheim W. Effect of high hydrostatic pressure on Ca-caseinate systems. *Milchwissenschaft* 1996;**51**:17–21.

113 Gaucheron F, Famelart MH, Mariette F, Raulot K, Michel F, Le Graët Y. Combined effects of temperature and high-pressure treatments on physicochemical characteristics of skim milk. *Food Chem* 1997;**59**:439–447.

114 Huppertz T, Fox PF, Kelly AL. Dissociation of caseins in high pressure-treated bovine milk. *Int Dairy J* 2004;**14**: 675–680.

115 Huppertz T, Kelly AL, Fox PF. High pressure-induced changes in ovine milk. 1. Effects on the mineral balance and pH. *Milchwissenschaft* 2006;**61**:285–288.

116 Huppertz T, Zobrist MR, Uniacke T, Upadhyat V, Fox PF, Kelly AL. Effects of high pressure on some constituents and properties of buffalo milk. *J Dairy Res* 2005;**72**: 226–233.

117 Gebhardt R, Doster W, Friedrich J, Kulozik U. Size distribution of pressure-decomposed casein micelles studied by dynamic light scattering and AFM. *Eur Biophys J* 2006; **35**:503–509.

118 Huppertz T, Kelly AL, De Kruif KG. Disruption and reassociation of casein micelles under high pressure. *J Dairy Res* 2006;**73**:294–298.

119 Ferrer MA, Alexander M, Corredig M. Does ultrafiltration have a lasting effect on the physico-chemical properties of the casein micelles? *Dairy Sci Technol* 2011;**91**:151–170.

120 Bastian ED, Collinge SK, Ernstrom CA. Ultrafiltration: partitioning of milk constituents into permeate and retentate. *J Dairy Sci* 1991;**74**:2423–2434.

121 Premaratne RJ, Cousin MA. Changes in the chemical composition during ultrafiltration of skim milk. *J Dairy Sci* 1991;**74**:788–795.

122 Renner E, Abd El-Salam MH. Ultrafiltration in milk. In: Renner E, Abd El-Salam MH (eds) *Application of Ultrafiltration in the Dairy Industry*. Elsevier Science Publishers Ltd, London, 1991, pp. 113–152.

123 Green ML, Scott KJ, Anderson M, Griffin MCA, Glover FA. Chemical characterization of milk concentrated by ultrafiltration. *J Dairy Res* 1984;**57**:267–278.

124 Surowka K, Celej A. Partitioning of protein and some trace elements during 3-step ultrafiltration of skim milk through membranes of decreasing permeability. *Milchwissenschaft* 1996;**51**:426–431.

125 Kaombe DD, Du Y, Lewis MJ. Mineral partitioning in milk and milk permeates at high temperatures. *J Dairy Res* 2012;**79**:1–6.

126 St-Gelais D, Haché S, Gros-Louis M. Combined effects of temperature, acidification, and diafiltration on composition of skim milk retentate and permeate. *J Dairy Sci* 1992;**75**:1167–1172.

127 Alexander M, Nieh MP, Ferrer MA, Corredig M. Changes in the calcium cluster distribution of ultrafiltered and diafiltered fresh skim milk as observed by small angle neutron scattering. *J Dairy Res* 2011;**78**:349–356.

128 De la Fuente MA, Montes F, Guerrero G, Juárez M. Total and soluble contents of calcium, magnesium, phosphorus and zinc in yoghurts. *Food Chem* 2003;**80**:573–578.

129 Delisle J, St-Amand N, Gosselin F, Pichette C, Amiot J. Assessment of soluble and ionic calcium released from milk and dairy products during simulated gastro-intestinal digestion. *Int Dairy J* 1993;**3**:49–59.

130 Ünal G, El SN, Kiliç S. *In vitro* determination of calcium bioavailability of milk, dairy products and infant formulas. *Int J Food Sci Nutr* 2005;**56**:13–22.

131 Scott KJ. Micronutrients in milk products. In: Renner E (ed.) *Micronutrients in Milk and Milk-Based Food Products*. Elsevier Science Publishers Ltd, New York, 1989, pp. 71–123.

132 Ahmed Z, Wang Y, Ahmad A *et al*. Kefir and health: a contemporary perspective. *Crit Rev Food Sci Nutr* 2013;**53**: 422–34.

133 Ozcan T. Determination of minerals in kefir by inductively coupled plasma-optical emission spectrometer (ICP-OES). *Asian J Chem* 2010;**22**:6589–6594.

134 Prieto B, Franco I, Prieto JG. Compositional and physicochemical modifications during the manufacture and ripening of Leon raw cow's milk cheese. *J Food Compos Anal* 2002;**15**:725–735.

135 Moreno-Rojas R, Cámara-Martos F, Sánchez-Segarra PJ, Amaro-López M. Influence of manufacturing conditions and discrimination of Northern Spanish cheeses using multi-element analysis. *Int J Dairy Technol* 2012;**65**: 594–602.

136 Cichoscki AJ, Valduga E, Valduga AT, Tornadijo ME, Fresno JM. Characterization of Prato cheese, a Brazilian semi-hard cow variety: evolution of physico-chemical parameters and mineral composition during ripening. *Food Control* 2002;**13**:329–336.

137 European Union. Council Directive 90/496/EEC of 24 September 1990 on nutrition labelling rules of foodstuffs. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31990L0496&from=EN.

138 European Union. Regulation (EC) No. 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:012:0003:0018:EN:PDF.

139 Martin JH, Zullo PA. Methods of increasing calcium in cottage cheese. *Cultured Dairy Prod J* 1991;**26**:11–14.

140 Pirkul T, Temiz A, Erdem YK. Fortification of yoghurt with calcium salts and its effect on starter microorganisms and yoghurt quality. *Int Dairy J* 1997;**7**:547–552.

141 Singh G, Muthukumarappan K. Influence of calcium fortification on sensory, physical and rheological characteristics of fruit yogurt. *LWT Food Sci Technol* 2008;**41**:1145–1152.

142 Tateo F, Bononi M, Testolin G, Ybarra L, Fumagalli M. Experiments in the use of calcium and magnesium lactates in milk enrichment. *Industrie Alimentari* 1997;**36**: 614–617.

143 Philippe M, Gaucheron F, Le Graët Y, Michel F, Garem A. Physicochemical characterization of calcium-supplemented skim milk. *Lait* 2003;**83**:45–59.

144 Perales S, Barberá R, Lagarda MJ, Farré R. Fortification of milk with calcium: effect on calcium bioavailability and interactions with iron and zinc. *J Agric Food Chem* 2006;**54**: 4901–4906.

145 Singh G, Arora S, Sharma GS, Sindhu JS, Kansal VK, Sangwan RB. Heat stability and calcium bioavailability of calcium-fortified milk. *LWT Food Sci Technol* 2007;**40**: 625–631.

146 Ranjan P, Arora S, Sharma GS, Sindhu JS, Kansal VK, Sangwan RB. Bioavailability of calcium and physicochemical properties of calcium-fortified buffalo milk. *Int J Dairy Technol* 2005;**58**:185–189.

147 Goldscher RL, Edelstein S. Calcium citrate: a revised look at calcium fortification. *Food Technol* 1996;**50**:96–98.

148 López-Huertas E, Teucher B, Boza JJ *et al*. Absorption of calcium from milks enriched with fructo-oligosaccharides, caseinophosphopeptides, tricalcium phosphate, and milk solids. *Am J Clin Nutr* 2006;**83**:310–316.

149 Kwak HS, Yang KM, Ahn J. Microencapsulated iron for milk fortification. *J Agric Food Chem* 2003;**51**:7770–7774.

150 Xia SQ, Xu SY. Ferrous sulfate liposomes: preparation, stability and application in fluid milk. *Food Res Int* 2005;**38**: 289–296.

151 Abbasi S, Azari S. Efficiency of novel iron microencapsulation techniques: fortification of milk. *Int J Food Sci Technol* 2011;**46**:1927–1933.

152 Hekmat S, McMahon DJ. Manufacture and quality of iron fortified yogurt. *J Dairy Sci* 1997;**80**:3114–3122.

153 Kim SJ, Ahn J, Seok JS, Kwak HS. Microencapsulated iron for drink yogurt fortification. *Asian Australas J Anim Sci* 2003;**16**:581–587.

154 Simova E, Ivanov G, Simov Z. Growth and activity of Bulgarian yogurt starter culture in iron-fortified milk. *J Ind Microbiol Biotechnol* 2008;**35**:1109–1115.

155 Zhang D, Mahoney AW. Effect of iron fortification on quality of Cheddar cheese. *J Dairy Sci* 1989;**72**:322–332.

156 Jackson LS, Lee K. Fortification of cheese with microencapsulated iron. *Cultured Dairy Prod J* 1992;**27**:4–7.

157 Rice WH, McMahon DJ. Chemical, physical, and sensory characteristics of Mozzarella cheese fortified using protein-chelated iron or ferric chloride. *J Dairy Sci* 1998;**81**: 318–326.

158 Kwak HS, Ju YS, Ahn HJ, Ahn J, Lee S. Microencapsulated iron fortification and flavor development in cheddar cheese. *Asian Australas J Anim Sci* 2003;**16**:1205–1211.

159 Gaucheron F. Iron fortification in dairy industry. *Trends Food Sci Technol* 2000;**11**:403–409.

160 Mehansho H. Iron fortification technology development: new approaches. *J Nutr* 2006;**136** :1059–1063.

161 Raouche S, Naille S, Dobenesque M *et al*. Iron fortification of skim milk: minerals and ^{57}Fe Mössbauer study. *Int Dairy J* 2009;**19**:56–63.

162 Raouche S, Dobenesque M, Bot A, Lagaude A, Marchesseau S. Casein micelles as a vehicle for iron fortification of foods. *Eur Food Res Technol* 2009;**229**:929–935.

163 Achanta K, Aryana KJ, Boeneke CA. Fat free plain set yogurts fortified with various minerals. *LWT Food Sci Technol* 2007;**40**:424–429.

164 Gulbas SY, Saldamli I. The effect of selenium and zinc fortification on the quality of Turkish white cheese. *Int J Food Sci Nutr* 2005;**56**:141–146.

165 Aquilanti L, Kahraman O, Zannini E *et al*. Response of lactic acid bacteria to milk fortification with dietary zinc salts. *Int Dairy J* 2012;**25**:52–59.

166 Kahraman O, Ustunol Z. Effect of zinc fortification on Cheddar cheese quality. *J Dairy Sci* 2012;**95**:2840–2847.

167 Raynal-Ljutovac K, Lagriffoul G, Paccard P, Guillet I, Chilliard Y. Composition of goat and sheep milk products: an update. *Small Ruminant Res* 2008;**79**:57–72.

168 Institute of Medicine. Dietary Reference Intakes (DRIs): Recommended Dietary Allowance and Adequate Intakes, Elements. National Academies Press, Washington, DC, 2013. Available at http://www.iom.edu/Activities/Nutrition/SummaryDRIs/~/media/Files/Activity%20Files/Nutrition/DRIs/New%20Material/2_%20RDA%20and%20AI%20Values_Vitamin%20and%20Elements.pdf (accessed 4 June 2013).

169 Pohl P, Prusisz B. Determination of Ca, Mg, Fe and Zn partitioning in UHT cow milks by two-column ion exchange and flame atomic absorption spectrometry detection. *Talanta* 2007;**71**:715–721.

170 Miquel E, Alegría A, Barberá R, Farré R. Speciation analysis of calcium, iron, and zinc in casein phosphopeptide fractions from toddler milk-based formula by anion exchange and reversed-phase high-performance liquid chromatography–mass spectrometry/flame atomic-absorption spectroscopy. *Anal Bioanal Chem* 2005;**381**: 1082–1088.

171 López-García L, Viñas P, Romero-Romero R, Hernández-Córdoba M. Liquid chromatography–electrothermal atomic absorption spectrometry for the separation and preconcentration of molybdenum in milk and infant formulas. *Anal Chim Acta* 2007;**597**:187–194.

172 Leiterer M, Truckenbrodt D, Franke K. Determination of iodine species in milk using ion chromatographic separation and ICP-MS detection. *Eur Food Res Technol* 2001;**213**: 150–153.

173 Navarro-Alarcón M, Cabrera-Vique C, Ruiz-López MD *et al*. Levels of Se, Zn, Mg and Ca in commercial goat and cow milk fermented products: relationship with their chemical composition and probiotic starter culture. *Food Chem* 2011;**129**:1126–1131.

174 O'Connor TP, O'Brien NM. Nutritional aspects of cheese. In: Fox PF, Guinee TP, Cogan TP, McSweeney PLH (eds) *Fundamentals of Cheese Science*. Aspen Publishers, Gaithersburg, MD, 2000, pp. 504–513.

175 Park YW. Comparison of mineral and cholesterol composition of different commercial goat milk products manufactured in USA. *Small Ruminant Res* 2000;**37**:115–124.

176 Marcos A, Esteban MA. Iberian cheeses. In: Fox PF (ed.) *Cheese: Chemistry, Physics and Microbiology*. Chapman & Hall, London, 1993, pp. 173–219.

CHAPTER 29
Mineral content of eggs

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Abstract: Studies on the mineral profile of bird and fish eggs are scarce despite the importance of eggs in human nutrition. The distribution of minerals in chicken eggs is concentrated in the shell, with a reduced mineral content in the yolk and white, with S followed by Na, K, Mg, Ca and Fe as the most abundant elements, Zn also being present in quail and ostrich eggs. With regard to fish eggs, it was found that P, K, Mg, Ca and Fe followed by S, Zn, Cu, Mn, Se and I. The analytical tools and sample pretreatment processes proposed in the literature are reviewed, paying attention to speciation studies and to future perspectives in the field.

Keywords: eggs, roes, mineral profile of bird and fish eggs, speciation

29.1 Mineral content of eggs

Eggs are one of the most important sources of proteins and thus their consumption is very common worldwide. Eggs are produced by birds, reptiles, amphibians and fish. Eggs from birds are traditionally consumed by humans, including those of chicken, duck, turkey and quail; however, fish roe and caviar are also consumed. Moreover, of the 76.2 billion eggs consumed in 2009, 30% were in the form of egg products [1]. In recent years, the intake of eggs has declined steadily in the UK [2], the USA [3] and Canada [4]; egg consumption in the UK was 4.8 eggs per person per week in 1968, but this figure had fallen to 2.9 by 1986 as a result of fears associated with the cholesterol content of eggs and other problems related to contamination with the *Salmonella* bacterium.

Poultry eggs are among the most nutritious foods on earth and can be an important and fundamental foodstuff for developing countries. Eggs provide an inexpensive and valuable source of nourishment. In weight terms, an egg contains about the same amount of animal protein as pork and poultry meat, about three-quarters that of beef and two-thirds that of whole milk cheese [5]. Because of their protein content, the United States Department of Agriculture (USDA) categorizes eggs as meats within the Food Guide Pyramid [6]. Moreover, liquid, frozen and dried egg products are widely used by the food service industry and as ingredients in other foods, such as prepared mayonnaise and ice cream.

This chapter focuses on the mineral composition of traditional poultry eggs consumed by humans, but for completeness the final section deals with the mineral composition of fish eggs like sturgeon, salmon and other fish roes.

The poultry egg comprises the shell, the white (or albumen) and the yolk. The weight of an egg and the weight distribution of the three parts differ considerably, depending on the type of egg and its age. The eggshell (0.2–0.4 mm thickness) is mainly composed of calcium carbonate. It can be divided into calcified shell and shell membranes, including inner and outer membranes, which function to prevent bacteria from entering the egg and also aid in preserving the quality of the albumen [7]. Chicken eggshells differ in colour from white-yellow to brown, while ducks shells are greenish to white and those of most wild birds are characteristically spotted. It has been demonstrated that there is little or no relationship between shell colour and nutritional content of the egg [8].

The egg white or albumen is an aqueous solution and consists of three different fractions varying in viscosity. A thin and very firm layer of albumen, termed the chalaziferous layer, closely surrounds the yolk and branches on opposites sides of the yolk into two chalazae that extend

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into the thick albumen. The chalazae serve to anchor the yolk to the centre.

The yolk is a fat-in-water emulsion with approximately 50% dry weight. It consists of alternate layers of dark- and light-coloured material arranged concentrically and is an important nutritional component of the avian egg, providing all the lipids for the developing embryo [9].

The average composition of the hen's egg is 61.5% white or albumen, 29.0% yolk and 9.5% shell. The whole egg contains water (75%), proteins (12%) and lipids (12%), as well as carbohydrates and minerals [10]. The proteins are distributed throughout the egg, with the major fraction found in the egg yolk and egg white, and a small proportion in the eggshell and shell membrane [11]. Lipids are found almost exclusively in the egg yolk, mainly in the form of lipoproteins [12]. Several minerals have also been found in eggs, most of them in the eggshell. Carbohydrates are a minor egg component, present throughout the egg, as both free and conjugated forms, attached to proteins and lipids.

29.2 The presence of minerals in eggs

In chicken eggs, minerals comprise 95.6% of the shell, 0.6% of the white and 1.1% of the yolk [13]. Figure 29.1 shows the distribution and concentrations of the different elements found in the different parts of a chicken egg. The most abundant elements in egg white are S (0.195% of the albumen), P (0.015–0.03%), Na (0.161–0.169%), K (0.145–0.167%), Mg (0.009%), Ca (0.008–0.02%) and Fe (0.0001–0.0002%). On the other hand, egg yolk contains approximately 1.1% minerals, P being the most abundant. More than 61% of the total phosphorus of egg yolk is contained in phospholipids [12]. The mineral elements present in the egg yolk are S (0.016% of the albumen), P (0.543–0.980%), Na (0.026–0.086%), K (0.112–0.360%), Mg (0.016%), Ca (0.121–0.262%) and Fe (0.0053–0.011%) [13].

Other minerals present in eggs at trace levels are Zn, Cu, Mn, Se and I. They are essential nutrients required in small amounts for normal growth and development

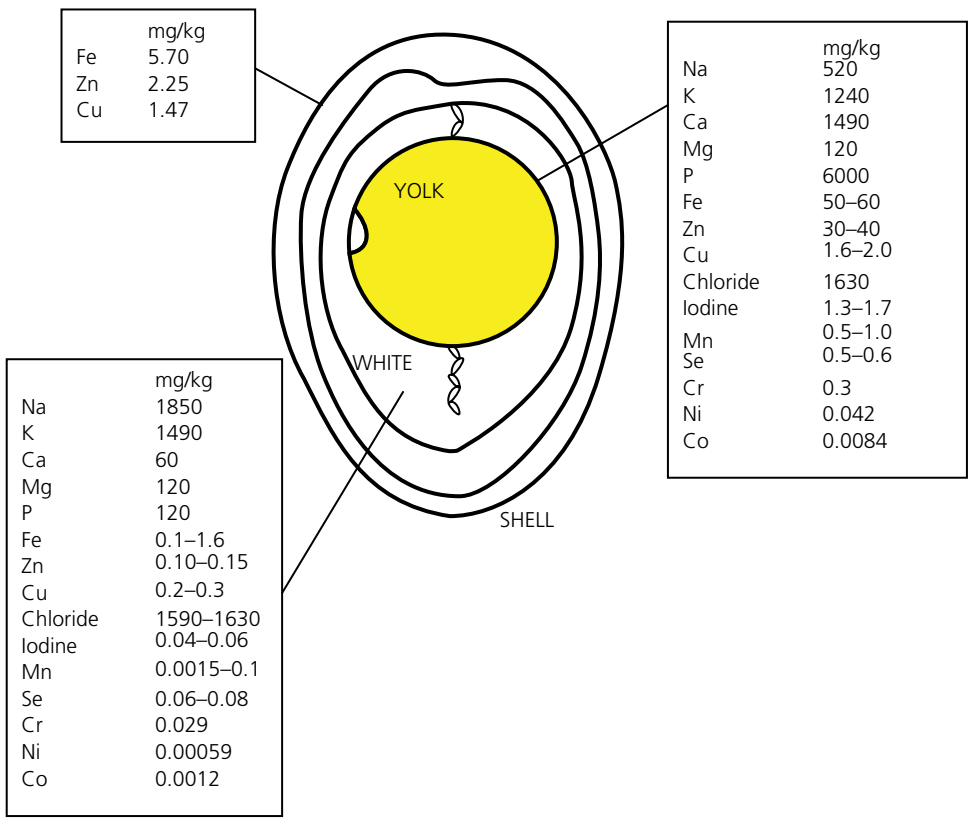


Figure 29.1 The structure of the chicken egg and the distribution of mineral elements in its different parts.

of the avian embryo [14]. In general, trace minerals function as either catalytic or structural cofactors in metal-containing enzymes and proteins that are contained in the cells of the embryo and its extraembryonic membranes. Deficiencies of specific trace minerals can be readily induced in developing avian embryos by feeding insufficient amounts of the mineral to laying hens, which in turn produce eggs that are depleted in trace minerals [15]. Some trace minerals exhibit a variable distribution among egg yolk and albumen depending on the chemical form of the element fed to the hen. Organically bound and natural forms of selenium, such as selenomethionine and selenized yeast, promote deposition of this mineral into egg albumen to a greater concentration than that found in the yolk. Conversely, inorganic forms such as selenite result in greater selenium concentrations in egg yolk than in albumen. Alternatively, feeding hens diets containing very high levels of zinc, copper and iron does not increase egg mineral content dramatically [16]. In fact, very high dietary levels of zinc [17] and copper [18] fed to laying hens will cause a rapid cessation of egg production.

29.3 Analytical techniques for mineral determination in eggs

Designing an experimental procedure involves selecting an appropriate method of analysis based on established criteria, such as accuracy, precision, sensitivity and limit of detection; analysis time; costs of a single analysis; the number of samples to be made per hour; and the amount of sample available for the analysis. The analytical method includes a number of different steps, such as identifying the problem and the context, selecting and defining the sampling and storage strategy, the sample treatment and the analytical method of determination, conducting the experiment, and evaluating the obtained data.

Once the problem has been identified (i.e. to determine the mineral composition of egg samples), a proper sampling and sample treatment strategy would ensure that samples are representative of the material from which they are taken. The following sections deal with the different sample treatments and methods of analysis routinely used in the literature for the mineral profile characterization of edible eggs.

29.3.1 Sampling and sample preparation

Once a representative sample has been obtained, most analyses usually start with sample drying. Many different methods are used for drying foods, such as lyophilization, the use of conventional ovens, desiccators and infrared ovens among others. Different drying methods can provide different sample composition due to differences in the loss of water and the volatile fraction of the matrix and elements to be determined.

After drying, the sample can be ground to reduce its particle size and facilitate intimate contact between the solvent employed to dissolve or extract the elements to be analysed and the sample. At this point, sample treatment can proceed by selective extraction of target elements or by total dissolution of samples. Extraction procedures are simple and rapid compared with total sample dissolution. However, for inorganic analytes, simple solvents cannot leach out trace elements and most inorganic analysis involves total sample dissolution prior to the mineral element determination.

Sample dissolution usually implies the digestion or mineralization of a sample to render it soluble and to totally destroy organic matter or other matrix material that may interfere in the recovery of the analytes under study. The destruction of the matrix can be carried out by wet or dry digestion. Selection of one of these methods depends on time of treatment, cost and number of samples to be analysed. Probably the simplest method for decomposition of the sample involves the use of a muffle furnace in which dry digestion can be made. However, it requires heating the sample in a silica or porcelain container with air at 400–800°C and risks the loss of volatile elements like Hg or other elements that form compounds easily lost at high temperatures. After decomposition, the residue is dissolved in acid and transferred to a tube. With the aforementioned sample treatment, the organic matrix is completely eliminated and inorganic components are pre-concentrated. Nevertheless, this method may cause losses of elements like Hg, Pb, Cd, Ca, As, Sb, Cr and Cu.

Acid digestion, called wet digestion, involves oxidation of the organic matrix by adding a mixture of strong acids in an open or closed container. HNO_3 and H_2SO_4 are frequently used; in some cases, H_2O_2 is also added to the acids. In open containers, when the dissolution has evaporated almost to dryness, a measured quantity of acid is added and diluted to a final known volume. In some cases, a final filtration step is necessary in order to

obtain a clear solution [19]. Digestion with acids does not lead to large losses by volatilization since the temperature of this treatment remains lower than that used for dry digestion. An additional advantage is that this sample pretreatment entails lower digestion times than dry ashing. Nevertheless, wet digestion requires constant attention of the operator due to the exposure of acids and the risks involved by their treatment at high temperatures.

In closed systems, the digestion can be done under pressure control and this provides a faster and more effective sample digestion than that in open systems, also increasing the volatilization temperature of acids and improving their ability to extract the mineral elements. Over recent decades, microwave-assisted digestion has gained acceptance in the field of mineral element determination. This technology, introduced in 1975 by Abu-Samra *et al*. [20], drastically reduces the time of digestion and avoids contamination risks, uses fewer reagents than those required in convective systems and avoids the loss of volatile elements when employed in hermetically sealed reactors. All these advantages are possible due to the direct heating of polar molecules, plus the use of high pressures, which allows augmentation of the acid boiling-point and thus facilitates decomposition of the samples [21]. The mixture of acids and sample is introduced to a Teflon or silica container that is heated according to a profile of temperatures or irradiation power. Microwave systems reach temperatures of around 300°C and pressures of 5.5 MPa (800 psi). Generally, these conditions are sufficient to completely digest all kinds of samples in reasonable treatment times and without the constant attention of the operator. After microwave oven treatment, reactors must be cooled and samples diluted to a known volume; in some cases, a filtration step can be necessary in order to obtain a clear solution, especially when samples contain large amounts of Si which is insoluble in acids.

29.3.2 Determination

In order to determine the mineral profile of eggs, different analytical techniques have been proposed. The techniques of choice to obtain the elemental profile of eggs are those with multi-element detection capability, such as inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES) [22]. ICP-MS typically provides a limit of detection (LOD) for 70 elements in the ppb and ppt range. Unfortunately, for a handful of elements (e.g. As, Se, Cr, Fe, Mn and Ca) conventional ICP-MS cannot provide the best LODs because of polyatomic interferences in the determination of the most abundant isotopes.

ICP-OES is a fast multi-element technique with an extended dynamic linear range and moderate to low LODs (0.2–100 ppb). The instrument uses a plasma source to dissociate the sample into its constituent atoms or ions, exciting them to a level where they emit light of a characteristic wavelength. Up to 60 elements can be screened per sample run in less than 1 min and trace element composition can be determined in a variety of aqueous or organic solutions. The possibility of working with axial and radial view provides high sensitivity, with low background avoiding interferences and involving minimal ionization effects. ICP-OES also allows the possibility of monitoring wavelengths down to 165 nm that provide high sensitivity for phosphorus and sulfur determinations.

Atomic absorption spectrometry (AAS) is also a technique of choice for determining the mineral composition of egg samples [23]. AAS is a sensitive and highly selective spectrometric tool suitable for the determination of many elements at trace and ultratrace levels. The techniques commonly employed in mineral composition of eggs include flame atomic absorption spectroscopy (FAAS), graphite furnace atomic absorption spectrometry, generally called ETAAS, and additionally cold vapour atomic absorption, used for Hg determination and hydride generation atomic absorption, suitable for the determination of covalent hydride-forming elements. The main advantages of FAAS with atomization in a common air–acetylene flame are low operational costs and good analytical performance. The use of FAAS for food analysis avoids possible spectral or polyatomic interferences, which can appear in plasma techniques. Furthermore, FAAS and flame emission are commonly used to determine alkaline and alkaline-earth elements (K, Ca, Na and Mg) that are generally minor components of food samples, avoiding high dilution to match the dynamic ranges of calibration curves. The ETAAS technique has also been employed for mineral characterization of egg samples, offering high sensitivity and selectivity at a reasonable cost [24]. The most important drawback of AAS techniques is the lack of multi-element analysis, which has been partially solved in the last few years by

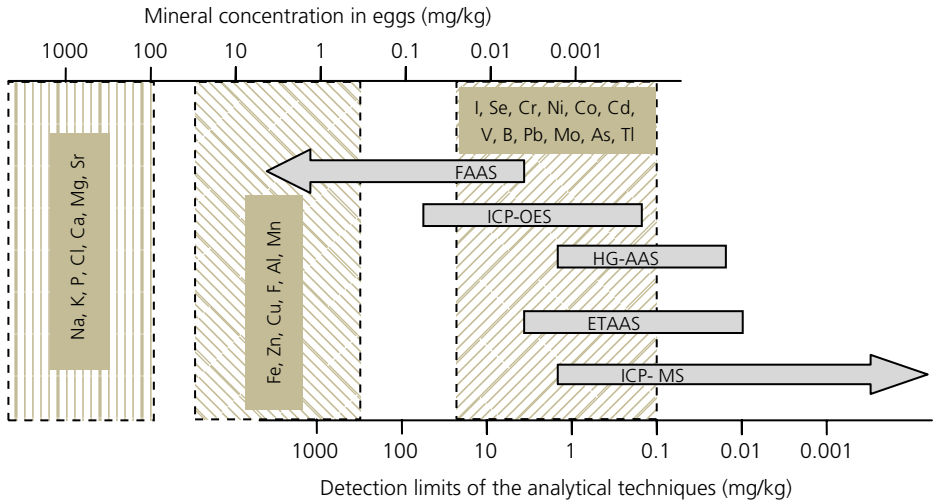


Figure 29.2 Limits of detection of the main analytical techniques used for mineral profile determination and the concentration of elements in egg samples.

the introduction of continuum sources, but it is far from a generally applied tool for food analysis [25].

Additionally, phosphorus has been determined by colorimetric procedures by using the molybdenum blue method [26], yellow vanadomolybdate complex method [27] and the malachite green method [28]. Hg has been determined by cold vapour AAS [29] due to its extreme sensitivity, simplicity, robustness and relative freedom from interferences. F is usually determined by gas chromatography and fluoride electrodes and Se by hydride generation atomic fluorescence spectroscopy.

Figure 29.2 shows the limit of detection of the different atomic analytical techniques compared with the normal concentration of the different minerals in eggs. As can be seen, macrominerals (Na, K, P, Cl, Ca, Mg and Sr) can be determined easily using any of the different atomic techniques. However, if the target elements are those with concentrations in the ppb range or lower, the analytical technique should be selected carefully or the use of pre-concentration steps could be mandatory.

In summary, the general trend observed worldwide is that the use of single-element techniques is being replaced by ICP-OES and ICP-MS for the simultaneous determination of as many elements as possible per sample, thus providing a complete picture of the essential and trace elements and also including the potentially toxic ones.

29.4 Mineral content of the edible part of eggs

Chicken eggs are the most consumed poultry eggs worldwide. The Department of Health in the UK undertook a rolling programme of nutrient analysis surveys to obtain the nutritional value of different foods in 2013 [30]. This survey included the nutrient composition of chicken eggs with regard to fat, fatty acids, protein and a full range of vitamins and minerals. To obtain these values different composite samples, made up of a number of different types of chicken egg (enriched cage, barn, free range and organic), were analysed. The mineral composition data obtained from these analyses have been summarized in Figure 29.3. The different analysed samples were digested with acid under oxidizing conditions, using sealed reactors inside automated microwave digestors, to prevent losses of volatile metals/inorganics. Na, K, Ca, Mg, Cu, Fe, Mn, Zn, P, Se and I were then determined by ICP-OES or by ICP-MS. Results revealed that mineral concentrations in yolk are higher than those in albumen. Moreover, the different processes used to cook eggs can also affect their mineral composition.

A similar study was performed in South Africa with hen eggs produced during the winter and summer of 1990 to obtain their nutrient content [31]. Proximate

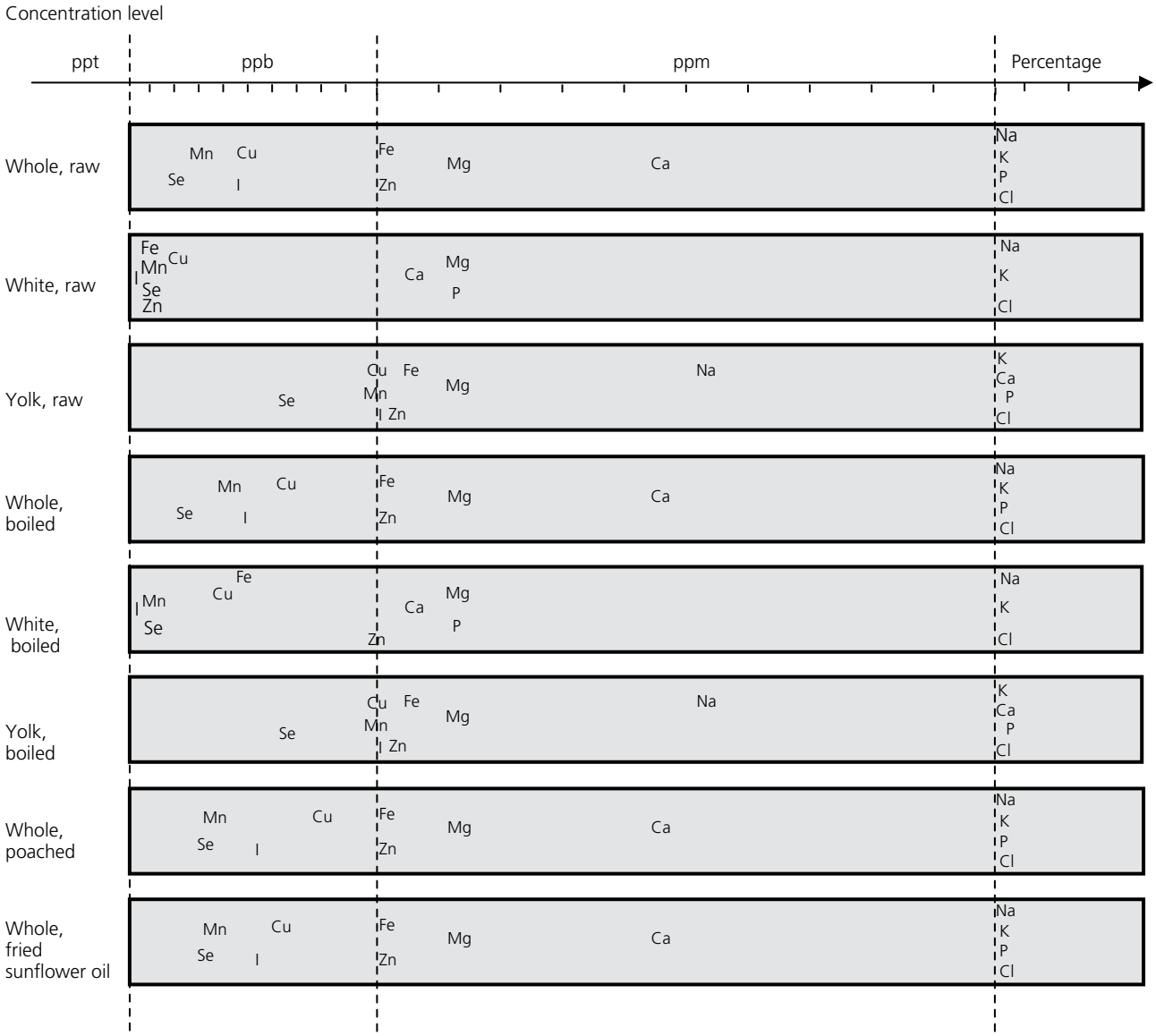


Figure 29.3 Concentrations of mineral components in chicken eggs obtained from raw and cooked eggs. Data summarized from Roe *et al.* [30].

analyses and vitamin, mineral, fatty acid and amino acid determinations were carried out on all the samples. Ca, Mg, Na, K, Cu, Fe, Zn and Mn levels were determined by AAS after dry ashing while P content was determined on the ash by a colorimetric method. The authors concluded that the analysed eggs contain more than 12% less niacin, folic acid, vitamin B_6, biotin, vitamin A, Ca, Mg, P, Fe and Zn than the values given in the South Africa Research Institute for Nutritional Diseases food tables. The reasons for these differences are not clear, but could be because of differences in hen feed or breed.

The husbandry system has also been evaluated in order to understand its effect on the trace mineral composition of chicken eggs in Greece [32]. The content of Se, Zn, Mn, Co, Cu, Mo, V, Cr, Ni, Tl, As and Cd in yolk and albumen of eggs of hens from three husbandry systems were analysed by ICP-MS. Dietary Se, Zn, Mn, Co and Cu concentrations were lower in courtyard compared with conventional and organic diets while Cr concentration was highest in courtyard. As can be seen in Figure 29.4, the highest content of Se in yolks was found in organic, followed by conventional, eggs. Zn contents were highest in courtyard yolk, followed by conventional, which in turn were higher than organic. Mn yolk contents were lowest in courtyard eggs; Cr contents were highest in courtyard eggs. The differences in albumen were in Zn and Cr values, which were highest in courtyard eggs. Thus, it can be concluded that significant differences among eggs from hens in different husbandry systems were observed.

A similar study regarding the effect of organic and conventional-housing systems on mineral content of chicken eggs was performed in Turkey [33]. Random samples of 12 eggs were collected in both housing systems. Eggshells and edible parts were analysed for ash, Ca, P, Mg, Fe, Zn and Cu contents. The P and Zn contents of the edible egg portion were lower in the organic eggs than in conventional eggs. Mg content of the eggshell was higher in organic eggs while Zn content showed a marked decrease in organic produced eggs. As far as Ca, Fe and Cu values were concerned, these did not differ between the eggs produced from organic and conventional systems. The results showed considerable differences in mineral content between the eggs from hens reared in organic and conventional systems, thus offering a good tool for the control of market products.

The levels of some selected mineral elements (As, Cd, Pb, Hg, Cu and Zn) have been evaluated in home-produced chicken eggs in Belgium, involving different spatiotemporal conditions [34]. Eggs were sampled in all provinces of Belgium in autumn 2006 and spring 2007. Trace elements were determined by ICP-MS except for Hg, which was determined by CV-AAS. The mean fresh weight concentrations in eggs in autumn and spring were, respectively, less than 8.0 and also less than 8.0 μg/kg for As, 0.5 and less than 0.5 μg/kg for Cd, 116 and 74 μg/kg for Pb, 0.43 and 0.52 mg/kg for Cu, 20.3 and 19.2 mg/kg for Zn, and 3.15 and 4.44 μg/kg for Hg. The concentration of these elements varied between seasons and regions in Belgium, the average concentrations of As, Cd, Hg, Cu and Zn being similar to those reported in other countries. Average Pb concentrations were high compared with concentrations in eggs from other countries and correlated well with Pb concentrations in soil, indicating that the soil is an important source of Pb for eggs.

The effect of different types of feeding of laying hens on the quality and mineral composition of eggs has been extensively studied. The mineral composition of eggs from laying hens fed with two marine macroalgae enriched with the microelements Cu(II), Zn(II), Co(II), Mn(II) and Cr(III) has been studied [35]. During feeding experiments, the weight of laying hens, weight of eggs, eggshell thickness and mineral content of blood, feathers, droppings and eggs (separately yolk and egg white) and eggshell were measured. Supplementing the diet of laying hens with biometallic additives resulted in an increase in microelement transfer to eggs and enhanced yolk colour. It was also found that the presence of macroalgae in laying hens diet advantageously influenced eggs weight, eggshell thickness and body weight of hens. Thus macroalgae enriched with microelement ions could be potentially used as mineral feed additives in laying hens.

The effect of spray-dried porcine blood plasma or spray-dried porcine blood cells on the plant protein diet of laying hens has also been evaluated [36]. The authors stated that the use of blood cell meal (2 or 4%) negatively influenced eggshell indices and yolk colour compared with control or spray-dried porcine blood plasma groups. Inclusion of spray-dried porcine blood cells enhanced the deposition of Mg and Mn in egg yolk. It was concluded that there was no clear justification for the use of spray-dried blood by-products as an alternative amino acid and mineral source in laying hen diets.

The eggs and broiler chickens originating from breeder flocks at the extremes of age have been studied [37]. Eggs were collected from breeder flocks at 27 and 62

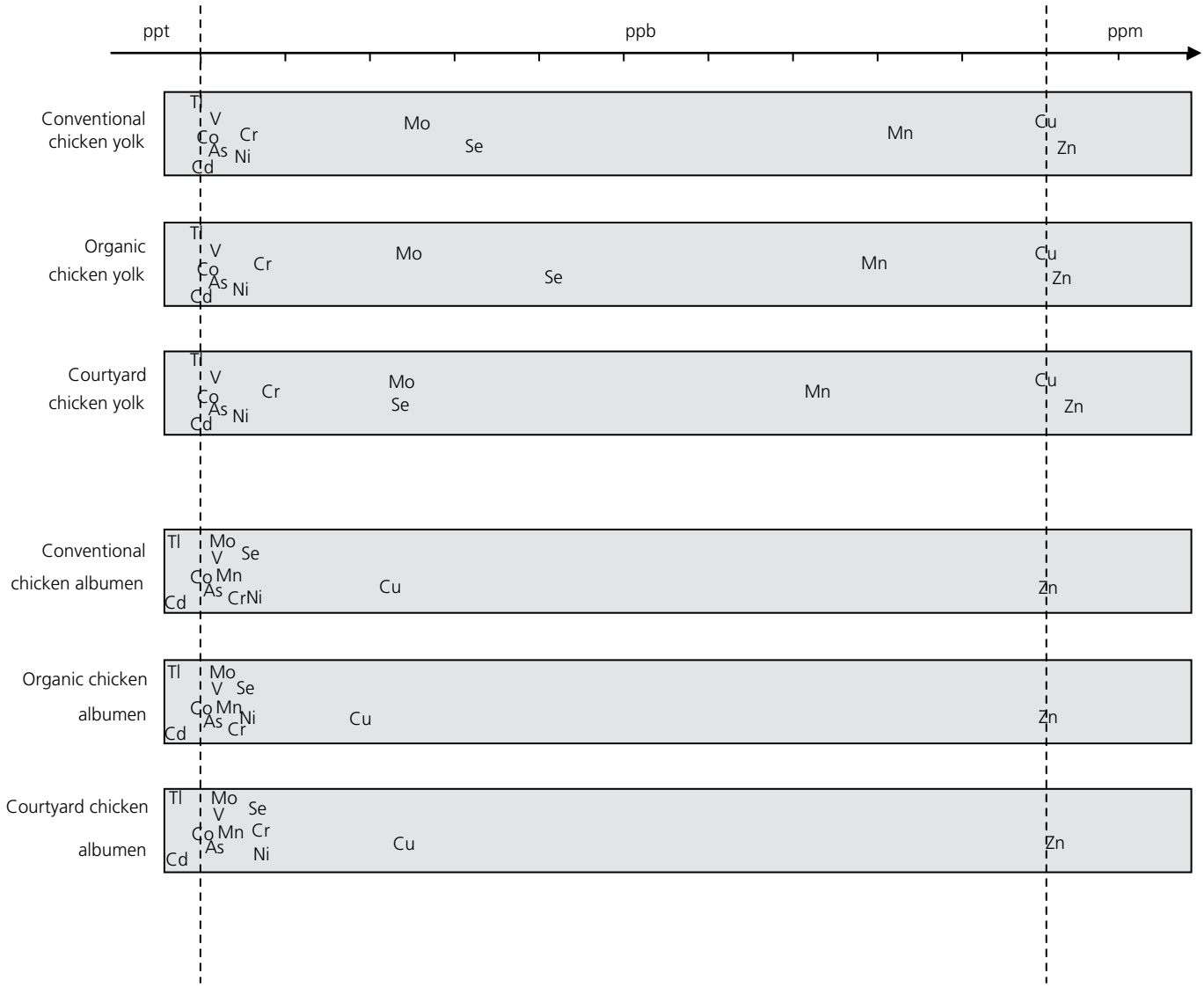


Figure 29.4 Influence of the husbandry system on the mineral composition of chicken eggs with regard to yolk and albumen concentrations. Data extracted from Giannenas *et al.* [32].

weeks of age. Yolks and albumens were separately pooled by flock, and the composites were lyophilized, ground and dried for 18 hours at 60 °C to obtain the dry matter content. Mineral analysis was performed on solubilized ash (6 N HC1) diluted with distilled water to accommodate ICP-OES. Proportions of amino acids and minerals in yolk sac and carcass were also similar at the extremes of age. On the other hand, P in the yolk sac of chicks from young breeders was lower than that found for old breeders.

A similar study was performed on heavy and light eggs from different strain breeder flocks [38]. Eggs were collected from breeder flocks aged between 36 and 45 weeks. Yolks and albumens were separately pooled by flock, and the composites were lyophilized, ground and dried. Mineral analysis was performed on HCl solubilized ash diluted with distilled water. Results obtained suggested that there was no difference in relative nutrients among chicks from extremes in egg weights or diverse strains.

Moreover, the mineral content of different modified egg products for human consumption has also been evaluated. For instance, the study of Kassis *et al.* [39] aimed at creating ω-3 fatty acid-fortified egg products via processing. Experimental egg products (egg whites, ω-3 oils and annatto), whole egg, and liquid egg products (Egg Beaters) were microwave-cooked, analysed and compared. Protein, fat and moisture contents of experimental egg products matched whole egg. As expected, cholesterol concentrations were highest in whole egg, around 1.35 g per 100 g dry weight basis; while it ranged from 0.01 to 0.24 g per 100 g (dry weight basis) in experimental egg products. On the other hand, the total ω-3 fatty acid content was lowest in whole egg (5.9% of total fatty acids) and highest in experimental egg products developed with krill oil (46.5%), flax (43.1%), algae (42.5–39.5%) and menhaden oil (27.6%). With regard to mineral content, whole egg contained more Ca, P and Fe than experimental products but less Mg than ω-3 fortified egg products.

Recently, other types of designer eggs, such as Se-enriched eggs, have come onto the market. Different studies [40–42] showed that the inclusion of organic selenium in the chicken diet could substantially increase the accumulation of this trace element in the eggs. Indeed, the production of Se-fortified eggs is extremely simple: Se supplementation of the diet at 0.4 mg/kg diet in the form of Se-enriched yeast produces eggs that contain approximately 30 mg of Se, which is about 50% of the daily requirement of this element for humans.

Another type of egg product is chicken eggshell powder, which could be considered an attractive source of Ca for human nutrition [43]. To study its nutritional value, minerals, amino acids and hormones were analysed in commercially available Slovakian eggshell powder. The mineral composition was compared with three Dutch samples that differed in feed and housing, one from Japan, refined $CaCO_3$ and an oyster shell supplement. Chicken eggshell powder contains high levels of Ca (401 ± 7.2 mg/g) and Sr (372 ± 161 μg/g) compared with recommended or estimated daily intakes for humans 51–70 years of age. Levels of potentially toxic Pb, Al, Cd and Hg were very low as were levels of V, B, Fe, Zn, P, Mg, N, F, Se, Cu and Cr. Large differences in the levels of F, Se, Cu, Cr and Sr in the Dutch and Slovakian powders indicated a strong influence of feed and environment. Compared with egg powders, refined $CaCO_3$ was found to contain increased levels of Cd, and the oyster shell supplement showed increased levels of Al and Cd. Therefore, the eggshell powder seems to have a beneficial composition, with about 39% of elemental Ca, relevant amounts of Sr, and low levels of Al, Pb, Cd and Hg.

The nutrition of breeding female birds can influence egg quality and is therefore extremely important for development of the embryo and the successful hatching of a high-quality chick. With this in mind, the effect of diet on the mineral content of other poultry eggs has also been evaluated. For instance, the effect of supplementation with minerals in their organic form on egg quality during storage and the deposition of minerals in eggs has been studied in Japanese laying quails [44]. Se-, Zn- and Mn-enriched diets were evaluated and compared with a control diet without mineral supplementation. Se concentrations were determined by ETAAS and the concentrations of Zn and Mn were determined by FAAS with air–acetylene flame. It was observed that supplementation with Se was able to maintain an unchanged egg yolk index over the storage period. However, supplementation with Zn and Mn is not effective for maintaining the quality of stored eggs. Supplementation with Se and Mn is effective at increasing the concentration of these minerals to 328.66% in the albumen and 74.47% in the yolk, respectively, while the different levels of Zn did not change egg composition.

The effect of vitamin E and Se on egg production, egg quality and the mineral content of egg yolk in Japanese

quails reared under normal and low ambient temperatures (18 and 6 °C) has also been evaluated [45]. Egg yolk concentrations of Fe, Zn and Mn were measured at specific wavelengths for each element using AAS. From the obtained results, it was observed that the performance, egg quality and mineral content of egg yolk were not influenced by supplemental vitamin E and Se in quails not exposed to cold stress. However, egg yolk concentrations of Zn, Fe and Mn increased with higher dietary vitamin E and Se in quails reared under cold stress.

Brand *et al.* [46] evaluated the effect of different combinations of dietary energy and protein on the composition of ostrich eggs. These authors investigated the effect of combining different levels of dietary energy and protein, with accompanied levels of amino acid, in the diets of female ostriches on the composition of their eggs. Fresh eggs were collected from each group at the end of the breeding season and the albumen and yolk separated and analysed for protein and lipid content and for amino acid and mineral composition. No significant interaction between energy and protein levels was observed, the conclusion being that despite some differences in egg composition and amino acid concentrations, different dietary energy and protein levels for the most part had little or no effect on the composition of ostrich eggs.

Figure 29.5 summarizes the mineral content of quail and ostrich eggs as compared with that of chickens, also indicating mineral distribution between yolk and albumen. As can be seen, Na, K, P and Ca are the main components, with Fe, Zn and Mg present at ppm levels. Ostrich eggs also contain relatively high concentrations (ppm levels) of Cu compared with chicken eggs.

Examination of trace element speciation in food is extremely important for understanding the biological activity of minerals. Unfortunately, while some trace elements are extensively studied (e.g. Se, As and Hg), others do not attract the attention of analysts [47]. Results from a study that examined Co speciation in chicken eggs [48] indicated that phosphitin from chicken egg yolks and ovalbumin from egg whites can be cobalophilins, which are responsible for the protection of naturally occurring vitamin B_{12} from degradation processes and enzymatic digestion. In many cases, the concentrations of Co in food extracts were extremely low; this made Co detection by size-exclusion chromatography (SEC) unattainable, even when using the sensitive technique of ICP-MS.

Lipiec *et al.* [49] used a similar instrumental set-up for the fractionation analysis of iodine. It was found that most of the I in chicken eggs was present as I^-.

29.5 Mineral content of fish eggs

Fish egg biomass is one of the most valuable food by-products from fishery sources for human consumption. Fish eggs, also known as roe, contain 11% albumins, 75% ovoglobulin and 13% collagen [50]. Furthermore, roe has a high content of nutritive lipids, particularly phospholipids and long-chain unsaturated fatty acids (LCUFAs) [51]. Roes from sturgeon and salmon, which are the most commonly consumed and valued, contained high concentrations of proteins (26–29%) and lipids (5–10%) [52]. The roes of sturgeon are known as 'original' caviar and in some countries only sturgeon caviar can be labelled as 'caviar'. Caviar from other species must be identified with a qualifying term that includes the common name of the fish from it was prepared. Caviar is an expensive product with exceptional flavour and high nutritional value, rich in vitamin A and complex vitamins B [53]. Demand for caviar on export markets is estimated at 500 tonnes per year. As a consequence, in 1997 the World Conservation Union (International Union for the Conservation of Nature or IUCN) listed all commercially utilized sturgeon species worldwide on Annex II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) regulations. Appendix II lists species currently not necessarily threatened with extinction, but which may become so unless trade is closely controlled. International trade in Appendix II species can be authorized to grant export permits or re-export certificates in order to increase protection. The decrease in supply caused by these regulations led to price increases, thereby increasing the feasibility of sturgeon aquaculture production for caviar [54].

The mineral and fatty acid composition of sturgeon eggs has been used to distinguish their source, i.e. farm-raised or wild fish [55]. The most notable difference was the higher concentrations of C18:2n-6 and eicosapentaenoic acid (C20:5n-3) in farm-raised eggs (6.5 and 5.56 mg per 100 g total lipid, respectively) compared with those in wild eggs (0.6 and 4.49 mg per 100 g total lipid, respectively). Moreover, the concentration of selenium was markedly higher in eggs from wild sturgeon

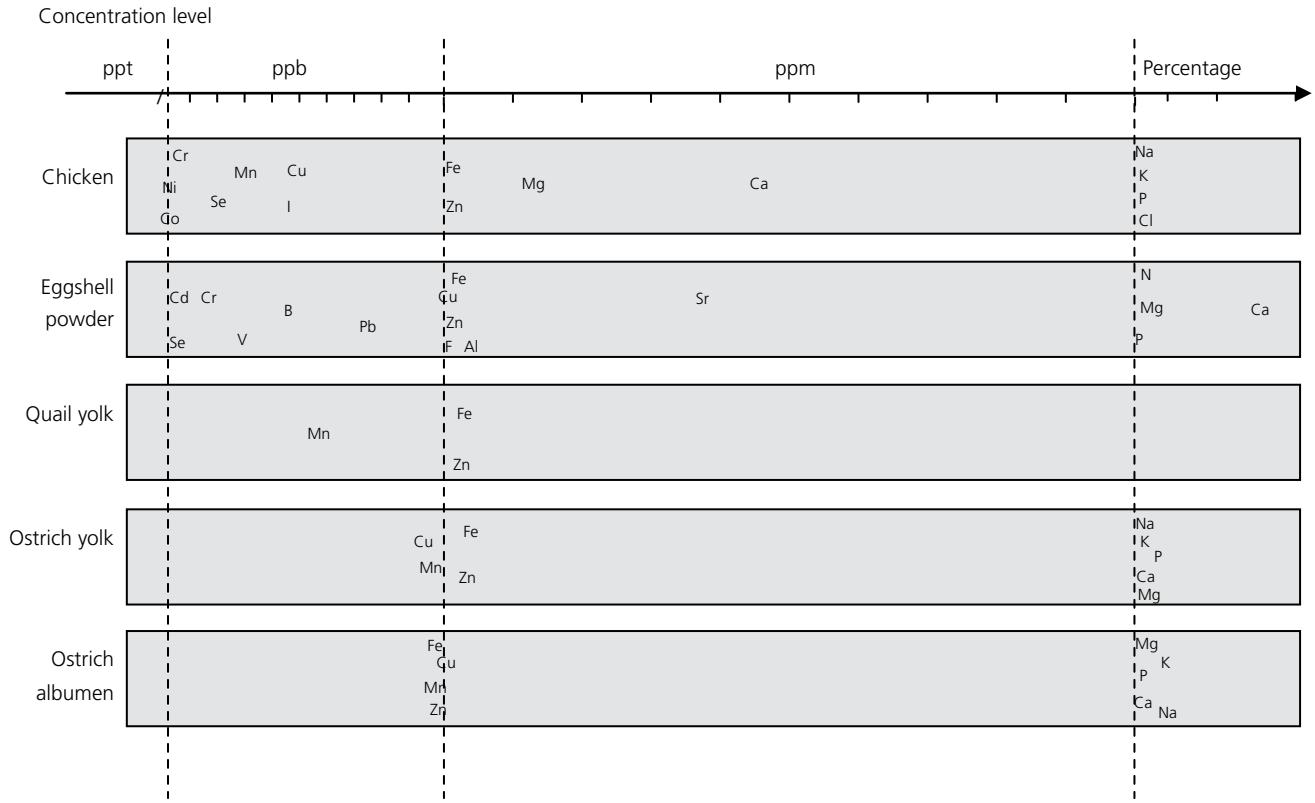


Figure 29.5 Mineral content of bird eggs indicating their concentrations in yolk, albumen and eggshell powder.

(10.0 mg/kg dry weight) than in eggs from farm-raised sturgeon (2.7 mg/kg dry weight), the concentrations of Fe, Zn, Cu, P, S, Ca and K being similar between both farm-raised and wild eggs. Additionally, the arsenic concentration in wild eggs was 3.3 mg/kg dry weight whereas arsenic was not detected in the farm-raised eggs. Thus it can be concluded that the fatty acid and mineral compositions of eggs differed significantly between farm-raised and wild sturgeon and these should be investigated further as biological markers for authentication of caviar origin.

On the other hand, caviar producers try to match the chemical composition of sturgeon eggs from wild and farm-raised fish by modifying feeding practices. According to the study of Gessner *et al*. [56] fatty acid composition in farm-raised sturgeon did not differ largely from that of wild-caught fish. The authors studied the concentration of selected heavy metals in the eggs of different species of sturgeon from different origins and concluded that the concentration of heavy metals observed in the caviar samples did not reveal large fluctuations between the different origins, leading to the conclusion that no accumulation of metals took place in these eggs.

The increase in salmon consumption around the world as a healthy food option has led to significant amounts of roe from salmon of different size and maturity as poorly utilized by-products of processing. Processed mature salmon roe has been used in many parts of the world for the manufacture of high-quality protein powder. Bekhit *et al*. [57] compared the chemical and biochemical properties of raw roe from mature and immature chinook salmon. Maturity had an impact on the profile of amino acids but not on fatty acids. Maturity affected mineral and trace elements, with high values of Ca, Co, Mg, P and S and low concentrations of Cu, Fe, Mn, Na and Zn in mature salmon roe. Immature roes had increased values of total and α-tocopherols but low concentrations of γ- and δ-tocopherols. Soluble proteins from mature and immature roes were similar, but the profiles of membrane-bound proteins differed. Thus maturity will affect functional and physical properties during processing and consumption of chinook salmon roes.

Other fish roes have been analysed from their mineral composition because of their use in human nutrition. The roe from *Mugil cephalus* from Mauritania is one example [53]. The concentrations of Zn and Cd were 11.35 and 0.004 mg per 100 g raw caviar and 12.24 and 0.005 mg per 100 g in waxed caviar, respectively. Fe concentration was 0.87 mg per 100 g, while Pb, Hg and Cu were below their detection limit.

Tuna roe, a by-product generated from fish processing (approximately 1.5–3.0% of total weight), is generally used as animal feed or for pet food preparation [58]. The chemical composition of roes from three tuna species including skipjack, tongol and bonito has been determined [59]. Tuna roes contained 72.2–73.0% moisture, 18.2–20.1% protein, 3.3–5.7% lipid and 1.8–2.1% ash. K, Na and P were the major elements in defatted tuna roes. Crude lipids from tuna roes contained phospholipids as the major component with high content of docosahexaenoic acid (C22:6n-3). Cholesterol contents of crude lipids from the roes of the tuna species were 172, 122 and 94 mg per 100 g lipids, respectively.

In short, it can be concluded that fish eggs generally provide large amounts of Na, K, P and S, also being richer in Mg than bird eggs and providing increased concentrations of trace metals like Cu, Fe, Zn and in some cases Se. Concentrations of Co, As, Cd and Pb occur at ppb levels in some fish species due to their ability to accumulate metals. Data reported in the literature are summarized in Figure 29.6.

29.6 Conclusions and future trends

From a nutritional point of view, it can be seen that eggs mainly contribute Na, K, P and Ca to the human diet, but also contain Mn, Fe, Zn, Cu and Mn at the ppm level, depending on their origin, with the possibility of improving the Se concentration in eggs by making changes in the feed of hens. On the other hand, from an analytical viewpoint, it is unfortunate that there are only a few examples of the determination of ultratrace elements in bird eggs and roes that are suitable for authentication studies and to prevent changes in animal feeding which could affect the dietary intake of both essential and toxic elements. Because of this, additional studies need to explore the possibilities offered by ICP-MS for the determination of as many elements as possible in eggs produced in different countries. Additionally, we are convinced that in the near future speciation studies will determine Se, As and Hg species in eggs produced from farms which use arsenic compounds such as roxarsone, an arsenic animal-feed additive widely used to promote growth and control disease [60], and in wild species.

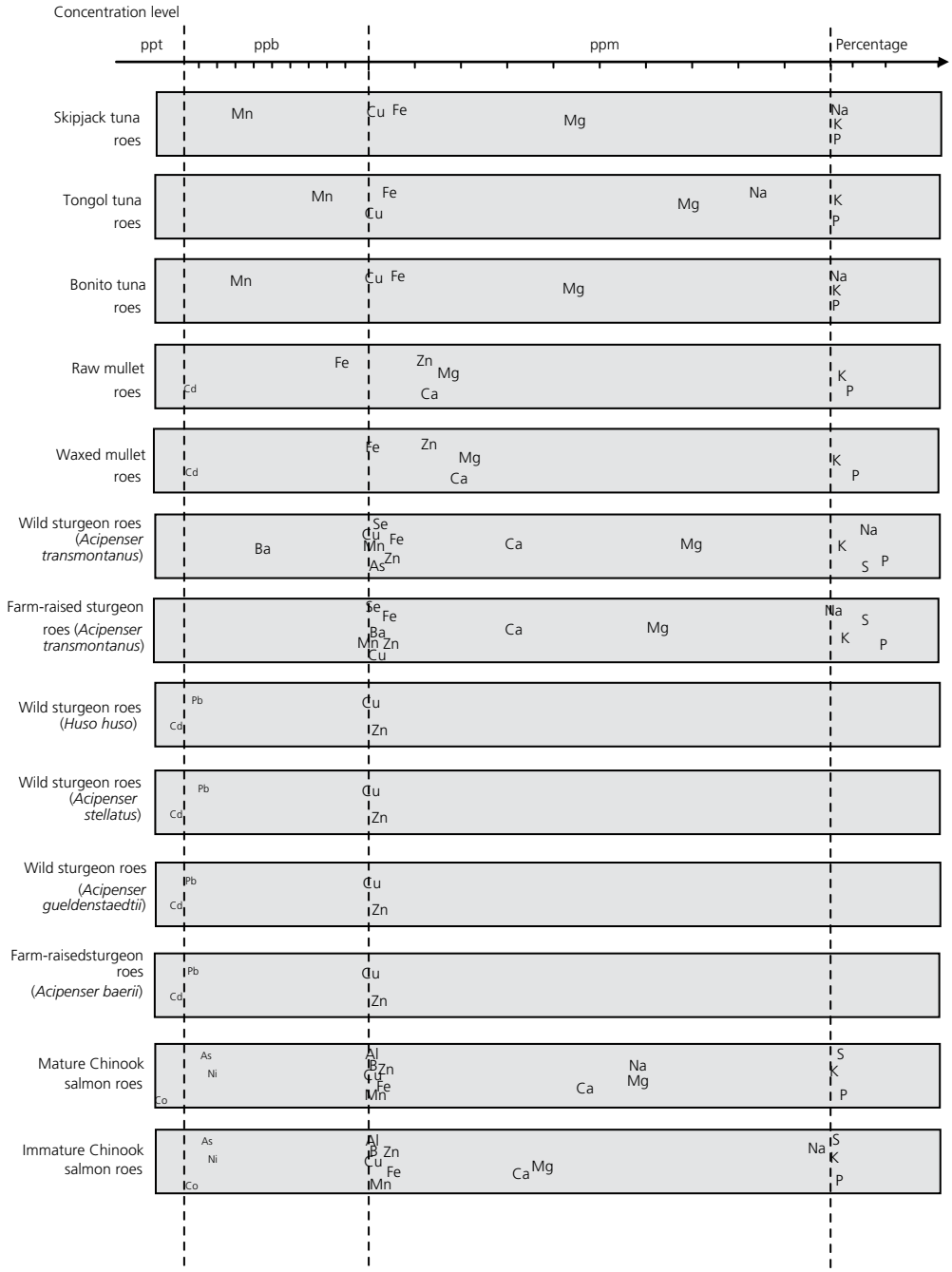


Figure 29.6 Mineral content of the eggs of different fish.

Abbreviations

CITES: Convention on International Trade in Endangered Species of Wild Fauna and Flora
ETAAS: Electrothermal atomic absorption spectroscopy
FAAS: Flame atomic absorption spectroscopy
GFAAS: Graphite furnace atomic absorption spectrometry
HG-AAS: Hydride generation atomic absorption spectrometry
ICP-MS: Inductively coupled plasma mass spectrometry
ICP-OES: Inductively coupled plasma optical emission spectrometry
IUCN: International Union for the Conservation of Nature
LOD: Limit of detection
LCUFA: Long-chain unsaturated fatty acid
SEC: Size-exclusion chromatography
USDA: United States Department of Agriculture

References

1 Egg products and food safety. Food Safety and Inspection Service, United States Department of Agriculture. http://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/egg-products-preparation/egg-products-and-food-safety/ct_index.
2 Walker ARP, Labadarios D, Benade AJS, Vorster HH, Glatthaar II. Natural foods: are current restrictions excessive? *S Afr Med J* 1991;**80**:311–312.
3 Wang Q, Jensen HH, Yen ST. Impact of cholesterol information on US egg consumption: evidence from consumer survey data. *Appl Econ Lett* 1996;**3**:189–191.
4 *Watt Poultry Yearbook: International Edition*. Watt Publishing Company, Rockford, IL, 1994.
5 Food and Agriculture Organization of the United Nations. *Egg Marketing: A Guide for the Production and Sale of Eggs*. FAO Agricultural Services Bulletin 150. FAO, Rome, 2003.
6 Welsh S, Davis C, Shaw A. A brief history of food guides in the United States. *Nutr Today* 1992;Nov–Dec:6–11.
7 Brady D, Lowe N, Gaines S, Fenelon L, McPartlin J, O'Farrelly C. Inhibition of *Streptococcus mutans* growth by hen egg-derived fatty acids. *J Food Sci* 2003;**68**:1433–1437.
8 Scott TA, Silversides FG. The effect of storage and strain of hen on egg quality. *Poultry Sci* 2000;**79**:1725–1729.
9 Kovacs-Nolan J, Phillips M, Mine Y. Advances in the value of eggs and egg components for human health. *J Agric Food Chem* 2005;**53**:8421–8431.
10 Burley RW, Vadehra DV. *The Avian Egg: Chemistry and Biology*. John Wiley & Sons, Inc., New York, 1989.
11 Mine Y. Recent advances in egg protein functionality in the food system. *World's Poultry Sci J* 2002;**58**:31–39.
12 Sugino H, Nitoda T, Juneja LR. General chemical composition of hen eggs. In: Yamamoto T, Juneja LR, Hatta H, Kim M (eds) *Hen Eggs: Their Basic and Applied Science*. CRC Press, Boca Raton, FL, 1997, pp. 13–24.
13 Belitz HD, Grosch W, Schieberle P. *Food Chemistry*, 3rd edn. Springer, Heidelberg, 2004.
14 Savage JE. Trace minerals and avian reproduction. *Fed Proc* 1968;**27**:927–931.
15 Richards MP, Steele NC. Trace element metabolism in the developing avian embryo: a review. *J Exp Zool* 1987;Suppl. **1**:39–51.
16 Stahl JL, Cook ME, Greger JL. Zinc, iron and copper contents of eggs from hens fed varying levels of zinc. *J Food Compos Anal* 1988;**1**:309–315.
17 Decuypere E, Helsen J, Van Gorp S, Verheyen G. The use of high zinc diets as forced molting method: effect on Zn uptake and egg Zn content. *Arch Geflugelkd* 1988;**52**:245–251.
18 Stevenson MH, Pearce J, Jackson NJ. The effects of dietary intake and of dietary concentration of copper sulphate on the laying domestic fowl: effects on laying performance and tissue mineral contents. *Br Poultry Sci* 1983;**24**:327–355.
19 Trampel DW, Imerman PM, Carson TL, Kinker JA, Ensley SM. Lead contamination of chicken eggs and tissues from a small farm flock. *J Vet Diagn Invest* 2003;**15**:418–422.
20 Abu-Samra A, Morri JS, Koityohann SR. Wet ashing of some biological samples in a microwave oven. *Anal Chem* 1975;**47**:1475–1477.
21 Dean JR. *Methods for Environmental Trace Analysis*. John Wiley & Sons Ltd, Chichester, 2003.
22 Broekaert JAC. *Analytical Atomic Spectrometry with Flames and Plasmas*. Wiley-VCH, Weinheim, 2002.
23 Dušová H, Trávníček J, Peksa Z, Falta D, Pálka V. Trace element content in market eggs in Bohemia. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* 2012;**6**:75–80.
24 Åstrand C, Jorhem L. Trace elements in foods. Round T-22, Scrambled eggs. Swedish National Food Administration, Proficiency Testing Programme, Food Chemistry, March/April 2011. Available at http://www.slv.se/upload/dokument/rapporter/pt_rapporter/2011/2011%20Livsmedelsverket%208%20-%20Proficiency%20testing,%20Trace%20Elements%20in%20Food,%20Round%20T-22.pdf.
25 Welz B. High-resolution continuum source AAS: the better way to perform atomic absorption spectrometry. *Anal Bioanal Chem* 2005;**381**:69–71.
26 Galhardo CX, Masini JC. Spectrophotometric determination of phosphate and silicate by sequential injection using molybdenum blue chemistry. *Anal Chim Acta* 2000;**417**:191–200.
27 Kitson RE, Mellon MG. Colorimetric determination of phosphorus as molybdivanadophosphoric acid. *Ind Eng Chem* 1944;**16**:379–383.
28 Motomizu S, Wakimoto T, Tôei K. Spectrophotometric determination of phosphate in river waters with molybdate and malachite green. *Analyst* 1983;**108**:361–367.
29 Jeng SL, Yang CP. Determination of lead, cadmium, mercury, and copper concentrations in duck eggs in Taiwan. *Poultry Sci* 1995;**74**:187–193.
30 Roe M, Pinchen H, Church S, Finglas P. *Nutrient Analysis of Eggs*. Department of Health, London, 2013.

31 Van Niekerk PJ, Van Heerden IV. The nutritional composition of South African eggs. *S Afr Med J* 1993;**83**:842–846.

32 Giannenas I, Nisianakis P, Gavriil A, Kontopidis G, Kyriazakis I. Trace mineral content of conventional, organic and courtyard eggs analysed by inductively coupled plasma mass spectrometry (ICP-MS). *Food Chem* 2009;**114**:706–711.

33 Küçükyılmaz K, Bozkurt M, Yamaner Ç, Çinar M, Çatli AU, Konak R. Effect of an organic and conventional rearing system on the mineral content of hen eggs. *Food Chem* 2009;**132**:989–992.

34 Waegeneers N, Hoenig M, Goeyens L, De Temmerman L. Trace elements in home-produced eggs in Belgium: levels and spatiotemporal distribution. *Sci Total Environ* 2009;**407**: 4397–4402.

35 Michalak I, Chojnacka K, Dobrzanski Z *et al*. Effect of macroalgae enriched with microelements on egg quality parameters and mineral content of eggs, eggshell, blood, feathers and droppings. *J Anim Physiol Anim Nutr* 2011;**95**:374–387.

36 Orda1 J, Jamroz D, Wiliczkiewicz A, Skorupińska J, Kubizna JK. The influence of porcine blood by-products on laying hen performance, egg quality, and yolk mineral content. *J Anim Feed Sci* 2012;**21**:107–121.

37 Vieira SL, Moran ET Jr. Eggs and chicks from broiler breeders of extremely different age. *J Appl Poultry Res* 1998;**7**:372–376.

38 Vieira SL, Moran ET Jr. Broiler chicks hatched from egg weight extremes and diverse breeder strains. *J Appl Poultry Res* 1998;**7**:392–402.

39 Kassis NM, Beamer SK, Matak KE, Tou JC, Jaczynski J. Nutritional composition of novel nutraceutical egg products developed with omega-3 rich oils. *LWT Food Sci Technol* 2010;**43**:1204–1212.

40 Surai PF. Organic selenium and the egg: lessons from nature. *Feed Compounder* 2000;**20**:16–18.

41 Surai PF. Organic selenium: benefits to animals and humans, a biochemist's view. In: Lyons TP, Jacques KA (eds) *Biotechnology in the Feed Industry*. Nottingham University Press, Nottingham, 2000, pp. 205–260.

42 Surai PF. Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. *Br Poultry Sci* 2000;**41**:235–243.

43 Schaafsma A, Pakan I, Hofstede GJH, Muskiet FAJ, Van Der Veer E, De Vries PJF. Mineral, amino acid and hormonal composition of chicken egg shell powder and the evaluation of its use in human nutrition. *Poultry Sci* 2000;**79**:1833–1838.

44 Gravena RA, Marques RH, Roccon J *et al*. Egg quality during storage and deposition of minerals in eggs from quails fed diets supplemented with organic selenium, zinc and manganese. *Rev Bras Zootec* 2011;**40**:2767–2775.

45 Sahin N, Sahin K, Onderci M. Vitamin E and selenium supplementation to alleviate cold-stress associated deterioration in egg quality and egg yolk mineral concentrations of Japanese quails. *Biol Trace Elem Res* 2003;**96**:179–189.

46 Brand Z, Brand TS, Brown CR. The effect of different combinations of dietary energy and protein levels on the composition of ostrich eggs. *S Afr J Anim Sci* 2003;**33**:193–200.

47 Ruzik L. Speciation of challenging elements in food by atomic spectrometry. *Talanta* 2012;**93**:18–31.

48 Lipiec E, Ruzik L, Zhou Y, Jarosz M, Połec-Pawlak K. Study of chicken egg protein influence on bioavailability of vitamin B12 by SEC ICP MS and ESI MS. *J Anal Atom Spectrom* 2011;**26**:608–612.

49 Lipiec E, Warowicka O, Ruzik L, Zhou Y, Jarosz M, Połec-Pawlak K. Investigation of iodine bioavailability from chicken eggs versus iodized kitchen salt with in vitro method. *Eur Food Res Technol* 2012;**234**:913–919.

50 Sikorski ZE. The contents of proteins and other nitrogenous compounds in marine animals. In: Sikorski ZE, Pan BS, Shahidi F (eds) *Seafood Proteins*. Chapman & Hall, New York, 1994, pp. 6–12.

51 Mahmoud KA, Linder M, Fanni J, Parmentier M. Characterisation of the lipid fractions obtained by proteolytic and chemical extractions from rainbow trout (*Oncorhynchus mykiss*) roe. *Process Biochem* 2008;**43**:376–383.

52 Al-Holy MA, Rasco BA. Characterisation of salmon (*Oncorhynchus keta*) and sturgeon (*Acipenser transmontanus*) caviar proteins. *J Food Biochem* 2006;**30**:422–428.

53 Olgunoglu IA, Olgunoglu MP. Concentrations of metal contaminants, vitamin and mineral in waxed caviar from *Mugil cephalus*. *Afr J Agric Res* 2011;**6**:1041–1046.

54 Logan SH, Johnston WE, Doroshov SI. Economics of joint production of sturgeon (*Acipenser transmontanus* Richardson) and roe for caviar. *Aquaculture* 1995;**130**:299–316.

55 DePeters EJ, Puschner B, Taylor SJ, Rodzen JA. Can fatty acid and mineral compositions of sturgeon eggs distinguish between farm-raised versus wild white (*Acipenser transmontanus*) sturgeon origins in California? Preliminary report. *Forensic Sci Int* 2013;**229**:128–132.

56 Gessner J, Wirth M, Kirschbaum F, Kruger A, Patriche N. Caviar composition, the influence of food sources and husbandry practice. *J Appl Ichthyol* 2002;**18**:665–672.

57 Bekhit AEA, Morton JD, Dawson CO, Zhao JH, Lee HYY. Impact of maturity on the physicochemical and biochemical properties of chinook salmon roe. *Food Chem* 2009;**117**: 318–325.

58 Altug G, Bayrak Y. Microbiological analysis of caviar from Russia and Iran. *Food Microbiol* 2003;**20**:83–86.

59 Intarasirisawat R, Benjakul S, Visessanguan W. Chemical composition and functional properties of mrigal (*Cirrhinus mrigala*) egg protein concentrates and their application in pasta. *Food Chem* 2011;**124**:1328–1334.

60 Rosal CG, Momplaisir GM, Heithmar EM. Roxarsone and transformation products in chicken manure: determination by capillary electrophoresis-inductively coupled plasma-mass spectrometry. *Electrophoresis* 2005;**26**:1606–1614.

CHAPTER 30

Mineral content of seasonings, salt and vinegar

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Abstract: Data on the available methodologies for determination of the mineral composition of seasoning products, including salt and vinegar, have been updated from the recently published papers. Data on the presence of major, minor, trace and ultratrace elements in spices, onion, garlic, salt and vinegar from different origins have been compiled to provide a picture of the state-of-the-art in the field and the potential contribution of the standard methods to the daily intake of minerals.

Keywords: spices, seasoning products, salt, vinegar, mineral content, elements, garlic, herbs, onion

30.1 Seasoning products

Since antiquity, spices, herbs and bulbs such as onions and garlic have been used throughout the world for flavouring and preserving food. In this chapter, the terms 'seasoning product' or 'condiment' will be used to group those vegetable products or mixtures, free from extraneous matter, used throughout the world for flavouring, seasoning or imparting aroma in foods [1].

Nowadays, there is continuous and expanding international demand for herbs, spices and essential oils. Social changes, including globalization of the traditional kitchen and food diversification, the desire for new flavours, the increasing importance of 'ethnic' food and the increased importance of processed food in the human diet which requires condiments and aromatic herbs for its preparation, are driving an increase in demand for seasoning. Worldwide trade in spices has increased continuously, from 209, 293 tonnes in 2000 to 323,163 tonnes in 2010, which correspond to a value of US$1.2 billion [2].

Spices can be classified as (i) dried fruits or seeds, such as fennel, mustard or black pepper; (ii) arils, such as mace; (iii) barks, such as cinnamon and cassia; (iv) dried flower buds, such as cloves; (v) stigmas, such as saffron; (vi) roots and rhizomes, such as turmeric, ginger and galingale; and (vii) resins, such as asafetida.

Spices provide aroma, colour and taste to food preparations and sometimes mask undesirable odours and help in food preservation. The organic composition of spices and herbs has been studied in depth. The volatile oils from spices supply the aroma and the oleoresins impart the taste. There is a growing interest in the theoretical and practical aspects of the biosynthetic mechanisms of the active principles present in spices, as well as in the relationship between the biological activity and the chemical structure of these secondary metabolites. The antioxidant properties of herbs and spices are of particular interest in view of the impact of oxidative modification of low-density lipoprotein cholesterol in the development of atherosclerosis. A range of bioactive compounds has been studied for anticarcinogenic properties in animals and humans. However, only a couple of decades ago, the scientific community evidenced a particular interest in the determination of trace and heavy metals in all food components including spices, which can contribute to the daily intake of minerals, and this chapter focuses on this point, also including data on the presence of mineral elements in salt and vinegar, two frequently added components for preservation and flavouring of foods.

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30.1.1 Analytical techniques

Atomic absorption spectrometry (AAS) using flame (FAAS), electrothermal (ETAAS) or cold vapour (CV) techniques, together with atomic fluorescence spectrometry (AFS) and inductively coupled plasma (ICP) techniques, are the most commonly used tools for determining the presence of mineral elements in seasonings. AAS is a sensitive and highly selective spectrometric tool suitable for the determination of selected elements at trace and ultratrace levels. The techniques commonly employed are ETAAS and CV atomic absorption, used for Hg determination and hydride generation (HG) atomic absorption, suitable for determining covalent hydride-forming elements. The general trend observed worldwide is that the use of AAS for single-element analysis is nowadays being replaced by the use of ICP optical emission spectrometry (ICP-OES) and ICP mass spectrometry (ICP-MS) for the elemental analysis of samples, the aim being to determine as many elements as possible. ICP-OES is one of the most widely used techniques for multi-element determinations at the part per million (ppm) level, ICP-MS being one of the most sensitive analytical techniques for fast multi-element determination of heavy metals at trace and ultratrace concentrations in different sample matrices [3]. The use of ICP-MS has grown in the last decade into a simultaneous multi-element detection technique. This methodology has excellent selectivity and also offers high sensitivity at the part per billion (ppb) level and the possibility of performing isotopic determinations.

Techniques based on X-ray fluorescence (XRF), such as wavelength-dispersive (WD) and energy-dispersive (ED) XRF, have been successfully applied to determine the elemental composition of plants [4] in many different fields including contamination [5], agriculture [6], forestry [7] and the elemental chemistry of medicinal herbs and their extracts [8], but the sensitivity achieved by the direct application of these techniques is quite limited.

Particle-induced X-ray emission (PIXE) and proton-induced gamma-ray emission (PIGE) are two of the most promising analytical tools to be used in trace element analysis. Because of their non-destructive nature, reliability, multi-element quantitative capability with excellent sensitivity and detection limits across a wide range of atomic number elements, minimal sample preparation, and reduced turnover time, these techniques have been widely used in various fields including analysis of medicinal plants [9, 10].

Instrumental neutron activation analysis (INAA) with thermal neutrons is a versatile multi-element technique [11]. The application of INAA to the determination of trace amounts of elements has been a standard technique for many years and has been applied in studies of several biological materials including medicinal plants [12]. Apart from the high sensitivity achieved for many elements, INAA has the advantage that it is a purely nuclear method and thus the results are not influenced by the chemical state of the elements under investigation.

Voltammetry techniques have also been used in the analysis of the mineral composition of plants [13]. Stripping voltammetry (SV) comprises a variety of electrochemical approaches, including a pre-concentration step on the electrode surface prior to voltammetric measurement. It possesses some intrinsic advantages such as high sensitivity due to the built-in pre-concentration step, selectivity, the use of inexpensive and portable instrumentation, and the ability to determine several elements accurately at trace and ultratrace levels [14]. The major advantage of SV compared with direct voltammetric measurements is the pre-concentration factor. In trace analysis of heavy metal ions, anodic stripping voltammetry (ASV) is the most popular stripping voltammetric technique. Other SV techniques include cathodic stripping voltammetry (CSV) and differential pulse anodic stripping voltammetry (DPASV). For the trace analysis of inorganic and organic compounds that cannot be accumulated by electrolysis, another stripping method was proposed: adsorptive stripping voltammetry (AdSV). In AdSV, the analyte is adsorbed onto the working electrode by means of a non-electrolytic process prior to the voltammetric scan.

In short, it can be concluded that nowadays there are many techniques suitable for the determination of the mineral composition of herbs and spices and selection of the most appropriate tool will depend on the available instrumentation, the concentration of the elements to be determined and the nature of the samples considered.

30.1.2 Mineral content of spices and herbs

Medicinal plants, spices and herbal products can be rich in trace elements. Despite the relatively small proportion of these plant products in the diet, their regular consumption can make a significant contribution to the daily recommended intake of minerals. It must to be taken into account, however, that only a proportion of the trace elements present in the spices and herbal products is

available for absorption in the digestive tract. Moreover, in-depth knowledge of the mineral composition of seasonings is required to evaluate their influence on the mineral intake of humans.

Firstly, we should emphasize that this section of the chapter is not a comprehensive review of all the papers previously published on the mineral content of herbs and spices. It is a truly difficult task (if not impossible) to identify all the analytical studies involving the mineral composition of herbs, spices (including some vegetables and peppers) and bulbs due to the high number of published studies on this topic and because data in market studies made by companies and policy-makers are not always available. The type of condiments analysed with regard to their mineral composition covers a number of different products [15]. The spices group includes, among others, vanilla, oregano, pepper (black, chilli, sweet red, etc.), cinnamon, cumin, ginger and turmeric; some of the herbal products are thyme, rosemary, mint, parsley, tea, peppermint, lemon balm, sage, hollyhock, linden, lavender, blackberry, rose, garlic and onion.

Figure 30.1 shows the concentration ranges of trace elements found in several species. As can be seen, potassium, ranging from 0.16 to 2.1% (w/w), is the element with the highest concentration. S is present in onion and black pepper at concentrations ranging from 300 to 1000 mg/kg. K and Ca are also present at concentrations between 100 and 6000 mg/kg. On the other hand Ga, Eu, Dy, Yb and Lu are at microgram per kilogram levels in garlic and at nanogram per kilogram levels in onion.

A huge number of studies in which the elemental composition of different spices and herbs around the world was determined can be found in the literature. Figure 30.1 summarizes the concentration of different elements as a function of the analysed product. As can be seen, other than K and Ca, Cl and Mg are in general at percentage levels. Moreover, Na, P and Fe exhibit some fluctuations between spices, their concentrations varying from milligram per kilogram to percentage levels. For example, Na values observed in parsley and cinnamon are 5260 and 119.75 mg/kg, respectively, and Fe values in mint and clove are 6580 and 131.3 mg/kg, respectively, thus showing the different nature of each spice and their different effect on the mineral composition of preserved or cooked food. It is worth pointing out that elements such as Ba, Br, Rb, Cu and Ni show a similar tendency in all spices studied, being found at milligram per kilogram levels. However, in the case of Al, large fluctuations in its concentration can be observed as a function of the product in which it has been determined: 5.2 mg/kg for parsley, 18.3 mg/kg for oregano, 117 mg/kg for cumin and 148 mg/kg for cinnamon. This variation has also been observed in the case of Zn, being present at 14.8 mg/kg in clove and up to 103.7 mg/kg in parsley. Non-essential elements like Pb and Cd show low concentrations in all the aforementioned spices, varying from 5 μg/kg to 5 mg/kg, except in some samples of parsley where Pb is higher than 5 mg/kg. The World Health Organization (WHO) [16] cites maximum permissible levels in raw plant materials only of 0.3 mg/kg for cadmium, 1 mg/kg for arsenic and 10 mg/kg for lead. In this context, the mineral content of medicinal plants is very important and they need to be screened for quality control.

Some examples of the determination of trace elements in spices include onion and pepper from Spain and Morocco [17], where the results confirmed that the content of metal residues depends on the particular type of food and its origin, probably due to different production, processing, storage and preservation methods but also the mineral composition of soils in which they are grown. The trace mineral content of different spice and herbal plant species from Turkey were determined by AAS [18] and ICP-OES [19] and values were in agreement with data reported in the literature. Moreover, the trace metal content of food spices from Pakistan has been evaluated by INAA [20]. Turmeric powder and ginger powder were found to contain relatively high amounts of essential elements and low amounts of toxic elements. Comparison of these values with previously reported data shows variation in trace element contents of red pepper, turmeric, coriander and ginger. Spices from the Orient [21], India [22], Pakistan [23], Nigeria [24], Italy [25], Austria [26] and worldwide [27] have also been analysed for their mineral composition.

On the other hand, it is well known that As, Cd, Hg and Pb are toxic for humans even at very low levels of intake and they are usually present in plants because of the increasing industrialization and associated pollution of the biosphere, uptake from the soil, pesticide treatments and other industrial and anthropogenic operations [28, 29]. Al has also been analysed in spices and medicinal herbs due to the increasing interest of the scientific community in potential toxic effects of this element [30]. Plants can accumulate these elements

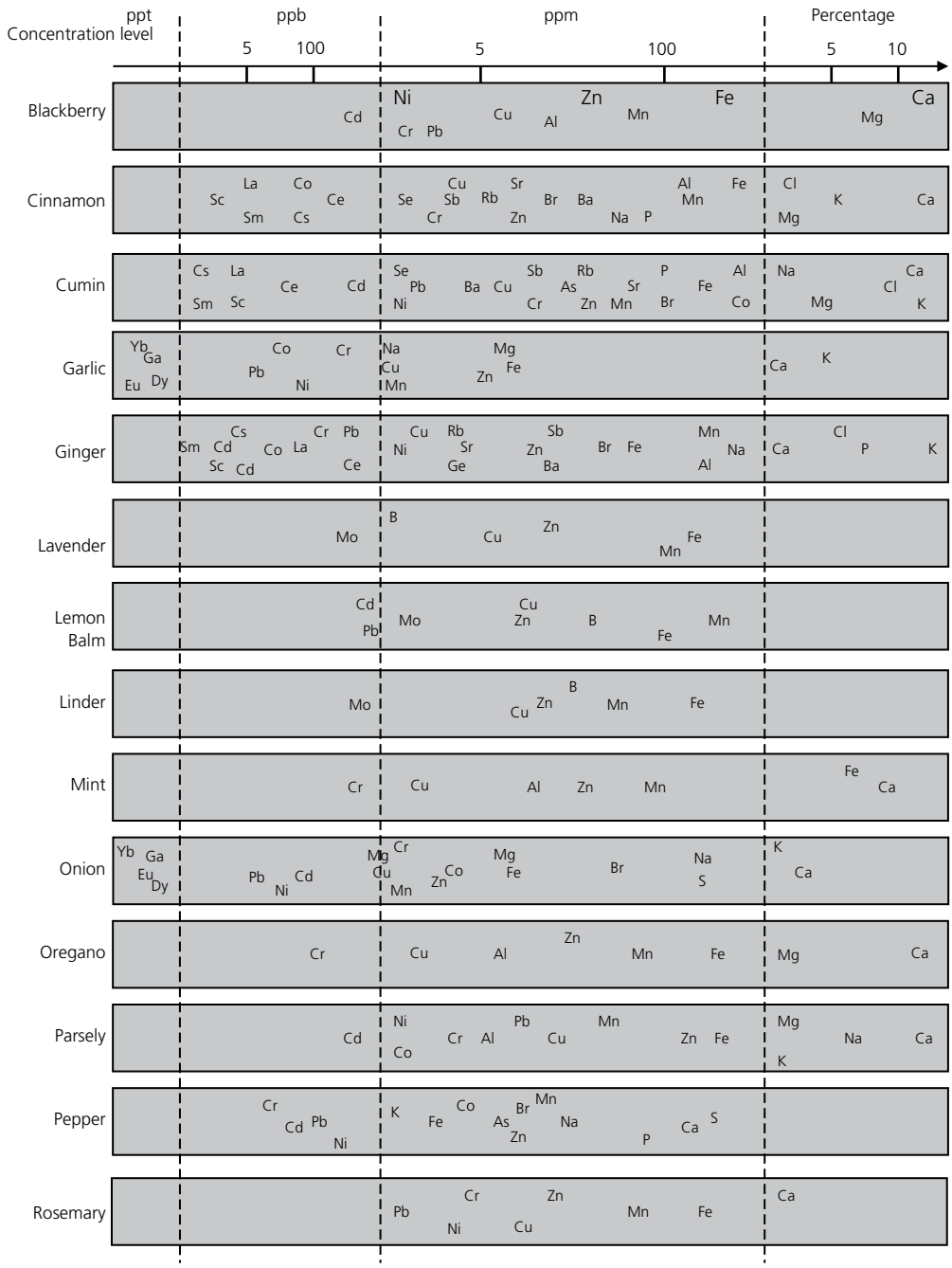


Figure 30.1 Mineral composition of different spices and herbs.

from the surrounding aerial or aquatic environment, enabling some plants to be used as biomonitors for environmental pollution [31–33]. The multi-element concentrations in various common vegetables, fruits and herbs grown near a motorway in London [34], in an old mining area of Romania [35], in urban vegetable gardens from Turkey [36] and in an industrial region of Crakow [37] have been evaluated.

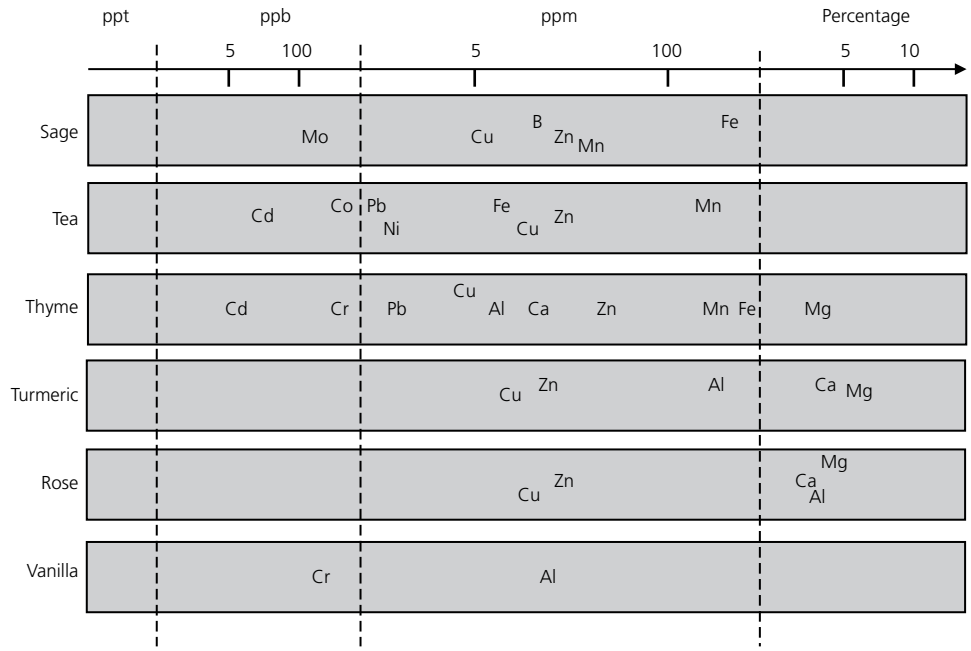


Figure 30.1 (Continued)

Therapeutic plants have always been valued as treatments for a variety of illnesses in folk cultures and have played a very important role in the discovery of modern medicines with novel chemical constituents [38]. However, the efficacy of medicinal plants for curative purposes is often accounted for in terms of their organic constituents like essential oils, vitamins and glycosides. Nowadays, it has been established that overdose or prolonged ingestion of medicinal plants leads to the chronic accumulation of different elements that cause various health problems [39]. In recent years, several studies have reported on the importance of the elemental constituents of herbal drug plants in China [40–42], Malaysia [43], India [44–46], Pakistan [47], Uzbekistan [48], Egypt [49], Nigeria [50, 51], Ghana [52], Turkey [53–56], Greece [57], Spain [58], Austria [59] and Brazil [60] and this has enhanced awareness about trace elements in these plants. Most of these studies concluded that essential metals can also produce toxic effects when minerals are in high concentrations, whereas non-essential metals are toxic even in very low concentrations.

Determination of the total concentration of trace elements in spices is important, but it is also necessary to determine the different chemical species of an element present in the samples in order to clearly evaluate their toxicity and or availability. The use of liquid chromatography hyphenated with ICP-MS offers an effective method for the speciation of Se; moreover, electrospray ionization tandem mass spectrometry (ESI-MS/MS) is also effective for the identification of unknown selenium compounds because it provides molecular information of the chromatographic peaks [61]. The same speciation techniques have been used for the determination of selenium compounds as selenite, selenate, Se-methylselenocysteine (MeSe-Cys) and γ-glutamyl-selenomethylselenocysteine (GluMeSe-Cys) [62], and also to determine Se-methionine and Se-cysteine/Se-cystine [63]. Anion- and cation-exchange chromatography were applied for the separation of selenium species (Se(IV), SeMet) with MS detection after two different sample preparation methods: NaOH and enzymatic digestion [64].

An alternative to the extraction of Se species is to use an ultrasound-assisted treatment. In this case, samples are homogenized by the action of ultrasound applied through a titanium probe. The ultrasound probe is able to reduce dramatically the extraction time of selected chemical forms, which is of critical importance for speciation studies in order to guarantee species stability and to avoid interconversion of spices. However, in order to improve the suitability of the ultrasound-assisted

extraction method, it is necessary to ensure species integrity during the whole extraction/separation/detection process [65]. In order to determine Se speciation, two different chromatographic mechanisms have been applied to the extracts of seasoning products, reversed-phase ion-pairing chromatography (RP-LP-HPLC) with heptafluorobutyric acid as ion-pairing agent and an HPLC column combining size-exclusion and ion-exchange mechanisms. In all the experiments total Se extraction efficiencies ranged from 75 to 122%.

As an example of non-chromatographic speciation, the simultaneous determination of As(III) and total As and Sb(III) and total Sb in Chinese herbal medicines by HG-double channel-AFS has been successfully performed [66]. On the other hand, species of elements such as As, Cd and Pb were determined in different extractable fractions in Bulgarian herbs by ICP-MS and ETAAS [67]. The major part of As and Pb existed in the biomacromolecular fraction. Cd appears to be present mainly in cationic form at pH 1, but at pH 7.6 there is a non-cationic fraction as well. A similar scheme of fractionation was used for the speciation of As [68] and Cu, Zn, Fe, Mg, Ca and Cr [69] in Chinese traditional herbs.

30.2 Salt

Rock salt or common sea salt is dominantly composed of mineral halite, with the chemical formula NaCl. It is found in deposits of rock salt, brines, saline lakes, marshes, sea water and saline earth [70] and is composed primarily of sodium chloride.

Depending on the formation, salts may be classified as (i) rock salt, extracted by conventional mining methodologies from underground or surface halite deposits; (ii) solar salt, obtained from sea water and saline lakes and recovered by solar evaporation; (iii) solution mining or salt brine, which is obtained by the dissolution of underground salt deposits, with the product used in the original form, as solution, or used to obtain the fourth class of salt; (iv) vacuum salt, a type of salt produced by mechanical evaporation technology; using this methodology it is also possible to make use of sea water or other sources of brine solutions [71]. In relation to solar salt, a subgroup is obtained by the evaporation of sea water and is an edible type called sea salt.

According to the United States Geological Survey from 2010, the principal end uses for salt are the chemical industry (42%), ice control (38%), agriculture (4%), food sector (4%), general industry (2%) and primary water treatment (2%).

Salt is one of the oldest, most ubiquitous food seasonings and salting is an important method of food preservation. Sodium chloride is an essential constituent of the human body as it is necessary for osmotic balance in human cells [72]; specifically, sodium is essential for transmission of the nerve impulse, hydration, pH and the absorption of nutrients while chloride enables the blood to carry carbon dioxide from tissue to the lungs. The World Health Organization (WHO) has recommended a reduction in salt intake to 5 g of NaCl (or 2 g of Na) per day because it is associated with blood pressure and cardiovascular problems [73]. Moreover, salt is an excellent carrier of iodine, an essential element in humans, in the form of potassium iodate, potassium iodide, sodium iodate and sodium iodide; thus iodinated salt is an ideal medium for the prevention of iodine deficiency disorders.

30.2.1 Analytical techniques

In 2003, the European Salt Producers' Association (Eusalt) listed a number of analytical protocols that can be adopted for the analysis of non-refined and refined salts [74]. The proposed standards are in agreement with the guidelines of the Codex Alimentarius Commission. FAAS, ICP-OES and ICP-MS are the most commonly used techniques for trace element determination. These techniques have been used to determine Pb, Cd and Cu in sea salts [75].

At present, concentrations of the components of the mother liquor and concentrated brine in the crystallizer are determined by means of densitometry [76] and chemical analysis [77]. However, densitometry cannot determine the concentrations of all the components individually and chemical analysis cannot offer rapid determination of the trace components. Additionally, an attenuated total-reflectance infrared (ATR-IR) spectrometry method has been proposed for determining the concentration of each component in the mother liquor and concentrated brine [78]. Mid- and near-infrared spectroscopy have been applied for the prediction of different constituents in brine solutions at different stages of salt crystallization [79, 80]. In this case it was demonstrated that NIR offers the ability to predict sulfate, hydrogen carbonate and phosphate [81].

The determination of trace metals in sea salt presents a challenging task for two main reasons: (i) interferences

of NaCl and (ii) the concentrations of metals in salt are normally very low. The most common analytical method for total Fe determination in salt is the photometric method with 1,10-phenanthroline (www.eusalt.com). A chromatographic method in which 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone (PMBP) was used as chelating agent for the simultaneous determination of Fe^{3+} and Al^{3+} in sea salts [82] and an automated method based on flow injection analysis (FIA) have been successfully applied for the determination of trace Fe in raw sea salt samples and in 'purified' salts [83]. In this case, the chromogenic reagent 2-(5-nitro-2-pyridylazo)-5-(*N*-propyl-*N*-sulfopropylamino) phenol, which forms water-soluble complexes with metal ions such as Fe^{2+}, was explored. Fe^{3+} was previously reduced to Fe^{2+} by ascorbic acid.

The determination of Mn in salt samples has been performed by manganese-catalysed oxidation of 3,4-dyhydroxybenzoic acid (DHBA) by hydrogen peroxide [84]. The recommended method for Hg determination in sea salts is the CV-AAS (www.eusalt.com). Electrothermal vaporization (ETV) ICP-MS was used to determine bromine in table and sea salt samples [85], concentrations ranging from 0.13 to 0.47 mg/g.

30.2.2 Mineral content of salt

Pure salt contains 39.3% sodium and 60.7% chlorine by weight and the chemical composition of sea salt includes Ca and Mg as chloride and sulfate salts and has approximately 3.5% moisture [86]. This composition varies with the concentrating conditions and affects the quality of the final product [87]. On the other hand, the composition of inland brine salt includes sodium chloride, sulfate, carbonate and bicarbonate.

Lake salts may also include some heavy metals, such as Fe (1.27–1.55 mg/L), Pb (0.33–0.67 mg/L), Mn (0.15–0.17 mg/L), Cu (0.11–0.20 mg/L) and Zn (0.02–0.08 mg/L) [88].

Gourmet salts from origins such as sea, river and mineral deposits have also been evaluated for their mineral composition including trace elements [89]. The highest concentrations of macroelements such as Ca (73–1400 mg/kg), Fe (2–500 mg/kg), K (600–29000 mg/kg), Mg (35–8600 mg/kg) and P (up to 380 mg/kg) were found in dark-coloured salts, primarily black salts, with important differences with respect to normal table salt. On the other hand, small concentrations of toxic elements were found in the gourmet salts tested. Negligible amounts of As, Cd and Hg were detected. The highest elemental concentrations of toxic elements were found in dark-coloured salts, with Cr levels of 2.6 mg/kg, Pb levels around 0.5 mg/kg and Tl concentrations lower than 1 mg/kg.

The chemical composition of rock salts from Kohar, Pakistan has been evaluated before and after a refinement process [90]. Chemical analysis showed that raw material was composed of water-insoluble impurities (6.90%), moisture (0.12%), sulfite (1.30%), Ca (1.66%), Mg (0.05%), Cl (54.40%), Na (33.91%) and K (1.58%). After the process, the composition of the product was moisture (0.12%), sulfite (0.010%), Na (39.23%), Cl (60.61%), K (0.002%) and traces of Pb, As and Fe.

Commercial table salt contains iodine at a recommended concentration of 20–40 mg/kg [91]. Worldwide, iodine deficiency affects about 2 billion people and is the leading preventable cause of mental retardation [92]. Deficiency also causes thyroid gland problems, including endemic goitre. In many countries, iodine deficiency is a major public health problem that can be cheaply addressed by purposely adding small amounts of iodine to the sodium chloride salt available in the markets.

It can be concluded that the main mineral contribution of salt to preserved and cooked foods corresponds to Na and Cl and, to a lesser extent, I as a function of the marine origin of samples and the national rules concerning the iodization of table salt.

30.3 Vinegar

Vinegar, from the French *vin aigre*, meaning 'sour wine', has been used for thousands of years as both a condiment and a preservative [93]. Vinegar is produced by alcoholic fermentation followed by acetic fermentation of any raw material that is rich in carbohydrates. Starting material may be derived from wine, molasses, dates, sorghum, apples, pears, grapes, berries, melons, coconut, honey, beer, maple syrup, potatoes, beets, malt, grains and whey. Initially, yeasts ferment the natural food sugars to alcohol. Next, acetic acid bacteria (*Acetobacter*) transform the alcohol to acetic acid. Commercial vinegar is produced by either fast or slow fermentation processes. Another method of production is chemical synthesis from natural gas and petroleum derivatives, resulting in a highly concentrated acetic acid solution without the presence of the flavouring components of alcohol fermentation [94].

The chemical and organoleptic properties of vinegars are a function of the starting material and the fermentation method. Acetic acid, the volatile organic acid that identifies the product as vinegar, is responsible for the tart flavour and pungent biting odour of vinegars. Specialty vinegars are grouped as herbal or fruit vinegars. Herbal vinegars consist of wine vinegars or white distilled vinegars, which may be seasoned with garlic, basil, tarragon, cinnamon, clove or nutmeg. Fruit vinegars are wine and white vinegars sweetened with fruit (lemon or raspberry) or fruit juice to produce a characteristic sweet–sour taste. Furthermore, they are used in cooking meat and fish as well as in the manufacturing of canned foods [95, 96].

30.3.1 Analytical techniques

AAS is the technique most frequently used for the quantification of mineral elements in vinegar with proper accuracy, sensitivity and precision [97]. Undesirable matrix effects can be avoided by using either a previous decomposition step [98] or the standard additions method [99].

An alternative that reduces the matrix effect problems is ICP-OES, a multi-element determination technique that allows the rapid determination of elements in complex samples [100]. However, this technique is also susceptible to matrix effects due to the presence of concomitants and acid medium [101]. The introduction of acid solutions with physical properties different from water in the plasma may modify the characteristics of the aspirated solution and consequently aerosol formation and transport processes. This latter effect is especially critical when organic acids are employed [102, 103]. The observed effects are attributed to the reduction of solution aspiration rate, an increase in aerosol transport efficiency and changes in plasma excitation conditions. A potential solution to avoid the aforementioned problems is to prepare the standard solutions with a pure acetic acid concentration similar to that found in commercial vinegar samples.

Axially viewed ICP-OES equipped with different sample introduction systems, such as a cross-flow nebulizer combined with a double-path spray chamber (CF-DP) and cone spray associated with a cyclone spray chamber (CS-CC), have been also employed for the determination of trace mineral composition of vinegar samples [104].

30.3.2 Mineral content of vinegar

Quantification of the mineral elements present in vinegars is important because of the role of metals in metabolism, the potential toxicity of certain elements, and for detection of adulteration of the product and characterization of the vinegar [105]. High concentrations of certain elements may induce undesirable properties, such as precipitation, colour changes and turbidity [106, 107]. The mineral composition of vinegar depends on the natural composition of the raw materials, the constituents formed during fermentation, contact with production and storage equipment, contamination of the production environment and, sometimes, the substances formed during the ageing process [93].

Data on the mineral content of vinegars have been extensively studied and reported due to their implications for organoleptic, hygienic and dietetic characteristics as well as their toxicological implications [104, 105, 108]. Figure 30.2 shows the trace mineral content of vinegar samples as a function of raw material employed according to data extracted from Akpinar-Bayizit *et al.* [109]. Wine and apple cider vinegar samples showed high concentrations of K and Na, which may be related to the raw material used in the production [105]. Ca, Mg and P, in minor concentrations, were also present in the raw materials. The presence of Mg in wine vinegars has been reported to be dependent on the natural content of grapes [104].

The concentration of microelements in vinegars is important because they can be used as an index of processes that could affect the properties and beneficial aspects of vinegars, such as the production process, environmental pollution, sanitary conditions and the quality of the raw material. Ni, Cd, Pb and Sn can be correlated with environmental pollution, while Cd, Cu and Fe with the release from alloys of the equipment used for vinegar production. The mineral element concentration of vinegars has been used in combination with chemometric techniques to classify samples as a function of their origin. It was successfully performed with the Italian Aceto Balsamico Tradizionale di Modena [98] and with wine vinegars from the south of Spain [110]. Therefore, information on the mineral content of vinegar is important for evaluating the potential health risks of vinegar consumption, and the quantification of these elements can serve as a tool for characterizing vinegar quality and authenticity using chemometrics [111].

Furthermore, the speciation of Sn species (butyltin compounds) has been performed in Chinese vinegars.

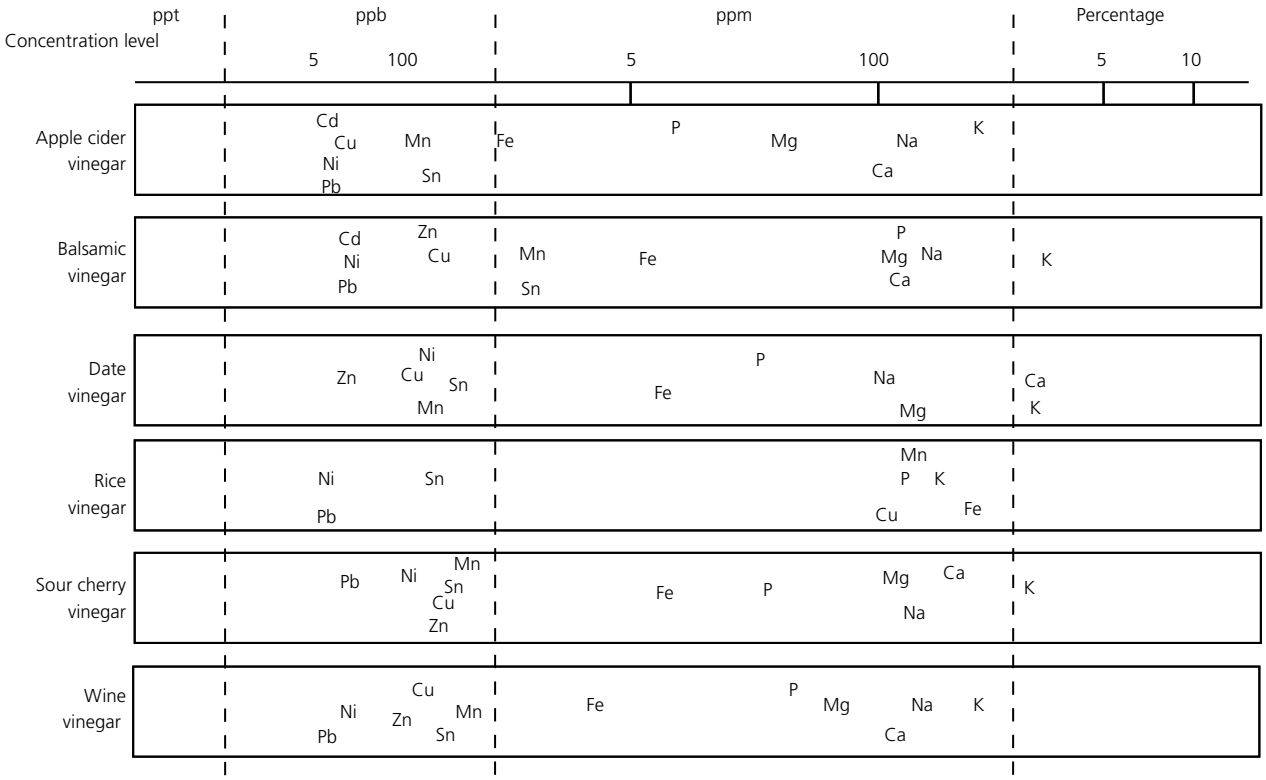


Figure 30.2 Influence of the raw material on the mineral composition of vinegars. Data extracted from Akpinar-Bayizit *et al.* [109].

The method used headspace solid-phase microextraction (HS-SPME) combined with gas chromatography (GC) and flame photometric detection (FPD) after *in situ* ethylation with sodium tetraethylborate. Butyltin species were detected in 16 vinegar samples and Sn concentrations ranged from 0.012 to 14.10 μg/L. The detection rate in white vinegar was higher than that in rice vinegar and mature vinegar [112].

The isotopic analysis of Pb has been demonstrated to be a useful tool for differentiating vinegar types [113]. The Pb isotopic fingerprints correspond to anthropogenic origin. This analysis revealed multiple contamination sources, including atmospheric pollution and post-harvest processing or storage contamination in vinegar production, thus providing complementary information on the samples.

From the data discussed above, it is clear that the contribution of vinegar to the mineral content of processed food is not as important as that found for many seasoning products and that vinegar analysis remains a challenge problem with regard to the determination of trace and especially ultratrace elements and speciation.

30.4 Conclusions and future trends on mineral composition of seasonings

The low concentration of mineral elements in seasoning products, except NaCl in salt and the use of limited qualities of the aforementioned compounds in food preparation and conservation, reduces the importance of salt, vinegar and seasonings as a source of minerals in the human diet. However, it is clear that controlling the presence of toxic elements in these samples is extremely important due to the global market of these products and their high added value. This is especially true for products such as garlic and onion, which are clear examples of the importance of seasoning in human food habits, but can also include additional herbal products very commonly employed in regional and national cooking practices.

However, the central importance of the analytical control of the mineral composition of seasonings concerns their origin and production control in order to protect the quality of raw and end products. Additionally, speciation of the mineral elements present in seasoning products remains a practically unexplored field, with improvements in sensitivity and selectivity of available methodologies being required in order to explore these aspects, which can be related to the identification of the fingerprint of special products and to prevent, in a global market, the introduction of inferior production practices in contaminated areas. Thus from the analytical point of view there is a need for fast, environmentally friendly and accurate methodologies to evaluate the mineral composition of seasonings devoted to their use in industrial food processes.

Abbreviations

AAS: Atomic absorption spectrometry
AdSV: Adsorptive stripping voltammetry
AFS: Atomic fluorescence spectrometry
ASV: Anodic stripping voltammetry
ATR-IR: Attenuated total-reflectance infrared
CF-DP: Cross-flow nebulizer combined with a double-path spray chamber
CS-CC: Cyclone spray chamber
CSV: Cathodic stripping voltammetry
CV: Cold vapour
DHBA: 3,4-Dihydroxybenzoic acid
DPASV: Differential pulse anodic stripping voltammetry
ED: Energy-dispersive
ESI-MS/MS: Electrospray ionization tandem mass spectrometry
ETAAS: Electrothermal atomic absorption spectrometry
ETV: Electrothermal vaporization
Eusalt: European Salt Producers' Association
FAAS: Flame atomic absorption spectrometry
FIA: Flow injection analysis
FPD: Flame photometric detection
GC: Gas chromatography
GluMeSe-Cys: γ-Glutamyl-selenomethylselenocysteine
HG: Hydride generation
HPLC: High performance liquid chromatography
HS-SPME: Headspace solid-phase microextraction
ICP: Inductively coupled plasma
INAA: Instrumental neutron activation analysis
IR: Infrared
MeSe-Cys: Se-methylselenocysteine
MS: Mass spectrometry
NIR: Near infrared
OES: Optical emission spectrometry
PMBP: 1-Phenyl-3-methyl-4-benzoyl-5-pyrazolone
PIGE: Proton-induced gamma-ray emission
PIXE: Particle-induced X-ray emission

RP-LP-HPLC: Reversed-phase ion-pairing chromatography
SV: Stripping voltammetry
WD: Wavelength-dispersive
WHO: World Health Organization
XRF: X-ray fluorescence

References

1 FAO and United Nations Industrial Development Organization. Herbs, spices and essential oils. Post-harvest operations in developing countries. FAO, Rome, 2005. Available at http://www.unido.org/fileadmin/user_media/Publications/Pub_free/Herbs_spices_and_essential_oils.pdf (accessed August 2013).

2 Food and Agriculture Organization of the United Nations. FAOSTAT. Available at http://faostat.fao.org/ (accessed August 2013).

3 Taylor HE. *Inductively Coupled Plasma-Mass Spectrometry: Practices and Techniques*. Academic Press, San Diego, CA, 2001.

4 Nguyen TH, Boman J, Leermakers M. EDXRF and ICP-MS analysis of environmental samples. *X-Ray Spectrom* 1998;**27**:265–276.

5 Carvalho ML, Silveira L, Casimiro A. Effects of cadmium on cation concentrations in sycamore cells (*Acer pseudoplatanus* L.): application of EDXRF. *Anal Bioanal Chem* 2002;**373**:827–829.

6 Vazquez C, Barbaro N, Lopez S. XRF analysis of micronutrients in endive grown on soils with sewage sludge. *X-Ray Spectrom* 2003;**32**:57–59.

7 Olsson M, Viksna A, Helmisaari HS. Multi-element analysis of fine roots of Scots pine by total reflection X-ray fluorescence spectrometry. *X-Ray Spectrom* 1999;**28**:335–338.

8 Lopez de Ruiz RE, Olsina RA, Masi AN. Different analytical methodologies for the preconcentration and determination of trace chromium by XRF in medicinal herbs with effects on metabolism. *X-Ray Spectrom* 2002;**31**:150–153.

9 Bhuloka Reddy S, John Charles M, Naga Raju GJ *et al*. Trace elemental analysis of cancer-afflicted intestine by PIXE technique. *Biol Trace Elem Res* 2004;**102**:265–282.

10 Nomita Devi K, Nandakumar Sarma H, Kumar S. Estimation of essential and trace elements in some medicinal plants by PIXE and PIGE techniques. *Nucl Instrum Method B* 2008;**266**:1605–1610.

11 Iyengar GV. *Elemental Analysis of Biological Systems: Biomedical, Environmental, Compositional and Methodological Aspects*. CRC Press, Boca Raton, FL, 1989.

12 Dim LA, Funtua II, Oyewale AO *et al*. Determination of some elements in *Ageratum conyzoides*, a tropical medicinal plant, using instrumental neutron activation analysis. *J Radioanal Nucl Chem* 2004;**261**:225–228.

13 Abu Zuhri AZ, Voelter W. Applications of adsorptive stripping voltammetry for the trace analysis of metals, pharmaceuticals and biomolecules. *Fresenius J Anal Chem* 1998;**360**:1–9.

14 Wang J. *Stripping Analysis: Principles, Instrumentation and Applications*. VCH Publishers, Deerfield Beach, FL, 1985.

15 Gonzalvez A, Armenta S, Cervera ML, de la Guardia M. Elemental composition of seasoning products. *Talanta* 2008;**74**:1085–1095.

16 World Health Organization. *Quality Control Methods for Herbal Materials*. WHO, Geneva, 1998. Available at http://libdoc.who.int/publications/2011/9789241500739_eng.pdf (accessed August 2013).

17 Bakkali K, Ramos Martos N, Souhail B, Ballesteros E. Determination of heavy metal content in vegetables and oils from Spain and Morocco by inductively coupled plasma mass spectrometry. *Anal Lett* 2012;**45**:907–919.

18 Divrikli U, Horzum N, Soylak M, Elci L. Trace heavy metal contents of some spices and herbal plants from western Anatolia, Turkey. *Int J Food Sci Technol* 2006;**41**:712–716.

19 Ozcan M. Mineral contents of some plants used as condiments in Turkey. *Food Chem* 2004;**84**:437–440.

20 Zaidi JH, Qureshi IH, Arif M, Fatima I. Trace element analysis of food spices by INAA. II. Solanaceae, Liliaceae, Zingiberaceae and Apiaceae families. *Int J Environ Anal Chem* 1992;**48**:33–40.

21 Al-Bataina BA, Maslat AO, Al-Kofahi MM. Element analysis and biological studies on ten oriental spices using XRF and Ames test. *J Trace Elem Med Biol* 2003;**17**:85–90.

22 Choudhury RP, Garg AN. Variation in essential, trace and toxic elemental contents in *Murraya koenigii*: a spice and medicinal herb from different Indian states. *Food Chem* 2007;**104**:1454–1463.

23 Sattar A, Wahid M, Durrani SK. Concentration of selected heavy metals in spices, dry fruits and plant nuts. *Plant Food Hum Nutr* 1989;**39**:279–286.

24 Obiajunwa EI, Adebiyi FM, Omode PE. Determination of essential minerals and trace elements in Nigerian sesame seeds, using TXRF technique. *Pakistan J Nutr* 2005;**4**:393–395.

25 Lombardi-Boccia G, Aguzzi A, Cappelloni M, Di Lullo G, Lucarini M. Total-diet study: dietary intakes of macro elements and trace elements in Italy. *Br J Nutr* 2003;**90**:1117–1121.

26 Chizzola R. Metallic trace elements in herbs and spices grown in Austria. *Acta Hort* 1989;**249**:89–96.

27 Miyamoto Y, Kajikawa A, Zaidi JH, Nakanishi T, Sakamoto K. Minor and trace element determination of food spices and pulses of different origins by NAA and PAA. *J Radioanal Nucl Chem* 2000;**243**:747–765.

28 Wang CF, Duo MJ, Chang HE, Yang JY. Essential and toxic trace elements in the Chinese medicine. *J Radioanal Nucl Chem* 1996;**211**:333–347.

29 Chen ZS. Metal contamination of flooded soils, rice plants and surface water in Asia. In: Adriano DC (ed.) *Biogeochemistry of Trace Metals*. CRC Press, Boca Raton, FL, 1992, p. 85.

30 Lopez FF, Cabrera C, Lorenzo ML, Lopez MC. Aluminium levels in spices and aromatic herbs. *Sci Total Environ* 2000;**257**:191–197.

31 Calliari I, Caniglia G, Nardi S, Tollardo AM, Callegaro R. EDXRS study of lichens as biomonitors and effects of washing procedure on element concentrations. *X-Ray Spectrom* 1995; **24**:143–146.

32 Viksna A, Selin-Lindgren E, Standzenieks P. Analysis of pine needles by XRF scanning techniques. *X-Ray Spectrom* 2001;**30**: 260–266.

33 Racz L, Bumbalova A, Harangozo M, Tolgyessy J, Tomecek O. Determination of cesium and selenium in cultivated mushrooms using radionuclide X-ray fluorescence technique. *J Radioanal Nucl Chem* 2000;**245**:611–614.

34 Ward NI, Savage JM. Metal dispersion and transportational activities using food crops as biomonitors. *Sci Total Environ* 1994;**147**:309–319.

35 Harmanescu M, Alda LM, Bordean DM, Gogoasa I, Gergen I. Heavy metals health risk assessment for population via consumption of vegetables grown in old mining area; a case study: Banat County, Romania. *Chem Cent J* 2011;**5**:64–74.

36 Tokalioglua S, Kartala S, Gultekina A. Investigation of heavy-metal uptake by vegetables growing in contaminated soils using the modified BCR sequential extraction method. *Int J Environ Anal Chem* 2006;**86**:417–430.

37 Krelowskakulas M. Determination of the level of certain trace-elements in vegetables in differently contaminated regions. *Nahrung* 1993;**37**:456–462.

38 Shirin K, Imad S, Shafiq S, Fatima K. Determination of major and trace elements in the indigenous medicinal plant *Withania somnífera* and their possible correlation with therapeutic activity. *J Saudi Chem Soc* 2010;**14**:97–100.

39 Nordic Council of Ministers. Cadmium Review. January 2003. Available at http://www.who.int/ifcs/documents/forums/forum5/nmr_cadmium.pdf (accessed August 2013).

40 Chen KS, Tseng CL, Lin TH. Trace elements in natural drugs determined by INAA. *J Radioanal Nucl Chem* 1993;**170**: 265–280.

41 Xiao L, Qin L. PIXE determination of essential trace elements in some traditional Chinese medicines. *Biol Trace Elem Res* 1990;**26/27**:751–756.

42 Wong MK, Tan P, Wee YC. Heavy metals in some of Chinese herbal plants. *Biol Trace Elem Res* 1993;**36**:135–142.

43 Ab Majid A, Sarmani S, Yusoff NI, Wei YK. Trace elements in Malaysian medicinal plants. *J Radioanal Nucl Chem* 1995; **195**:173–183.

44 Sharma KR, Agrawal M, Marshall MF. Heavy metals in vegetables collected from production and market sites of a tropical urban area of India. *Food Chem Toxicol* 2009;**47**: 583–591.

45 Naga Raju GJ, Sarita P, Ramana Murty GAV *et al*. Estimation of trace elements in some anti-diabetic medicinal plants using PIXE technique. *Appl Radiat Isotopes* 2006;**64**:893–900.

46 Singh V, Garg AN. Availability of essential trace elements in ayurvedic Indian medicinal herbs using instrumental neutron activation analysis. *Appl Radiat Isotopes* 1997;**48**: 97–101.

47 Jabeen S, Tahir Shah M, Khan S, Qasim Hayat M. Determination of major and trace elements in ten important folk therapeutic plants of Haripur basin, Pakistan. *J Med Plant Res* 2010;**4**:559–566.

48 Mukhammedov S, Tillaeva Kh, Badlov NB. Determination of the content of elements in some wild medicinal herbs of Uzbekistan. *Atom Energy* 1983;**61**:446–447.

49 Sheded GM, Pulford ID, Hamed IA. Presence of major and trace elements in seven medicinal plants growing in the South-Eastern Desert, Egypt. *J Arid Environ* 2006;**66**: 210–217.

50 Ajasa MA, Bello OM, Ibrahim OM, Ogunwande AI, Olawore ON. Heavy trace metals and macronutrients status in herbal plats of Nigeria. *Food Chem* 2004;**85**:67–71.

51 Obiajunwa EI, Adebajo AC, Omobuwajo OR. Essential and trace element contents of some Nigerian medicinal plants. *J Radioanal Nucl Chem* 2002;**252**:473–476.

52 Serfor-Armah Y, Nyarko BJB, Akaho EHK *et al*. Activation analysis of some essential elements in five medicinal plants used in Ghana. *J Radioanal Nucl Chem* 2001;**250**:173–176,

53 Koe H, Sari H. Trace metal contents of some medicinal, aromatic plants and soil samples in the Mediterranean region, Turkey. *J Appl Chem Res* 2009;**8**:52–57.

54 Basgel S, Erdemoglu SB. Determination of mineral and trace elements in some medicinal herbs and their infusions consumed in Turkey. *Sci Total Environ* 2006;**359**:82–89.

55 Sekeroglu N, Ozkutlu F, Kara SM, Ozguven M. Determination of cadmium and selected micronutrients in commonly used and traded medicinal plants in Turkey. *J Sci Food Agr* 2008;**88**:86–90.

56 Ekinci N, Ekinci R, Polat R, Budak G. Analysis of trace elements in medicinal plants with energy dispersive X-ray fluorescence. *J Radioanal Nucl Chem* 2004;**260**:127–131.

57 Kanias GD, Loukis A. Determination and correlation of active consitituents and trace elements in the medicinal plants *Thymus capitatus* Hffm, Link. *Frezenius Z Anal Chem* 1987;**327**:355–357.

58 Queralt I, Ovejero M, Carvalho ML, Marques AF, Llabres JM. Quantitative determination of essential and trace element content of medicinal plants and their infusions by XRF and ICP techniques. *X-Ray Spectrom* 2005;**34**:213–217.

59 Chizzola R, Michitsch H, Franz C. Monitoring of metallic micronutrients and heavy metals in herbs, spices and medicinal plants from Austria. *Eur Food Res Technol* 2003;**216**:407–411.

60 Yamashita CI, Saiki M, Vasconcellos MBA, Sertié JAA. Characterization of trace elements in *Casearia* medicinal plant by neutron activation analysis. *Appl Radiat Isotopes* 2005;**63**:841–846.

61 McSheehy S, Yang WJ, Pannier F *et al*. Speciation analysis of selenium in garlic by two-dimensional high-performance liquid chromatography with parallel inductively coupled plasma mass spectrometric and electrospray tandem mass spectrometric detection. *Anal Chim Acta* 2000;**421**:147–153.

62 Ogra Y, Ishiwata K, Iwashita Y, Suzuki KT. Simultaneous speciation of selenium and sulfur species in selenized odorless garlic (*Allium sativum* L. Shiro) and shallot (*Allium ascalonicum*) by HPLC-inductively coupled plasma-(octopole reaction system)-mass spectrometry and electrospray ionization-tandem mass spectrometry. *J Chromatogr A* 2005;**1093**: 118–125.

63 Wrobel K, Wrobel K, Kannamkumarath SS *et al*. HPLC–ICP-MS speciation of selenium in enriched onion leaves: a potential dietary source of Se-methylselenocysteine. *Food Chem* 2004;**86**:617–623.

64 Kapolna E, Fodor P. Speciation analysis of selenium enriched green onions (*Allium fistulosum*) by HPLC-ICP-MS. *Microchem J* 2006;**84**:56–62.

65 Montes-Bayon M, Molet M, Gonzalez E, Sanz-Medel A. Evaluation of different sample extraction strategies for selenium determination in selenium-enriched plants (*Allium sativum* and *Brassica juncea*) and Se speciation by HPLC-ICP-MS. *Talanta* 2006;**68**:1287–1293.

66 Sun HW, Qiao FX, Suo R, Li LX, Liang SX. Simultaneous determination of trace arsenic(III), antimony(III), total arsenic and antimony in Chinese medicinal herbs by hydride generation-double channel atomic fluorescence spectrometry. *Anal Chim Acta* 2004;**505**:255–261.

67 Arpadjan S, Celik G, Taskesen S, Gucer S. Arsenic, cadmium and lead in medicinal herbs and their fractionation. *Food Chem Toxicol* 2008;**46**:2871–2875.

68 Wang X, Zhuang Z, Sun D *et al*. Trace metals in traditional Chinese medicine: a preliminary study using ICP-MS for metal determination and As Speciation. *Atom Spectrosc* 1999; **20**:86–91.

69 Shen X, Zhang Y, Yang C *et al*. Speciation analysis of trace elements in Radix Astragali by flame atomic adsorption sepctrophotometry. *Chinese J Anal Chem* 2006;**34**:396–398.

70 Geo ER. The age of the saline series of the Punjab and Kohat, in symposium on the age of saline series in the salt range of Punjab. *Proc Natl Acad Sci India* 1944;**14**:269–312.

71 Kostick DS. Salt US Geological Survey 2010 Minerals Yearbook. Available at http://minerals.usgs.gov/minerals/pubs/commodity/salt/myb1-2010-salt.pdf (accessed August 2013).

72 Salt Institute. http://www.saltinstitute.org (accessed August 2013).

73 Brown IJ, Tzoulaki I, Candeias V, Elliott P. Salt intakes around the world: implications for public health. *Int J Epidemiol* 2009;**39**:1–23.

74 European Salt Producers' Association. www.eusalt.com (accessed August 2013).

75 Ji S, Yabutani T, Itoh A, Chiba K, Haraguchi H. Distributions of elements into natural salt from coastal seawater in natural-salt preparation process. *Bunseki Kagaku* 2000;**49**:111–119.

76 Hasegawa M, Ito H. Control of composition of solution in an industrial crystallizer by pressure difference method. *Bull Soc Sea Water Sci Japan* 1997;**51**:369–374.

77 Nihon Senbai Kōsha. JTS Methods for Salt Analysis. Japan Tobacco & Salt Public Corporation, 1982.

78 Yoshikawa N, Sato H, Ohya H. A method for determining the composition of aqueous highly concentrated salt mixture solutions by attenuated total-reflectance IR spectrometry. *Anal Sci* 1998;**14**:803–808.

79 Galvis-Sánchez AC, Santos IC, Mesquita RBR, Lopes JA, Rangel AOSS, Delgadillo I. Application of infrared spectroscopy for the control and chemical evaluation of brine solutions and traditional sea salts. *Food Anal Method* 2013;**6**:470–480.

80 Chen JY, Matsunaga R, Ishikawa K, Zhang H. Main inorganic component measurement of seawater using near-infrared spectroscopy. *Appl Spectrosc* 2003;**57**:1399–1406.

81 Galvis-Sánchez AC, Lopes JA, Delgadillo I, Rangel AOSS. Fourier transform near-infrared spectroscopy application for sea salt quality evaluation. *J Agric Food Chem* 2011;**59**: 11109–11116.

82 Akama Y, Tong A. High-performance liquid chromatographic determination of aluminium and iron(III) in solar salt in the form of their 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone chelates. *J Chromatogr A* 1993;**633**:129–133.

83 Yamane T, Yamada H. Flow-injection spectrophotometric determination of trace iron in various salts. Elimination of blank peak effect and use of 2-(5-nitro-2-pyridylazo)-5-(*N*-propyl-*N*-sulfopropylamino)phenol as chromogenic agent. *Anal Chim Acta* 1995;**308**:433–438.

84 Yamane T, Koshino K. Flow-injection systems for determination of trace manganese in various salts by catalytic photometric detection. *Talanta* 1996;**43**:963–969.

85 Kataoka H, Tanaka S, Konishi C, Okamoto Y, Fujiwara T, Ito K. Sensitive determination of bromine and iodine in aqueous and biological samples by electrothermal vaporization inductively coupled plasma mass spectrometry using tetramethylammonium hydroxide as a chemical modifier. *Rapid Commun Mass Spectrom* 2008;**22**:1792–1798.

86 Saral R. MSc thesis. Gebze Institute of Technology, Kocaeli, Turkey, 2000.

87 Yoshikawa N, Hasegawa M. Comparison of the operating method in the salt-making facilities between the U.S.A. and Japan. *Bull Soc Sea Water Sci Japan* 1991;**45**:353–359.

88 Kilic AM. In: V Natl Environment Eng. Congress, Ministry of Environment, Ankara, 2003, p. 584.

89 Atkins P. (2012) Analysis of gourmet salts for the presence of heavy metals. SPEX CertiPrep, Metuchen, NJ. Available at http://www.spexcertiprep.com/knowledge-base/files/AppNote_GourmetSalts.pdf.

90 Qadir H. Farrukh MA, Aurangzaib M. Production of table salt from Kohat rock salt. *J Appl Sci* 2005;**5**:12–14.

91 Internatinal Council for the Control of Iodine Deficiency Disorder. Current IDD Status Database. http://www.people.virginia.edu/~jtd/iccidd/mi/cidds.html (accessed August 2013).

92 World Health Organization. Comparative analysis of progress on the elimination of iodine deficiency disorders. WHO Regional Office for Europe, Copenhagen, 2000.

Available at http://www.euro.who.int/__data/assets/pdf_file/0009/119772/E68017.pdf?ua=1.

93 Solieri L, Giudici P. Vinegar processing: the role of fermentation. In: Solieri L, Giudici P (eds) *Vinegars of the World*. Springer-Verlag, New York, 2009, pp. 7–13.

94 Adams MR. Vinegar. In: Woods BJB (ed.) *Microbiology of Fermented Foods*, Vol. **1**. Blackie Academic and Professional, London, 1998, pp. 1–44.

95 Parrondo J, Herrero M, García LA, Díaz M. A note: production of vinegar from whey. *J Inst Brew* 2003;**109**:356–358.

96 Horiuchi JI, Kanno T, Kobayashi M. Effective onion vinegar production by a two-step fermentation system. *J Biosci Bioeng* 2000;**90**:289–293.

97 Dean JR, Ma R. Atomic absorption, atomic emission, and inductively coupled plasma spectroscopies in food analysis. In: Otles S (ed.) *Handbook of Food Analysis Instruments*. CRC Press, Boca Raton, FL, 2008, pp. 319–346.

98 Corradini F, Marcheselli L, Marchetti A, Preti C, Biancardi C. Analysis of heavy metals in Aceto Balsamico Tradizionale di Modena by flame atomic absorption spectroscopy. *J AOAC Int* 1994;**77**:714–718.

99 Gonzalez AMT, Chozas MG. Metallic contaminants in Andalusian vinegars. *Nahrung* 1988;**32**:743–748.

100 Dolan SP, Capar SG. Multi-elements analysis of food by microwave digestion and inductively coupled plasma atomic emission spectroscopy. *J Food Compos Anal* 2002;**15**:593–615.

101 Dubuisson C, Poussel E, Mermet JM. Comparison of axially and radially viewed inductively coupled plasma atomic emission spectrometry in terms of signal-to-background ratio and matrix effects. Plenary Lecture. *J Anal Atom Spectrom* 1997;**12**:281–286.

102 Todoli JL, Mermet JM. Acid interferences in atomic spectrometry: analyte signal effects and subsequent reduction. *Spectrochim Acta B* 1999;**54**:895–929.

103 Roncevic S, Siroki M. Effects of low acetic acid concentrations in inductively coupled plasma atomic emission spectrometry. *J Anal Atom Spectrom* 1994;**9**:99–104.

104 Da Silva JCJ, Cadore S, Nobrega JA, Baccan N. Dilute-and-shoot procedure for the determination of mineral constituents in vinegar samples by axially viewed inductively coupled plasma optical emission spectrometry (ICP OES). *Food Addit Contam* 2007;**24**:130–139.

105 Artiles AA, Romero CD, De La Torre AH. Concentration levels of metals in different types of vinegars. *Nahrung* 1993;**37**:72–73.

106 Guerrero MI, Heredia FJ, Troncoso AM. Characterization and differentiation of wine vinegars by multivariate-analysis. *J Sci Food Agric* 1994;**66**:209–212.

107 Joshi VK, Sharma S. Cider vinegar: microbiology, technology and quality. In: Solieri L, Giudici P (eds) *Vinegars of the World*. Springer-Verlag, New York, 2009, pp. 197–208.

108 Guerrero MI, Herce-Pagliai C, González AG, Heredia FJ, Troncoso AM, Cameán AM. Mineral elements in wine vinegar made by traditional methods. *Sci Aliment* 1996;**16**:143–149.

109 Akpinar-Bayizit A, Turan MA, Yilmaz-Ersan L, Taban N. Inductively coupled plasma optical-emission spectroscopy determination of major and minor elements in vinegar. *Not Bot Hort Agrobot Cluj* 2010;**38**:64–68.

110 Guerrero MI, Herce-Pagliai C, Cameán AM, Troncoso AM, González AG. Multivariate characterization of wine vinegars from the south of Spain according to their metallic content. *Talanta* 1997;**45**:379–386.

111 Del Dignore A, Campisi B, Di Giacomo F. Characterization of balsamic vinegar by multivariate statistical analysis of trace element content. *J AOAC Int* 1998;**81**:1087–1095.

112 Cui ZY, Zhnag KG, Zhou QF, Liu JY, Jiang GB. Butyltin compounds in vinegar collected in Beijing: species distribution and source investigation. *Sci China Chem* 2012;**55**:323–328.

113 Ndung'u K, Hibdon S, Véron A, Flegal AR. Lead isotopes reveal different sources of lead in balsamic and other vinegars. *Sci Total Environ* 2011;**409**:2754–2760.

CHAPTER 31
Other foods of plant origin

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Abstract: Infusions prepared from tea, coffee and other plants are widely consumed by the population and they generally play an important social role. Their contribution of nutrients such as protein, vitamins, fibre and carbohydrate is low but their mineral composition is important. This chapter provides a review of essential and toxic minerals for the principal infusions prepared from these foods of plant origin. It considers factors that may affect their concentrations (raw material, industrial processing and infusion preparation conditions) and the quantities of minerals that they provide to the human organism, bearing in mind nutrition requirements and intoxication risks.

Keywords: infusions, tea, coffee, essential minerals, toxic minerals

31.1 Tea infusions

A tea infusion is one of the non-alcoholic drinks most widely consumed by the world population. The infusion is prepared by steeping dried leaves of *Camellia sinensis* in hot or boiling water. There are different types of tea, depending on the manufacturing process applied to the leaves of *C. sinensis*, the most popular being black, green and oolong tea. Green tea is obtained by steaming freshly harvested leaves and then rolling and drying them [1]. To make black tea, the leaves are air-dried, bruised and cut. This starts a fermentation process which can be stopped by drying when conditions are considered optimal. Oolong tea is obtained by a shorter fermentation than the process applied to black tea. Fermentation alters the aroma, colour and taste of tea as a result of enzymatic oxidation of polyphenols. Other types of tea, consumed less frequently and sometimes locally, are pu-erh tea and brick tea.

A tea infusion contains little in the way of protein, vitamins, fibre or carbohydrate but may be a rich source of flavonoids and some minerals and trace elements essential to human health. It also provides some non-essential and undesirable toxic trace elements. This chapter does not review commercially available tea leaves and concentrates solely on the minerals present in tea infusions, since it is the consumption of the beverage that affects human daily intake of these elements. In recent years there have been excellent systematic reviews of minerals and trace elements in tea infusions, examining contents, analysis methods, speciation and bioavailability [1–6]. The articles in which the analysis of minerals in tea infusions is reported are listed in Table 31.1.

The methods employed for analysing minerals in tea use sample preparation procedures and instrumental techniques that have been established in routine analysis of minerals and trace elements in food. The alternatives for elemental analysis of tea leaves are very extensive, as shown by recent reviews by Szymczycha-Madeja *et al*. [4] and Welna *et al*. [6]. The sample preparation step is considerably simplified in the case of tea infusions, since they can be analysed directly by spectrophotometric methods after simple centrifugation and/or filtration procedures and subsequent acidification [7–9]. However, for tea infusions there are also reports of the use of ultrasonic-assisted pseudo-digestion with $HNO_3 + H_2O_2$ [10], dry ashing [11–13], wet digestion (HNO_3 + HCl, $HNO_3 + H_2O_2$; $HNO_3 + HClO_4$) [10, 14] and microwave digestion (HNO_3, $HNO_3 + H_2O_2$) [15–19]. Pre-concentration steps based on the formation of complexes have also been used for the analysis of Cd and Pb [20, 21]. Little work has been done on developing new sample preparation methods; one example is ultraviolet (UV)

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Table 31.1 Summary of published data about mineral contents in *C. sinensis*.

Reference	Element	Analytical procedure
[8]	Al, As, Pb, Cr, Cd, Ni, Fe, Zn, Cu, Mn, Ca, Mg	ICP-AES
[10]	Al, As, Ca, Cd, Fe, K, Mg, Ni, Pb, Zn	FAAS, HGAAS, ETAAS
[15]	Mg, Al, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Sr, Sb, Ba, As, Cd, Hg, Pb	ICP-MS
[16]	As, Cr, Pb, Cd, Co, Cu, Fe, Mg, Zn, Se	ICP-MS
[17]	Al, Ca	ICP-AES
[18]	Zn, Ni, Fe, Mn, Cu, Na, K	FAAS
[28]	K, Ca, P, Mg, Al, Mn, Fe, Na, Zn, Sr, Ba, Cu, Ni, Sn, Pb, Co	ICP-MS, ICP-AES
[31]	Al	ICP-AES
[36]	Ni	ICP-MS
[41]	Al, Ba, Cu, Mg, Fe, Na, K, Ca, Mn	ICP-OES
[42]	Mn, Zn	FAAS
[43]	Al, Cu, Mn	ICP-AES
[45]	F, Al, As, Cd, Cr, Co, Cu, Mn, Ni, Sr, Zn	ICP-MS
[46]	F, As, Cd, Cr, Cu, Pb	ICP-OES
[52]	As	HG-AFS
[55]	As, Cd, Pb, Sn, Mn, Fe, Cr, Co, Ni, Cr, Cu, Zn, Al	ICP-MS

photolysis digestion (H_2O_2, 2 min, 65°C), proposed by Dash *et al*. [22].

To determine the total concentrations of elements in tea infusions the customary spectrometric techniques have been used: flame atomic absorption spectrometry (FAAS), graphite furnace AAS (GFAAS), hydride generation AAS (HG-AAS), hydride generation atomic fluorescence spectrometry (HG-AFS), inductively coupled plasma atomic emission spectrometry (ICP-AES) and ICP mass spectrometry (ICP-MS). In contrast with these sample-consuming techniques, Zhang *et al*. [23] applied non-destructive techniques for the analysis of tea infusions. After the tea leaves have been dried, the samples are pulverized and multiple trace elements are determined by neutron activation analysis. For fluoride, different analysis methods are used; it is generally quantified by ion-specific electrode (ISE) potentiometry [24, 25] and it is only necessary to adjust the ionic strength of the sample before the analysis. There are also reports of the use of ion chromatography with conductivity detection [26] and molecular absorption spectrometry after electrothermal vaporization in a graphite tube furnace [27] for the determination of fluoride in tea infusions, although far fewer results have been obtained by these techniques than by ISE. The laboratory's choice of method for the analysis of tea infusions is bound to be determined by the equipment available and the aims of the study (multi-element or single-element analysis, sensitivity). There are numerous certified reference materials for trace elements in tea leaves which facilitate the quality assurance and quality control of the procedure [4]. When analysing infusions for which no reference materials exist, recovery experiments are the only alternative.

The speciation of minerals in tea infusions requires new analytical developments and is an area in which little research has been done. Meija *et al*. [3] reviewed the use of plasma spectrometry-based hyphenated techniques for elemental speciation of tea. Size-exclusion liquid chromatography (SEC) and high-performance liquid chromatography (HPLC) are the separation techniques most frequently used with these samples, and they all permit estimation of the molecular size of the metal–organic complexes that are present [1, 3, 28]. Other alternatives have been tried for the speciation of Al: anion-exchange fast protein liquid chromatography (FPLC) coupled to ICP-OES [29], and SEC or cation exchange with on-line UV/visible detection [30, 31]. Off-line approaches have also been described: cloud point extraction (CPE) and FAAS detection for Mn speciation [32]; ultrafiltration and ICP-OES detection for Ca, Cu, Fe, Mg, Mn, K, Na and Zn [33]; and sorption on ion-exchange resins and GFAAS or FAAS detection for Al [34, 35]. A few studies include molecular identification in the speciation analysis: electrospray ionization tandem mass spectrometry, ES-MS/MS, for As speciation [29] and quadrupole time-of-flight mass spectrometry, Q-TOF-MS, for Ni speciation [36].

The concentrations of elements in tea plants vary with growing conditions (soil, water, altitude, plant protection agents, fertilizers, anthropogenic activity in cultivated areas) and the age of the tea leaves when they are harvested [1]. Tea is only grown on acid soils, with pH values ranging between 5.0 and 5.6 [10], conditions that favour the solubility of most heavy metals [37]. Tea leaves contain elements such as Ca, Na, K, Mg, F, Al and Mn at milligram per gram levels, whereas elements such

as Cr, Fe, Co, Ni, Zn, Cd, Pb, As and Hg are found at levels of a few nanograms per gram.

The mineral element composition of the tea leaves influences the concentrations of these elements in the infusion. Three groups of elements have been identified, depending on the efficiency of the extraction from tea leaves to the infusion: less extractable (<10%; Ba, Ca, Fe, Mo, Sr, V), moderately extractable (10–50%; Al, B, Co, Cu, Mg, Mn, P, Si, Zn) and highly extractable (>50%; K, Na, Ni, F) [28, 38]. Some elements (Ag, As, Be, Bi, Cd, Cr, Pb, Se) are poorly extractable and their concentrations are low or even undetectable [4]. These extraction efficiencies can be partially explained on the basis of cellular distribution and ionic and covalent characteristics of minerals [17, 28]. Particle size plays a part in extraction [17], as also do the conditions used for preparing the infusion (leaf/water ratio, temperature, brewing time). Solubility is high after 5 min of infusion, although the efficiency of the process may increase with increasing brewing time [4, 38–40].

The essential macroelements appear in tea infusions in very variable concentrations. Ca, Na, K, P and Mg vary between less than 1 and 10 mg/L [10, 16, 18], although much higher concentrations have been reported (102.5 mg/L for Na; 198 mg/L for K; 305 mg/L for Ca; 738 mg/L for Mg) [8, 18, 28, 41]. The order of the concentrations of the elements varies among the infusions analysed, but K and P tend to have the highest concentrations [15, 18, 22, 41].

Mn, an essential microelement, has been detected in tea infusions in concentrations of 0.56–11 mg/L [2, 15, 18, 31, 42, 43], the concentrations in black tea infusions being higher than in green tea infusions [42, 43]. Speciation studies, all using the fractionation approach, indicate that a high percentage of Mn (53–95%) is in the unbound state, basically complexed inorganic Mn(II) species, while the remaining percentage is bound to polyphenol compounds [32, 42, 44]. Powell *et al*. [33] applied an *in vitro* gastrointestinal digestion which showed that 40% of the Mn in the infusions is ultrafiltrable (<3 kDa), and this fraction is considered bioavailable.

The essential microelements Fe, Ni, Zn and Cu appear in much lower concentrations than Mn. The concentrations of the first three are normally less than 1 mg/L [15, 28, 31, 36, 42, 45], although higher values have been reported in some samples (Ni, 4.4 mg/kg; Fe, 6.8 mg/kg) [10]. For Cu, concentrations range between 0.003 and 0.640 mg/L [15, 31, 41, 43, 45, 46], but much higher concentrations were found in all the samples of black tea analysed by Salahinejad *et al*. [8] (2.38–9 μg/g).

Al and F are the non-essential trace elements that appear with the highest concentrations in tea infusions (milligram per litre levels). Tea is one of the few plants that accumulate Al, and because of the possible negative health effects it has been the object of numerous studies [1–3, 43]. The samples analysed present differences with regard to origin, type of tea and infusion conditions, but the Al concentrations do not vary much (between 1 and 7 mg/L) and are similar to those reported for Mn [1, 2, 43]. However, higher concentrations have been found, sometimes as much as 41 mg/L [1, 10, 15, 36]. Speciation studies of Al in tea infusions [1, 3, 29] indicate that it is mostly (>90%) bound to organic matter and strongly complexed to relatively large organic molecules. Al complexes with organic molecules, with very different molecular mass values having been reported (<3000 Da, 4000–8500 Da, <20,000 Da), the differences being attributed to the speciation methods employed [1]. It is assumed that polyphenols are the most important Al-complexing ligands because of the large number of potential complexing sites provided by the phenolic hydroxyl groups. Other complexes are possible, such as negatively charged Al-citrate [29]. The bioavailability of Al is affected by the changes in pH that take place during gastrointestinal digestion. The increase in pH during the intestinal digestion step increases the insoluble species and the high-molecular-weight soluble species, thereby reducing the bioavailability [1]. It is estimated that only between 5 and 9% of the Al in a tea infusion is absorbed in the intestine.

Fluoride contents in tea infusions range between 0.32 and 8.80 mg/L [2, 5, 45–48], although infusions with much higher concentrations, as much as 33.4 mg/L, have been reported [49]. In brick tea, not considered good-quality tea and commonly made from old tea leaves and even branches and fruits which are fermented in a similar way to black tea, concentrations can be very high, as much as 878 mg/kg [38], although the infusions are characterized by having normal fluoride concentrations (up to 7.3 mg/L [48]) because of low leaching efficiency. F leaching is also slower in post-fermented pu-erh tea than in other types of tea [39]. F concentrations are higher in infusions obtained from granulated tea and tea bags than in leaf tea infusions, but this effect depends on the concentration initially present in the tea, not on the leaching efficiency [39].

Ni is a toxic trace element that has not been studied much in tea infusions [2]. It is not detected [18] or appears in low concentrations (0.001–0.269 mg/L). The infusions analysed by Salahinejad *et al*. [8] and Jalbani *et al*. [10] are exceptions which considerably exceed that range (with values of 1.86–7.65 μg/g), corresponding to samples consumed in Iran and Pakistan. Other toxic trace elements are found in infusions in much lower concentrations: As (0.002–230 μg/L), Cd (0.002–2 μg/L), Cr (0.7–50 μg/L) [2, 16, 46, 50–52]. However, high concentrations of As (1.1–4.4 mg/kg) and Cd (0.12–0.99 mg/kg) have been found in samples marketed in Pakistan [10]. For Pb, concentrations of 1.40–740 μg/L have been reported [2, 10], although in some infusions they reach 1.25–6 mg/L [2, 46]. In addition to the information about total concentrations, there are a few studies on speciation of As, Ni and Cr in tea infusions. For As, most of the infusions analysed by Yuan *et al*. [50] contained only As(V), although other species [As(III), MMA(V) and DMA(V)] were detected in a small number of samples. For Ni, quinic acid was found to be the ligand that complexes this element in *C. sinensis* infusions with a slightly acidic pH. This speciation is pH-dependent, with Ni appearing in divalent ionic form in infusions with a neutral pH [36]. For Cr, Mandiwana *et al*. [51] detected Cr(VI) in infusions, with much higher concentrations in black tea than in green tea.

Most of the studies mentioned so far prepare infusions of tea with distilled water. However, when brewing tea, consumers use mains water with a mineral composition that may significantly alter the composition of the infusions. This may cause changes in leaching of elements (an increase in Ca in water reduces extraction of Al [17]) or provide an anomalous contribution of contaminants, as happens in arsenic- and fluoride-endemic areas [53, 54]. A further factor to be considered is migration of the metals present in some traditional metal teapots into the tea solutions. A substantial increase in concentrations of Pb, Ni, Zn, Cu, Cr and Sn has been shown in infusions prepared in brass teapots compared with tea brewed in Pyrex glass vessels [14, 55]. There has been little study of the changes produced by current consumption patterns; it has been reported that Al species change after the addition of milk or lemon to the infusion [29] and that the use of lemon when the infusion is made in traditional metal teapots considerably increases migration of Pb and Ni [14].

There has been a substantial increase in information about minerals in tea infusions in recent years, and this is helping to establish baselines for concentrations in this beverage. From the viewpoint of evaluating the risk–benefit associated with tea consumption, we discuss the results in relation to current legislation and dietary intake. Many countries have specific legislation for toxic elements, and where this is not the case the maximum permissible levels in drinking water established by WHO guidelines are used. Pb, F and Al are the elements for which the values reported substantially exceed the maximum permissible levels [55]. With regard to the calculation of intakes, Table 31.2 lists the papers in which the impact of this beverage on health is considered. There are notable differences between the tea consumption used in different studies (250–1250 mL), reflecting the habits of the populations being considered. This, in combination with the different concentrations found in the infusions, gives rise to large variations in the contribution of minerals to the respective daily dietary intakes. In general, Al, F and Mn are the elements that contribute most to the corresponding daily dietary intakes, and therefore tea infusions are considered an important dietary source of these elements. Among contaminants, intakes of Pb, Ni and Cd sometimes exceed the toxicological reference values, being considerably influenced by the quality of the material in which the infusion in prepared.

31.2 Other herbal tea products

In addition to *C. sinensis*, other herbal tea products are widely consumed by the world's population, often for medical purposes. Among them is maté, the most traditional tea-like beverage of South America, extensively consumed in Argentina, Brazil, Uruguay and Paraguay. It is made from leaves of *Ilex paraguariensis* and plays a very important social role, similar to that of tea for some oriental cultures [56]. Studies on this herbal tea infusion have increased in recent years, concentrating on biochemically active compounds [56] and mineral contents (Table 31.3). Other herbal tea products, some of very local consumption, that have also been objects of study (Table 31.3) include peppermint, fennel, *Hibiscus sabdariffa*, *Matricaria chamomilla*, *Rosa canina*, *Ginkgo biloba*, *Cymbopogon citratus*, *Aloe vera*, *Panax ginseng*, *Gynostemma pentaphyllum*, *Morus alba*, *Erythroxylum coca*, *Cyclopia intermedia* and *Athrixia*

Table 31.2 Intake of mineral elements from tea of *C. sinensis*.

Reference	Infusion intake	Reference daily dietary intake (%)
[8]	4.38 g per day	Ca, Mg, Mn, Cu, Zn, Fe, Al, Ni, Pb, Cr, Cd, As: < Reference daily intake
[10]	10 cups per day	As: 20–40% Ni: 50–70% Al: 45–75% Intakes of Ni, Mg, Zn, Pb, Ca, K, Fe
[14]	800 mL per day	Zn, Cu, Fe <1% Pb: 36–8961% Ni: <1 to 1500%
[15]	300 mL	Mg, Al, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Sr, Sb, Ba, As, Cd, Hg, Pb: daily intakes below safety levels
[18]	250 mL per day	Mn: 51.1% K, Cu ≈1% Zn, Na, Fe <1%
[23]	3 g per day	Intake values for Ca, Na, Cl, K, Fe, Zn, Mn, As, Al, Cr
[43]	1 L	Al: 103.2–109.2% Cu: 5.28–6.40% Mn: 89.5–290.3%
[55]	300 mL per 800 mL	Pb: 0.5–940% Ni: 24–244% Cu: 0.07–6% Zn: 0.2–3.6% Cr: 0.2–6.3% Cd: 0.2–88%

phylicoides. The methods used for analysing minerals do not differ from those described for *C. sinensis* tea, either in sample treatment or in quantification techniques. The only alternative described is the use of the capillary ion electrophoresis method with indirect UV detection reported by Carducci *et al*. [57].

Most of the studies concentrate solely on analysing concentrations in the plant and in the infusion. The influence of factors such as processing of the plant and infusion preparation conditions on the mineral contents have only been studied for *Ilex paraguariensis*. It has been shown that the roasting step employed in industrial processing of green maté contributes to a decrease in the concentration of Al in the infusion [58]. Also, hot water helps leaching of K and Cl from this plant [59], so that concentrations of these two elements are higher in maté infusion (hot water) than in maté prepared in the form of *tereré* (with water at a temperature between 5 and 10°C). The percentage solubilization of each element also varies widely, depending on the plant considered. Wróbel *et al*. [60] showed that leaching of Al, Cr, Cu, Fe and Mn to infusions of hibiscus and maté was inversely proportional to the tannin content in the beverage. It would be of interest to extend this study to other infusions and elements.

The contribution of minerals from these herbal tea infusions is considerable, although the small number of studies conducted for each type of infusion does not permit the definition of concentration ranges or the establishment of baselines for contents. The great differences between the various studies with regard to the sample/water ratio used when preparing the infusion makes it hard to standardize data, as is reflected in the review provided by Malik *et al*. [61]. However, several studies compare minerals in infusions prepared with the same sample/water conditions [15, 22, 60–63], revealing differences in the mineral profiles of the infusions and making it possible to establish various tendencies: rooibos and honeybush have considerably lower concentrations of most minerals than maté, coca and *C. sinensis* tea [63]; maté infusion provides higher Mn contents than hibiscus [60]; hibiscus supplies higher concentrations of Cu and Fe than maté [60] and higher concentrations of B, K and Mn than ginseng, aloe vera and ginkgo [62]. With the exception of Al, the toxic trace elements in these infusions have scarcely been studied. The Al content is high in most of the infusions, with values that generally exceed the Al guideline in drinking water (recommended daily allowance 0.2 mg/L), and that exceed 1 mg/L in some infusions of hibiscus, maté, aloe vera and *G. pentaphyllum* [15, 58, 61, 64–66].

With regard to the dietary reference intakes for these minerals, the studies suggest that chamomile and maté could be interesting sources of B, Ca and Mg [62]. The quantity of Mn provided by maté is also very significant, and the results of some infusions indicate that the required daily intake for adult women is achieved with only one and a half cups [63]. On the other hand, some studies advise avoiding excessive consumption of herbal plant teas such as hibiscus because of their high Al contents [61], although it would be necessary to study the bioaccessibility of Al from these infusions to make a proper evaluation of the risk, as has been recommended for *C. sinensis*.

Table 31.3 Summary of published data about mineral contents in herbal plant teas other than *C. sinensis*.

Reference	Herbal plant	Element	Analytical procedure
[15]	*Gynostemma pentaphyllum* *Morus alba*	Mg, Al, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Sr, Sb, Ba, As, Cd, Hg, Pb	ICP-MS
[57]	*Ilex paraguariensis*	K, Na, Ca, Mg, Mn	CIE-UV
[58]	*Ilex paraguariensis*	Al	FAAS
[60]	*Hibiscus sabdariffa* *Ilex paraguariensis*	Al, Cr, Cu, Fe, Mn, Ni	ET-AAA
[62]	*Ilex paraguariensis* Rooibos *Aspalathus linearis* *Cyclopia intermedia* *Matricaria recutita* L.	Al, B, Cu, Fe, Mn, P, Zn, Ca, K, Mg	ICP-OES, FAAS
[61]	*Hibiscus sabdariffa* *Rosa canina* *Ginkgo biloba* *Cymbopogon citratus* *Aloe vera* *Panax ginseng*	Al, B, Cu, Fe, Mn, Ni, P, Zn, Ca, K, Mg	ICP-OES, FAAS
[63]	*Ilex paraguariensis* *Erythroxylum coca* *Aspalathus linearis* *Cyclopia intermedia* *Athrixia phylicoides*	Na, K, Ca, Mg, P, S, Fe, Mn, Zn, Cu, Al	ICP-OES
[64]	*Ilex paraguariensis*	P, K, Ca, Mg, S, B, Cu, Fe, Mn, Ni, Zn, Al, Cd, Co, Cr, Na, Pb	ICP-OES
[65]	*Ilex paraguariensis*	Al	FAAS
[66]	*Ilex paraguariensis*	Cd, Pb, Cu, Zn, Al, Fe, Cr, Mn, Mo, Ag	ETAAS

31.3 Coffee

Coffee is a beverage that is consumed by millions of people, both because of its sensory characteristics and for social reasons. *Coffea* sp. plants are grown on soils with an acid pH (4.2–5.1) in many tropical and subtropical countries. The arabica and robusta species are the ones that are most used for making coffee beverages. There is information about levels of macronutrients, micronutrients and toxic elements in coffee beans of different origins, but there has been little study of the leaching of these components into the infusion, the concentrations in the beverage and their influence on the daily intake of nutrients and toxins. The information available has increased in recent years but is still much less than the extensive information about tea infusions.

Coffee is available in the market in the form of roasted beans, ground, or as soluble/instant powder [67]. Little is known about the effect of industrial processing on the mineral contents of the infusion. It has been reported that leaching of Al into the infusion might be affected by the grinding of coffee beans and additional factors, such as the degree of roasting [68]. It has also been shown that the process of decaffeinating coffee beans does not significantly affect Fe contents in the infusion [69].

The rate of transfer of Al from ground coffee into the infusion is 2.8% [7], much less than the rate reported for *C. sinensis* infusions. This leaching might also be affected by the coffee brewing method employed. Fraňkova *et al*. [68] showed that the Al content in a coffee infusion is significantly influenced by the method chosen; infusions brewed in a moka pot, ibrick or single-cup filter contained at least four times more Al than those prepared by simple extraction. The leaching factor for other minerals has not been studied much, since most research has concentrated on studying instant coffee. The quality of the cookware used must also be

Table 31.4 Summary of published data about mineral contents in coffee.

Reference	Sample	Element	Analytical procedure
[67]	Instant pure coffee and coffee substitutes	Ca, Mg, K, Na, Fe, Mn, Cr, Ni	HR-CS-FAAS, HR-CS-GFAAS
[68]	Coffee infusions	Al	ICP-OES
[69]	Instant coffee	F	ISE
[7]	Coffee infusions	Al	GFAAS
[71]	Instant coffee	Ca, K, Mg, P, S, Zn, Se	ICP-OES
[72]	Instant coffee	As, Se	HG-ICP-AES
[73]	Instant pure coffee and chicory-blended coffee	Ca, Cr, Fe, K, Mg, Mn, Ni, Sr, Zn, Pb, Cd, Cu	FAAS, DPASV
[74]	Instant coffees	Cr, Mn, Fe, Co, Zn, Ba, Na, K, Sb, Se, Hg,	INAA
[75]	Coffee infusions	Al, Cd, Cr, Cu, Mn, Ni, Pb, U, Zn	ICP-MS, ICP-OES
[76]	Coffee infusions	Al	AES

considered, since there have also been reports of leaching of Al [68] and Ni [70] into coffee infusions.

The methods used for analysing instant coffee include slurry preparation [71], dry ashing [72], wet digestion with aqua regia extraction and microwave-assisted digestion [71, 72], and a combination of dry ashing and microwave digestion [73]. The minerals are subsequently quantified by traditional spectroscopic techniques (Table 31.4), with the exception of the high-resolution continuum source flame (HR-CS-FAAS) and graphite furnace (HR-CS-GFAAS) methods used recently by Oliveira *et al*. [67]. Non-destructive techniques such as instrumental neutron activation analysis (INAA) [74] have been applied less frequently. Coffee infusions are analysed directly without any special preparation [7, 67, 68], although the use of a preliminary dry ashing step has been described [75].

Various studies report mineral contents in coffee infusions and analyse their results on the basis of data provided by other authors (Table 31.4). There is considerable variability in the contents reported. K is the element that appears with the highest concentrations (19–33 mg/g). There are smaller but appreciable concentrations of P (260–429 mg per 100 g), Mg (270–554 mg per 100 g) and Ca (49–128 mg per 100 g). Other elements appear in much lower concentrations: Fe (15.5–3.2 μg/g), Mn (6.6–23.2 μg/g), Zn (2.4–37.7 μg/g) and Al (1.6–19 μg/g). There are very few data for toxic trace elements: Pb (21–308 ng/g), Hg (9–22 ng/g), Cd (1–29 ng/g), Sb (34–52 ng/g), Cr (62–800 ng/g).

The consumption values used in the studies of minerals provided in coffee infusions vary considerably, between 2 and 10 g of soluble powder per day [67, 73, 74]. The daily intakes of minerals that these studies obtain vary, but they all agree that coffee infusions are not an important source of nutrients or contaminants in the human diet [67, 73, 74]. Oliveira *et al*. [67] suggest daily intakes ranging between 0.4% (Ca) and 9.5% (Na) of the recommended daily allowances (RDA). Mn and Mg have intermediate values, about 5% of their respective RDAs [67]. Other studies have evaluated the relative contribution of coffee to total intake. The only appreciable contributions are Al (6%), Cr (8%), Fe (1.7%) and Ni (3.9%), which coffee provides for populations of Germany [7], Brazil [75] and India [73]. For other elements the contribution is less than 1%.

Finally, in order to reduce intake of caffeine, coffee substitutes (barley, malt, chicory, rye) are often consumed, either with or without coffee. It has been shown that these substitutes, especially the ones containing barley, have mineral contents less than those of pure instant coffees [67, 73]. The significant positive relationships found between most of the elements and the labelled coffee percentage has led to a suggestion to analyse Mg contents in order to estimate coffee amounts in cereal blends [67].

31.4 Conclusions

The consumption of infusions of tea, herbal plants and coffee provides variable quantities of essential minerals and toxic elements to humans. As this chapter has shown, the mineral contents depend on many factors; one that must always be considered is the way in which the infusion is prepared, since it can significantly alter the quantities of minerals that are leached. The mass/volume ratio, type of utensils and infusion time, among other aspects, may vary considerably among the numerous cultures that consume these infusions.

Information for infusions of *C. sinensis* is abundant, but scarce for other herbal plants and coffee. It would be desirable to increase the number of samples of these products that are analysed in order to obtain a broad baseline that includes varieties, origin and the effect of infusion preparation methods. Future research should adopt a unified way of expressing mineral contents. The existing studies report values in terms of m/m or m/v, and sometimes omit the sample/water ratio used for preparing the infusions, which makes comparison difficult and reduces the information that can be used to establish a baseline.

With regard to daily recommended intakes, the consumption of these infusions provides very variable quantities of minerals. The quantities of Mn, Mg, Al and F may be considerable. For a more accurate evaluation of the risk–benefit associated with these products it would be necessary to consider not only the mineral composition but also aspects such as their speciation and bioavailability; these are decisive factors for risk evaluation, as has been shown for other foods. Such studies, now beginning for Al in tea infusions, should be extended to other infusions and include other essential and toxic macroelements and micronutrients.

Abbreviations

CPE: Cloud point extraction
ES-MS/MS: Electrospray ionization tandem mass spectrometry
FAAS: Flame atomic absorption spectrometry
FPLC: Fast protein liquid chromatography
GFAAS: Graphite furnace atomic absorption spectrometry
HG-AAS: Hydride generation atomic absorption spectrometry
HG-AFS: Hydride generation atomic fluorescence spectrometry
HPLC: High-performance liquid chromatography
HR-CS-FAAS: High-resolution continuum source flame atomic absorption spectrometry
HR-CS-GFAAS: High-resolution continuum source graphite furnace atomic absorption spectrometry
ICP-AES: Inductively coupled plasma atomic emission spectrometry
ICP-OES: Inductively coupled plasma optical emission spectrometry
ICP-MS: Inductively coupled plasma mass spectrometry
INAA: Instrumental neutron activation analysis
ISE: Ion-specific electrode
RDA: Recommended daily allowance
SEC: Size-exclusion liquid chromatography

References

1 Flaten T. Aluminium in tea: concentrations, speciation and bioavailability. *Coord Chem Rev* 2002;**228**:385–395.
2 Karak T, Bhagat RM. Trace elements in tea leaves, made tea and tea infusion: a review. *Food Res Int* 2010;**43**:2234–2252.
3 Meija J, Mounicou S, Caruso JA. Plasma spectrometry for elemental speciation and characterization in beverages. *J AOAC Int* 2004;**87**:205–224.
4 Szymczycha-Madeja A, Welna M, Pohl P. Elemental analysis of teas and their infusions by spectrometric methods *Trends Anal Chem* 2012;**35**:165–181.
5 Mehra A, Lynch P, Saikat S, Chan L. Trace elements in tea (*Camellia sinensis*) and their bioavailability. In: Preedy V (ed.) *Tea in Health and Disease Prevention*. Academic Press, London, 2012, pp. 257–273.
6 Welna M, Szymczycha-Madeja A, Pawel P. A comparison of samples preparation strategies in the multi-elemental analysis of tea by spectrometric methods. *Food Res Int* 2013; **53**:922–930.
7 Müller M, Anke M, Illing-Günther H. Availability of aluminium from tea and coffee. *Z Lebensm Unters Forsch A* 1997;**205**:170–173.
8 Salahinejad M, Aflaki F. Toxic and essential mineral elements content of black tea leaves and their tea infusions consumed in Iran. *Biol Trace Elem Res* 2010;**134**:109–117.
9 Pękal A, Biesaga M, Pyrzynska K. Trace metals and flavonoids in different types of tea. *Food Sci Biotechnol* 2013;**22**: 925–930.
10 Jalbani N, Kazi T, Arian B, Jamali MK, Afridi H. Evaluation of total contents of Al, As, Ca, Cd, Fe, K, Mg, Ni, Pb, Zn and their fractions leached to the infusions of different tea samples. A multivariate study. *Chem Spec Bioavailab* 2007;**19**:163–173.
11 Kjellevold Malde M, Bjorvatn K, Julshamn K. Determination of fluoride in food by the use of alkali fusion and fluoride ion-selective electrode. *Food. Chem* 2001;**73**:373–379.
12 Gallaher RN, Gallaher K, Marshall AJ, Marshall AC. Mineral analysis of ten types of commercially available tea. *J Food Compos Anal* 2006;**19**:S53–S57.
13 Ipeaiyeda AR, Dawodu M. Leaching of manganese, iron, copper and zinc from tea (*Camellia sinensis*) in tea mug. *Elec J Environ Agric Food Chem* 2011;**10**:2240–2247.
14 Bolle F, Brian W, Petit D, Boutakhrit K, Feraille G, Van Loco J. Tea brewed in traditional metallic teapots as a significant source of lead, nickel and other chemical elements. *Food Addit Contam A* 2011;**28**:1287–1293.
15 Nookabkaew S, Rangkadilok N, Satayavivad J. Determination of trace elements in herbal tea products and their infusions

consumed in Thailand. *J Agric Food Chem* 2006;**54**: 6939–6944.
16 Shen FM, Chen HW. Element composition of tea leaves and tea infusions and its impact on health. *Bull Environ Contam Toxicol* 2008;**80**:300–304.
17 Mossion A, Potin-Gautier M, Delerue S, Le Hécho I, Behra P. Effect of water composition on aluminium, calcium and organic carbon extraction in tea infusions. *Food Chem* 2008; **106**:1467–1475.
18 Aksuner N, Henden E, Aker Z, Engin E, Satik S. Determination of essential and non-essential elements in various tea leaves and tea infusions consumed inTurkey. *Food Addit Contam B* 2012;**5**:126–132.
19 Rocha RA, de la Fuente B, Clemente MJ, Ruiz A, Vélez D, Devesa V. Factors affecting the bioaccessibility of fluoride from seafood products. *Food Chem Toxicol* 2013;**59**:104–110.
20 Marchisio PF, Sales A, Cerutti S, Marchevski E, Martinez LD. On-line preconcentration/determination of lead in *Ilex paraguariensis* samples (mate tea) using polyurethane foam as filter and USN-ICP-OES. *J Hazard Mater* 2005;**124**:113–118.
21 Han WY, Zhao FJ, Shi YZ, Ma LF, Ruan JY. Scale and causes of lead contamination in Chinese tea. *Environ Pollut* 2006; **139**:125–132.
22 Dash K, Manjusha R, Thangavel S, Arunachalam J. UV photolysis-assisted digestion of tea (*Camellia sinensis*) and tulsi (*Ocimum sanctum*) and their infusions: comparison of available trace elements. *Atom Spectrosc* 2008;**29**:56–62.
23 Zhang N, Fu N, Fang Z, Feng Y, Ke L. Simultaneous multichannel hydride generation atomic fluorescence spectrometry determination of arsenic, bismuth, tellurium and selenium in tea leaves. *Food Chem* 2011;**124**:1185–1188.
24 Emekli-Alturfan E, Yarat A, Akyuz S. Fluoride levels in various black tea, herbal and fruit infusions consumed in Turkey. *Food Chem Toxicol* 2009;**47**:1495–1498.
25 Balcerzak M, Janiszewska J. Fluorides in tea products and analytical problems with their determination. *Crit Rev Anal Chem* 2013;**43**:138–147.
26 Kumar SD, Narayan G, Hassarajani S. Determination of anionic minerals in black and kombucha tea using ion chromatography. *Food. Chem* 2008;**111**:784–788.
27 Morés S, Monteiro GC, Santos FDS, Carasek E, Welz B. Determination of fluorine in tea using high-resolution molecular absorption spectrometry with electrothermal vaporization of the calcium mono-fluoride CaF. *Talanta* 2011;**85**:2681–2685.
28 Matsuura H, Hokura A, Katsuki F, Itoh A, Haraguchi H. Multielement determination and speciation of major-to-trace elements in black tea leaves by ICP-AES and ICP-MS with the aid of size exclusion chromatography. *Anal Sci* 2001;**17**:391–398.
29 Kralj B, Krizaj I, Bukovec P, Slejko S, Milacic R. Speciation of aluminium in tea infusions by use of SEC and FPLC with ICP-OES and ES-MS-MS detection. *Anal Bioanal Chem* 2005; **383**:467–475.
30 Flaten AK, Lund W. Speciation of aluminium in tea infusions studied by size exclusion chromatography with detection by post-column reaction. *Sci Total Environ* 1997;**207**:21–28.
31 Street R, Drabek O, Szakova J, Mladkova L. Total content and speciation of aluminium in tea leaves and tea infusions. *Food Chem* 2007;**104**:1662–1669.
32 Yalçín S, Filik H, Apak R. Speciation analysis of manganese in tea samples using flame atomic absorption spectrometry after cloud point extraction. *J Anal Chem* 2012;**1**:47–55.
33 Powell J, Burden T, Thompson R. *In vitro* mineral availability from digested tea: a rich dietary source of managanese. *Analyst* 1998;**123**:1721–1724.
34 Alberti G, Biesuz R, Profumo A, Pesavento M. Determination of the total concentration and speciation of Al(III) in tea infusions. *J Inorg Biochem* 2003;**97**:79–88.
35 Erdemoglu S, Pyrzyniska K, Seref G. Speciation of aluminum in tea infusion by ion-exchange resins and flame AAS detection. *Anal Chim Acta* 2000;**411**:81–89.
36 Scancar J, Zuliani T, Zigon D, Milacic R. Ni speciation in tea infusions by monolithic chromatography: ICP-MS and Q-TOF-MS. *Anal Bioanal Chem* 2013;**405**:2041–2051.
37 Desideri D, Meli MA, Roselli C, Feduzi L. Polarized X ray fluorescence spectrometer (EDPXRF) for the determination of essential and non essential elements in tea. *Microchem J* 2011;**98**:186–189.
38 Fung KF, Zhang ZQ, Wong JWC, Wong MH. Fluoride contents in tea and soil from tea plantations and the release of fluoride into tea liquor during infusion. *Environ Pollut* 1999;**104**:197–205.
39 Koblar A, Tavčar G, Ponikvar-Svet M. Fluoride in teas of different types and forms and the exposure of humans to fluoride with tea and diet. *Food Chem* 2012;**130**:286–290.
40 Malinowska E, Inkielewicz I, Czarnowski W, Szefer P. Assessment of fluoride concentration and daily intake by human from tea and herbal infusions. *Food Chem Toxicol* 2008;**46**:1055–1061.
41 Froes RES, Neto VB, Beinner MA, Nascentes CC, da Silva JBB. Determination of inorganic elements in teas using inductively coupled plasma optical emission spectrometry and classification with exploratory analysis. *Food Anal Method* 2014;**7**:540–546.
42 Pohl P, Prusisz B. Fractionation analysis of manganese and zinc in tea infusions by two-column solid phase extraction and flame atomic absorption spectrometry. *Food Chem* 2007; **102**:1415–1424.
43 Mehra I, Baker CL. Leaching and bioavailability of Al Cu and Mn from tea (*Camellia sinensis*). *Food Chem* 2007;**100**:1456–1463.
44 Ozdemir Y, Guçer S. Speciation of manganese in tea leaves and tea infusions. *Food Chem* 1998;**61**:313–317.
45 Sofuoglu SC, Kavcar P. An exposure and risk assessment for fluoride and trace metals in black tea. *J Hazard Mater* 2008;**158**:392–400.
46 Lv H-P, Lin Z, Tan JF, Guo L. Contents of fluoride, lead, copper, chromium, arsenic and cadmium in Chinese Pu-erh tea. *Food Res Int* 2013;**53**:938–944.
47 Chan L, Mehra A, Saikat S, Lynch P. Human exposure assessment of fluoride from tea (*Camellia sinensis* L.): a UK based issue? *Food Res Int* 2013;**51**:564–570.

48 Wong MH, Fung KF, Carr HP. Aluminium and fluoride contents of tea, with emphasis on brick tea and their health implications. *Toxicol Lett* 2003;**137**:111–120.

49 Yi J, Cao J. Tea and fluorosis. *J Fluorine Chem* 2008;**129**: 76–81.

50 Yuan C, Gao E, He B, Jiang G. Arsenic species and leaching characters in tea (*Camellia sinensis*). *Food Chem Toxicol* 2007;**45**:2381–2389.

51 Mandiwana K, Panichev N, Panicheva S. Determination of chromium (VI) in black, green and herbal teas. *Food Chem* 2011;**129**:1839–1843.

52 El-Hadri F, Morales-Rubio A, de la Guardia M. Determination of total arsenic in soft drinks by hydride generation atomic fluorescence spectrometry. *Food Chem* 2007;**105**:1195–1200.

53 Del Razo LM, Garcia-Vargas GG, Garcia-Salcedo J *et al*. Arsenic levels in cooked food and assessment of adult dietary intake of arsenic in the Region Lagunera, Mexico. *Food Chem Toxicol* 2002;**40**:1423–1431.

54 Viswanathan G, Gopalakrishnan S, Siva Ilango S. Assessment of water contribution on total fluoride intake of various age groups of people in fluoride endemic and non-endemic areas of Dindigul District, Tamil Nadu, South India. *Water Res* 2010;**44**:6186–6200.

55 Petit D, El Houari W, Jacobs K, Baeyens W, Leermakers M. Trace element content in tea brewed in traditional metallic and stainless steel teapots. *Environ Monit Assess* 2013;**185**: 8957–8966.

56 Bracesco N, Sanchez AG, Contreras V, Menini T, Gugliucci A. Recent advances on *Ilex paraguariensis* research: minireview. *J Ethnopharmacol* 2011;**136**:378–384.

57 Carducci CN, Dabas PC, Muse JO. Determination of inorganic cations by capillary ion electrophoresis in *Ilex paraguariensis* (St. H.), a plant used to prepare tea in South America. *J AOAC Int* 2000;**83**:1167–1173.

58 Gomes Da Costa AM, Nogami EM, Visentainer JV, De Souza NE, Egea Garcia E. Fractionation of aluminum in commercial green and roasted yerba mate samples (*Ilex paraguariensis* St. Hil.) and in their infusions. *J Agric Food Chem* 2009;**57**: 196–200.

59 Giulian R, Iochims Dos Santos CE, De Moraes Shubeita S, Manfredi da Silva L, Ferraz Dias J, Luä Cia Yoneama M. Elemental characterization of commercial mate tea leaves (*Ilex paraguariensis* A. St.-Hil.) before and after hot water infusion using ion beam techniques. *J Agric Food Chem* 2007;**55**: 741–746.

60 Wróbel K, Wróbel K, Colunga Urbina EM. Determination of total aluminum, chromium, copper, iron, manganese, and nickel and their fractions leached to the infusions of black tea, green tea, *Hibiscus sabdariffa*, and *Ilex paraguariensis* (Mate) by ETA-AAS. *Biol Trace Elem Res* 2000;**271**:271–280.

61 Malik J, Frankova A, Drabek O, Szakova J, Ash C, Kokoska L. Aluminium and other elements in selected herbal tea plant species and their infusions. *Food Chem* 2013;**139**:728–734.

62 Malik J, Szakova J, Drabek O, Balik J, Kokoska L. Determination of certain micro and macroelements in plant stimulants and their infusions. *Food Chem* 2008;**111**: 520–525.

63 Olivier J, Symington EA, Jonker CZ, Rampedi IT, van Eeden TS. Comparison of the mineral composition of leaves and infusions of traditional and herbal teas. *S Afr J Sci* 2012;**108**:1–5.

64 Heinrichs R, Malavolta E. Mineral composition of a commercial product from mate-herb (*Ilex paraguariensis* St. Hil.). *Ciência Rural* 2001;**31**:781–785.

65 Pereira Tatsch FR, Gonçalves VC, Meurer EJ. Total and soluble aluminum in samples of commercial mateherb in Rio Grande do Sul state, Brazil. *Scientia Agraria* 2010;**11**:83–86.

66 Cordoba Bragança VL, Melnikov P, Zanoni LZ. Trace elements in different brands of yerba mate tea. *Biol Trace Elem Res* 2011;**144**:1197–1204.

67 Oliveira M, Casal S, Morais S *et al*. Intra- and interspecific mineral composition variability of commercial instant coffees and coffee substitutes: contribution to mineral intake. *Food Chem* 2012;**130**:702–709.

68 Fraňkova A, Drábek O, Havlík J, Szakova J, Vaněk A. The effect of beverage preparation method on aluminium content in coffee infusions. *J Inorg Biochem* 2009;**103**:1480–1485.

69 Warren DP, Henson HA, Chan JT. Comparison of fluoride content in caffeinated, decaffeinated and instant coffee. *Fluoride* 1996;**29**:147–150.

70 Berg T, Petersen A, Pedersen GA, Petersen J, Madsen C. The release of nickel and other trace elements from electric kettles and coffee machines. *Food Addit Contam* 2000;**17**:189–196.

71 Asfaw A, Wibetoe G. Simultaneous determination of hydride (Se) and non-hydride-forming (Ca, Mg, K, P, S and Zn) elements in various beverages (beer, coffee, and milk), with minimum sample preparation, by ICP–AES and use of a dual-mode sample-introduction system. *Anal Bioanal Chem* 2005;**382**:173–179.

72 Santos EJ, de Oliveira E. Evaluation of arsenic and selenium in Brazilian soluble coffee by inductively coupled plasma atomic emission spectrometry with hydride generation. *Braz Arch Biol Technol* 2001;**44**:233–238.

73 Suseela B, Bhalke S, Vinod Kumar AV. Daily intake of trace metals through coffee consumption in India. *Food Addit Contam* 2001;**18**:115–120.

74 Zaidi JH, Fatima I, Arif M, Qureshi IH. Determination of trace elements in coffee beans and instant coffee of various origins by INAA. *J Radioanal Nucl Chem* 2006;**267**:109–112.

75 Santos EE, Lauria DC, Porto da Silveira CL. Assessment of daily intake of trace elements due to consumption of foodstuffs by adult inhabitants of Rio de Janeiro city. *Sci Total Environ* 2004;**237**:69–79.

76 Rajwanshi P, Singh V, Gupta MK *et al*. Studies on aluminium leaching from cookware in tea and coffee and estimation of aluminium content in toothpaste, baking powder and paan masala. *Sci Total Environ* 1997;**193**:243–249.

CHAPTER 32
Baby foods

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Abstract: The mineral profile of baby food is very important in order to establish the levels of essential and non-essential elements and their contribution to adequate intake (AI) and estimated average requirement (EAR) for providing adequate nutrition during infancy. This is essential to ensure the growth, health and development of children to their full potential, because poor nutrition increases the risk of illness in adulthood. This chapter discusses different strategies for determining the mineral profile in most common childhood foods, with regard to both sample preparation and analytical method employed for determination. The results in the literature on the mineral content of baby foods used from 6 to 12 months of age are also updated.

Keywords: baby foods, infant foods, mineral composition, essential elements

32.1 Baby food and essential element requirements

The first year of human life is a critical period in the development of a child. It is a period of rapid growth, with major changes in motor and cognitive function. Adequate nutrition during infancy and childhood is essential to ensure the growth, health and development of children. Malnutrition during the first 2 years of life increases the risk of illness in adulthood, principally chronic malnutrition and not attaining full growth potential. High intake of food can also have negative consequences, such as childhood obesity. Thus it is vital to thoroughly survey the nutrient intake of infants, since nutrition at this age marks the future quality of life.

Some authorities encourage the timely introduction of complementary foods during infancy, which may be necessary for both nutritional and development reasons, and to enable the transition from breast milk to infant foods, because the ability of breast milk to meet requirements for macronutrients and micronutrients becomes limited with increasing age of the baby [1]. Early diet has an immediate effect on the health of infants in economically developing countries because of factors such as the lack of availability of suitable alternatives to breast milk, microbial contamination of foods and beverages, displacement of breast milk by less nutritious alternatives, and the physiological maturity of gastrointestinal and renal function [2].

Agencies such as the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) have produced recommendations that encourage exclusive breastfeeding for 6 months (180 days) and nutritionally adequate and safe complementary feeding starting from the age of 6 months with continued breastfeeding up to 2 years of age or beyond [3, 4].

From the age of 6 months, infant needs for energy and nutrients start to exceed that provided by breast milk and thus complementary feeding becomes necessary to fill the energy and nutrient gap [5]. The stage between 6 and 24 months of age is the most critical in terms of malnutrition, since children cease taking all the nutrients from breast milk and the nutrient composition of other foods becomes the key problem. This introduces many factors that affect infant nutrition, such as the amount of supplementary feed and its composition, consistency and bioavailability of nutrients.

In general, up to 6 months of age babies fed only on breast milk are supplied with the necessary micronutrients, including Fe and Zn, which are present in low concentration but with high bioavailability and absorption. After 6 months, the child can eat solid or semi-solid food

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in the form of porridge or mashed foods. These foods are usually milk mixed with cereals or baby rice or mixed vegetables with meat or fruit porridge. After 9 months of age, baby food includes many types of meat and the child is introduced to fish and eggs, which are not included in the diet until 1 year of age. Additionally, the consistency of food must be such that babies can pick up the food. After 12 months, the child can start eating family foods, but chopped or mashed [2, 3].

Various national and international health agencies recommend the levels of mineral elements needed for appropriate baby nutrition. Table 32.1 compares the levels recommended by different agencies for each essential element. Recommended values for 12 essential elements (Ca, Cr, Cu, Fe, Mg, Mn, Mo, P, Se, Na and Zn), together with the daily intake recommendations according to infant nutrition at three different ages (0–5, 6–11 and 12–23 months), were taken from different organizations in the USA and Canada [6], Australia and New Zealand [7], Europe [8] and the WHO [9]. From the data summarized in Table 32.1 it can be concluded that the recommended values are very similar among the different organizations. Additionally, it can be seen that essential elements can be classified according to their required amounts: K and Na at levels of around 1 g and 0.4 g/day, respectively; Ca and P at levels in the order of hundreds of milligrams per day; Mg, Fe, Zn and Mn also required at milligram per day levels; Cu at the hundreds of micrograms per day level; and Se, Mo and Cr in the low micrograms per day level. Because of this, all these elements must be carefully controlled in baby foods.

The main parameters are reported as follows.

- Estimated average requirement (EAR): the average daily nutrient intake that meets the needs of 50% of healthy individuals in a particular age and gender group.
- Recommended daily allowance (RDA), equivalent to recommended nutrient intake (RNI): the average daily nutrient intake sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a particular life stage and gender group.
- Adequate intake (AI): the recommended average daily intake based on observed or experimentally determined approximations or estimations of nutrient intake by a group of apparently healthy people that are assumed to be adequate.
- When an RDA cannot be determined, tolerable upper intake level (UL): the highest average daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population [9, 10].

32.2 Methods employed for determination of the mineral composition of baby foods

The main analytes to be determined in baby foods are those that can cause problems for the child's health, either by excessive or insufficient intake. For example, iron and zinc are the most problematic elements for baby's health, because of their relatively low bioavailability in infant food. Sodium content can also be a problem for infants, because despite its essential character an excess of this element may cause hypertension. The most common baby food samples are purées of different foods, as mixtures of meat or fish with vegetables or mixtures of fruits. The nutritional content of these purées is usually about 80–85% water, 10% carbohydrate, 3.2% protein, 2% fat, 1% fibre and 0.1% sodium.

Baby food samples have high water content, and samples can be analysed either wet or after being dried. The drying process allows pre-concentration of the elements to be determined and ensures better preservation of samples than that achieved for wet foods. The drying process can be accomplished by application of temperature in an oven (normally about 100°C), by applied vacuum or by lyophilization. For oven drying, Carbonell-Barrachina *et al.* [12] suggest placing the samples in an oven for 78 hours at 80°C. Lyophilization involves very low temperatures and pressures of around –50°C and 0.05 mbar, respectively [13], and these conditions are highly recommended in order to avoid the loss of highly volatile elements and chemical forms of minerals like Hg and chloride compounds.

The most common measurement techniques for the analysis of mineral composition in baby foods are atomic absorption and emission spectrometry. However, to carry out the mineral analysis using these methodologies requires liquid samples, so that baby food samples need previous dissolution or digestion. Figure 32.1 shows the methodology used to determine mineral elements in infant food, and also shows how mineral speciation could be accomplished using prior separation of the different mineral compounds of the same element by chromatographic or non-chromatographic techniques.

Table 32.1 Recommended values for essential element intake of infants obtained from different national and international agencies.

Element	Age (months)	Food and Nutrition Board (USA and Canada) [6]				Australia and New Zealand [7]				WHO [9]		Europe [8]
		EAR	RDA	AI	UL	EAR	RDI	AI	UL	RDI	UL	RDA
Ca (mg/day)	0–5			200	1000			210		300		
	6–11			260	1500			270		400		450
	12–23	500	700		2500	360	500		2500	500	3000	550
Cr (μg/day)	0–5			0.2				0.2				
	6–11			5.5				5.5				10
	12–23			11				11				20
Cu (μg/day)	0–5			200				200				
	6–11			220				220				400
	12–23	260	340		1000			700	1000	500*	1500*	500
Fe (mg/day)	0–5			0.27	40			0.2	20			
	6–11	6.9	11		40	7	11		20	7.7†		8
	12–23	3.0	7		40	4	9		20	4.8†		8
K (g/day)	0–5			0.4				0.4				
	6–11			0.7				0.7				0.7
	12–23			3.0				2				1.0
Mg (mg/day)	0–5			30				30		26		
	6–11			75				75		54		75
	12–23	65	80			65	80			60	65	80
Mn (mg/day)	0–5			0.003				0.003				
	6–11			0.6				0.600				0.7
	12–23			1.2	2			2.0				1.2
Mo (μg/day)	0–5			2				2				
	6–11			3				3				15
	12–23	13	17		300	13	17		300			25
P (mg/day)	0–5			100				100				
	6–11			275				275				350
	12–23	380	460		3000	380	460		3000			550

(Continued)

Table 32.1 (Continued)

Element	Age (months)	Food and Nutrition Board (USA and Canada) [6]				Australia and New Zealand [7]				WHO [9]		Europe [8]
		EAR	RDA	AI	UL	EAR	RDI	AI	UL	RDI	UL	RDA
Se (μg/day)	0–5			15	45			12	45	6		
	6–11			20	60			15	60	10		15
	12–23	17	20		90	20	25		90	17		20
Na (g/day)	0–5			0.12				0.12				
	6–11			0.37				0.17				0.3
	12–23			1.0	1.5			0.2–0.4	1			0.4
Zn (mg/day)	0–5			2	4			2.0	4	2.8‡		
	6–11	2.5	3		5	2.5	3.0		5	4‡		4
	12–23	2.5	3		7	2.5	3.0		7	4‡		5

EAR, estimated average requirement; RDA, recommended daily allowance; RDI, recommended daily intake; AI, adequate intake; UL, tolerable upper intake level.

*World Health Organization [11].

†Assuming 12% bioavailability.

‡Assuming moderate bioavailability.

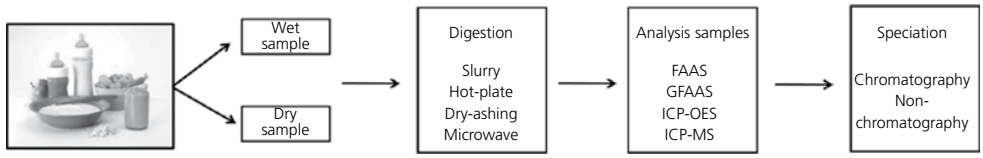


Figure 32.1 Scheme of general methodology used for mineral element analysis in baby food.

32.2.1 Mineralization and dissolution of samples

The organic nature of baby foods makes samples insoluble in water and therefore digestion of baby food samples with acid is mandatory in order to obtain a solution of the mineral compounds. Figure 32.2 shows the parameters (temperature, pressure, reagents) required for complete digestion of baby foods in order to destroy the matrix without loss of minerals.

Mineral elements can be dissolved by simply using reagents at room temperature, thus providing solutions or slurries of samples. It is true that many of the elements to be analysed are easily soluble in water or dilute acids, but such dilution of baby food samples with aqueous extraction solutions causes problems related to matrix effects during measurement and compels the use of highly sensitive techniques for determination of trace and ultratrace elements.

Dry ashing or wet ashing are the main strategies for digestion of baby food samples. Dry ashing uses basically temperature and total decomposition of the matrix, thus providing a high pre-concentration of samples to be analysed. However, it is clear that the simple use of temperature and time causes the loss of volatile elements and compounds and so ashing aids have been proposed to reduce these problems. Indeed, the combined use of temperature and acids is the most useful technique for complete dissolution of the food ash. The use of pressure is probably the best way to accelerate food sample digestion. Nowadays, pressurized microwave-assisted digestion is the most commonly used digestion process.

In some cases, direct analysis of solid samples can be performed by using simple laser ablation of samples or by direct atomization of sample slurry in a graphite furnace. Methods employed for mineralization of baby food samples are summarized in Table 32.2 and it can be seen that sample slurries, digestion with HNO_3 and HCl on a hot plate, dry ashing and microwave-assisted digestion are the most commonly used procedures.

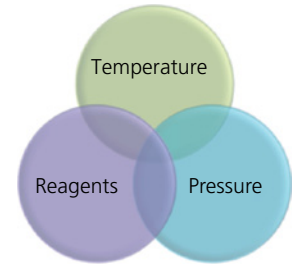


Figure 32.2 Key parameters used for the digestion of baby food samples.

Slurry sampling

The simplest sample preparation is direct analysis of samples without applying temperature or pressure or the addition of any reagent, although no studies of this kind where infant food was measured directly have been found in the literature in recent years. However, it is quite common to prepare sample slurries in water when analysing infant food, in which case it is only necessary to add aqueous solutions of acids or surfactants and then homogenize the sample. Slurry sampling has many advantages, such as reduced sample preparation time, decreased analyte losses, reduced risk of contamination, and elimination of hazards associated with the use of acids at high temperatures. Slurries can then be nebulized into a flame (FAAS) or plasma (ICP-OES/MS) or deposited in a graphite tube for GFAAS.

Baby food slurry is usually composed of a small amount of sample plus Triton X-100, nitric acid and an antifoam agent. The addition of nitric acid facilitates extraction of the analyte or analytes into the liquid phase of the slurry, while Triton X-100 serves as a dispersing agent of solid particles. Ozbek and Akman [14] reported a method for slurry analysis of baby food for Fe and Zn determination using FAAS. To prepare the slurry, 0.5 g of sample were weighed and suspended in 20 mL of a diluent containing 0.05% Triton X-114 and 0.1 M nitric acid. The slurries were homogenized using an Ultra-Turrax at 15,000 rpm for 5 min. This method

Table 32.2 Methods employed in the analytical literature for the mineralization of baby food samples.

Sample	Digestion process	Reference
Baby food reference (TUGAP 1201 R)	Slurry (Triton X-114/HNO_3) MW (HNO_3/H_2O_2)	[14]
Commercial baby food based on chicken, hake, vegetables and fruit	Slurry (Triton X-100/ HNO_3/H_2O_2) MW (HNO_3/H_2O_2)	[15]
Commercial baby food, fish based	Slurry (Triton X-100/ HNO_3/H_2O_2) MW (HNO_3/H_2O_2)	[16]
Commercial infant rice, puréed infant foods (hake and chicken based)	Dry ashing	[19]
Commercial and home-made baby food of lamb, beef and chicken	Dry ashing	[21]
Home-made baby food, meat based (chicken and veal)	Dry ashing	[23]
Commercial baby food, vegetable based	Hot-plate (HNO_3/HCl)	[17]
Total diet survey	HNO_3/HCl	[18]
Commercial infant cereals, baby food based on chicken, hake and infant rice	MW(HNO_3/H_2O_2)	[12]
Foods consumed by children	MW (HNO_3/H_2O_2)	[25]
Commercial baby food of vegetables and fruits	MW (HNO_3/H_2O_2)	[26]
Commercial baby food of meat and vegetables	MW (HNO_3/H_2O_2)	[27]
Commercial baby food of meat, fish, vegetables and fruit	MW (HNO_3)	[28]
Commercial baby food	MW (HNO_3)	[29]
Infant food	MW (HNO_3)	[30]
Commercial baby food of chicken and fish	MW (HNO_3/HCl/H_2O_2)	[31]

MW, microwave assisted.

was compared with microwave-assisted digestion, using GBW-08503 Wheat flour and IRMM-804 Rice flour, obtaining recovery percentages of 95% and 98% for Fe and 103–104% and 101–102% for Zn, respectively for slurry and microwave digestion. The total time for slurry preparation was 20 min, while the time required for microwave digestion was 135 min. For the determination of Cu, Co, Ni, Mn, Se, Pb and Cd in samples of meat, fish, vegetable and fruit baby foods and fish baby foods, Viñas *et al*. [15, 16] weighed the samples and prepared suspensions by diluting with 5 mL of a solution containing 0.1% w/v Triton X-100, 1% v/v nitric acid and 30% w/v hydrogen peroxide (1% w/v ammonium dihydrogenphosphate only for Cu), with measurement by GFAAS. Slurries were homogenized by movements of the plunger, which took about 5 min. The suspensions were sonicated for a few minutes and stirred with a magnetic stirrer before measurement by GFAAS. The accuracy of the method was verified by using the reference materials HDP Total diet, DORM-2 Dogfish muscle, SRM 1566a Oyster tissue, SRM 1577b Bovine liver, SRM 1568a Rice flour and SRM 1572 Citrus leaves. This sample preparation method was compared with microwave-assisted digestion; Student's *t*-test revealed no difference between the results obtained using either of the assayed procedures at a level of significance of 0.05.

Wet digestion

Wet digestion of baby foods uses mixtures of acids, generally nitric acid mixed with other acids or with hydrogen peroxide, which are heated on a hot plate. In a study involving the determination of eight essential elements (Ca, Cu, Fe, K, Mg, Mn, P and Zn) by ICP-OES [17], the decomposition of different weaning foods was made on a hot plate, with 0.1 g of sample being digested in 125-mL beakers with 20 mL concentrated nitric acid and 1 mL concentrated perchloric acid. Samples were covered with glass and set on a hot-plate at 120°C for 1 hour. After that, the temperature was increased to 150°C and samples refluxed overnight. Then, samples were taken to almost dryness in order to reduce the volume to approximately 1 mL, at the same temperature. Dried samples were treated with 2.5 mL of nitric/perchloric acid (4 : 1). After cooling, solutions were transferred to tubes and diluted to a 50-mL final volume with deionized water.

Thomson *et al*. [18] determined Na and Fe in the New Zealand Total Diet Survey by ICP-OES by also using wet digestion. In this study, 0.5–2.5 g of the homogenized food were digested with nitric and hydrochloric acids. The accuracy of the method was verified by measurement of NIST 1549 Non-fat milk powder, SRM 1577b

Bovine liver, RM 8414 Bovine muscle, SRM 1568a Rice flour, SRM 1515 Apple leaves and SRM 1566b Oyster tissue. The accuracy of the determinations, based on recovery from certified reference materials, was better than 85%. These results indicate the suitability of wet digestion at atmospheric pressure for the determination of trace elements in baby foods. However, the atmospheric pressure wet digestion is time-consuming, analyte contamination and losses occur, and often this kind of digestion does not allow complete decomposition of the sample matrix.

Dry ashing

Dry ashing has been commonly used in baby food analysis. This method allows ash dissolution in small amounts of reagents, whereas wet digestion uses large amounts of reagents. On the other hand, dry digestion has the drawback of time (1 day or more) and requires attention from the analyst.

Burló *et al*. [19] determined As in baby food using dry ashing at 450°C for 12 hours and HGAAS. In this method, 1 g of dry sample was weighed, adding 1 mL ashing aid and 5 mL nitric acid 50% v/v, then the mixture was evaporated on a sand bath to total dryness. Samples were mineralized as described [20]. Ashes from the digested samples were dissolved in 5 mL HCl 50% (v/v) and 5 mL KI-ascorbic acid. After 30 min, solutions were diluted to the volume with HCl 50% (v/v) and filtered. Okesli *et al*. [21] used an official method [22] for the determination of sodium by flame atomic emission spectrometry and previous incineration of the samples in a muffle furnace at 525°C, while Van den Boom *et al*. [23] used the dry digestion official method [24] for the determination of Na, K, Ca, Mg, Fe and Zn by atomic spectrometry.

Microwave-assisted digestion

Wet sample digestion can be performed on a heating plate at atmospheric pressure or inside closed vessels by microwave heating at high pressure. The use of microwave-assisted sample digestion has increased considerably in the last few decades. This technique is rapid and allows total decomposition of sample with minor risks of contamination and minor analyte losses. In the majority of published papers nitric acid and hydrogen peroxide were used for the digestion of baby food samples. The volume of nitric acid varies from 2 to 8 mL and hydrogen peroxide from 0.5 to 2 mL, depending on the study. For example, Bosscher *et al*. [25, 26] used 2 mL of nitric acid and 0.5 mL of H_2O_2 for the digestion of 0.4 g of baby food to be analysed by FAAS for determination of Ca, Fe and Zn. The accuracy of the method was verified by measurement of NIST 1573a Tomato leaves, NBS 1549 Non-fat milk powder and NBS 1571 Orchard leaves, obtaining recovery of 94, 114 and 107% for Ca, Fe and Zn, respectively. Carbonell-Barrachina *et al*. [12] used 2 mL of HNO_3 and 2 mL of H_2O_2 to digest meat and fish baby food samples, with digestion of 30 min at 95°C. The method was validated using NIST SRM 1568a Rice Flour, obtaining recoveries of 64.2–99.5% for 13 analytes studied by ICP-MS. Ozbek and Akman [14] proposed the use of 7 mL of HNO_3 and 1 mL of H_2O_2, with a digestion step at 190°C for 15 min. IRMM-804 Rice Flour and GBW-08503 Wheat flour were used as certified reference materials for evaluation of the effectiveness of the digestion. The recovery percentages obtained were 98% for Fe and 101–102% for Zn. Zand *et al*. [27] used 5 mL of HNO_3 and 0.5 mL of H_2O_2 to digest 0.5 g of wet baby food sample. The recoveries obtained for Ca, Cu, Mg, Fe, Zn, K, Na and Se were 90–112%. Other authors [28–30] used only HNO_3 for sample digestion, with acid volumes of 3–8 mL.

As can be seen, microwave digestion is very versatile, provides a digestion time lower than that required for digestion with acids at atmospheric pressure or for dry ashing, and reagent consumption is not very high, especially when working with sealed reactors. It allows fast and complete dissolution of both dry and wet baby food samples.

32.2.2 Analytical methods

Various factors should be considered when choosing an appropriate measurement technique for determination of the mineral profile in baby foods. Such factors include detection capability and sensitivity, availability of instrumentation, speed of analysis, cost and application, as well as classical features like accuracy, precision and selectivity. Table 32.3 shows a comparison of the characteristics of the main measurement techniques used to determine mineral elements in baby food. The analytical methods employed in the literature to determine minerals in baby food are indicated in Table 32.4, from which it can be seen that minor elements like Fe, Zn and Ca have been frequently determined by FAAS. Trace elements like Cu, Co, Ni, Mn, Se, Cd and Pb were analysed using GFAAS and a more or less complete mineral profile of baby foods was established using ICP-OES or ICP-MS.

Table 32.3 Comparison of the main characteristics of measurement techniques commonly employed for the determination of the mineral profile of baby foods.

Technique	Multi-element	LOD (μg/kg)	Precision (%)	Cost
FAAS	No	1–100	0.1–1	Low
GFAAS	No	0.01–1	1–5	Moderate
ICP-OES	Yes	1–100	0.1–1	High
ICP-MS	Yes	0.001–0.1	0.2–2	High

Table 32.4 Analytical methods employed in the recent literature to determine mineral elements in baby food.

Sample	Elements	Analytical technique	Reference
Baby food reference (TUGAP 1201 R)	Fe, Zn	FAAS	[14]
Foods consumed by children	Fe, Zn	FAAS	[25]
Commercial baby food of vegetables and fruits	Ca, Fe, Zn	FAAS	[26]
Commercial baby food based on chicken, hake, vegetables and fruit	Co, Cu, Ni, Mn	GFAAS	[15]
Commercial baby food, fish based	Cd, Se, Pb	GFAAS	[16]
Foods consumed by children	Cu	GFAAS	[25]
Commercial infant cereals, baby food based on chicken, hake and infant rice	Ca, Co, Cr, Cu, Fe, Mn, Na, Ni, Se, Zn	ICP-MS	[12]
Commercial baby food of meat and vegetables	Ca, Cu, Fe, K, Mg, Na, Se, Zn	ICP-OES/ICP-MS	[27]
Commercial baby food of meat, fish, vegetables and fruit	Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mo, Na, Ni, Zn	ICP-MS	[28]
Commercial baby food	Ca, Cu, Fe, Mn, Ni, Se, Zn	ICP-OES	[29]
Commercial baby food of chicken and fish	Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Se, Zn	ICP-OES/ICP-MS	[31]

Flame atomic absorption spectrometry

FAAS is a well-known technique for the analysis of trace elements in baby food samples. However, despite being a simple and robust tool for the analysis of a wide range of elements, it is a single-elemental technique.

Fe and Zn have been determined by FAAS in vegetable and fruit baby foods [25, 26]. Samples were digested with HNO_3 and H_2O_2 in a microwave oven and analysed using the method of standard addition from 0 to 1 mg/L for Fe, and from 0 to 0.4 mg/L for Zn, with a dilution factor of 4 for Fe and 5 for Zn. This method shows linearity between 0 and 10 mg/L and between 0 and 0.5 mg/L for Fe and Zn, respectively, with good detection limits of 0.06 mg/L and 0015 mg/L (Table 32.5). The reproducibility of the method for 10 measurements of vegetable jars was 2.5–15% for Fe and 8–9.4% for Zn, and for fruit jars was 4.7–14.4% and 8.9–15.8% for Fe and Zn, respectively.

Ozbek and Akman [14] used FAAS for Fe and Zn determination in baby food samples. In this case, slurry sampling with Triton X-114 and nitric acid was used for the direct analysis of these elements. For the determination of Fe a wavelength of 256.8 nm was used, with a limit of detection (LOD) of 5.46 mg/kg. In the case of Zn, a wavelength of 213.9 nm was used, with an LOD of 3.37 mg/kg (Table 32.5). These LODs are quite high, but bearing in mind that samples were not digested and were directly determined in a sample slurry, it must be accepted that the sensitivity is sufficient for screening purposes.

In infant food analysis, Fe and Zn are the two most important elements, and their lack in infant nutrition often creates greater deficiency problems in childhood. Thus it can be concluded that FAAS is a good technique if only Fe and Zn are to be measured, as it has good sensitivity and accuracy and low cost, which makes this an adequate methodology for small laboratories.

Table 32.5 Limits of detection reported in the recent literature for the determination of mineral elements in baby foods.

Element	[12] LOD (mg/kg)	[13] LOD (mg/kg)	[14] LOD (mg/kg)	[25] LOD (mg/L)	[29] LOD (mg/kg)	[31] LOD (mg/kg)
Al		0.15 (ICP-OES)				0.1 (ICP-MS)
As	0.006 (ICP-MS)	0.3 (ICP-OES)				0.003 (ICP-MS)
Ba		0.009 (ICP-OES)				0.02 (ICP-MS)
Ca	10 (AAS)	0.10 (ICP-OES)			2 (ICP-OES)	1.56 (ICP-OES)
Cd	0.007 (ICP-MS)	0.02 (ICP-OES)				0.006 (ICP-MS)
Co	0.037 (ICP-MS)	0.05 (ICP-OES)				0.0015 (ICP-MS)
Cr	0.005 (ICP-MS)	0.04 (ICP-OES)				0.0078 (ICP-MS)
Cu	0.11 (ICP-MS)	0.03 (ICP-OES)		0.0005 (GFAAS)	0.2 (ICP- OES)	0.036 (ICP-MS)
Fe	7 (AAS)	0.05 (ICP-OES)	5.46 (FAAS)	0.06 (FAAS)	0.2 (ICP- OES)	0.15 (ICP-OES)
K		3.0 (ICP-OES)				0.03 (ICP-OES)
Mg		0.03 (ICP-OES)				0.36 (ICP-OES)
Mn	0.02 (ICP-MS)	0.003 (ICP-OES)			0.04 (ICP- OES)	0.014 (ICP-MS)
Mo		0.03 (ICP-OES)				0.0069 (ICP-MS)
Na	10 (AAS)	0.5 (ICP-OES)				0.33 (ICP-OES)
Ni	0.038 (ICP-MS)	0.07 (ICP-OES)			0.2 (ICP- OES)	0.024 (ICP-MS)
Pb	0.039 (ICP-MS)	0.3 (ICP-OES)				0.0042 (ICP-MS)
Se	0.013 (ICP-MS)	1.3 (ICP-OES)			0.2 (ICP- OES)	0.0066 (ICP-MS)
Zn	0.16 (ICP-MS)	0.2 (ICP-OES)	3.37 (FAAS)	0.015 (FAAS)	0.2 (ICP- OES)	0.15 (ICP-OES)

Graphite furnace atomic absorption spectrometry

GFAAS provides a parts per billion detection capability using only microlitre samples deposited inside a graphite tube. This technique is a versatile and highly sensitive tool for food analysis. Viñas *et al*. [15] reported a method for the analysis of meat, fish, vegetable and fruit baby food slurries with regard to the determination of Cu, Co, Ni and Mn. Optimum atomization temperatures for the analytes were 1800°C for Cu, 2600°C for Co, 2600°C for Ni and 2000°C for Mn. The wavelength used to determine Cu, Co, Ni and Mn in baby food were 324.8, 240.7, 232.0 and 279.5 nm, respectively. To evaluate the precision of the method, the relative standard deviation (RSD) values were 3.7–11.2% for Cu, 0.7–1.7% for Mn, 2.4–9.9% for Ni and 6.2–9.6% for Co. Lower RSD values were found for jars of vegetables and fruits, and the highest RSD values were obtained for meat and fish jars.

Viñas *et al*. [16] also employed baby food slurries to determine toxic elements (Cd and Pb) by GFAAS. They used wavelengths of 283.3 and 228.8 nm for Pb and Cd analysis, respectively. LODs obtained by this method were 0.4 μg/kg for Cd and 3.4 μg/kg for Pb, and the corresponding RSD values were 2.5 and 2.7%.

Determination of Cu in samples of food for children aged 2–3 years was made by GFAAS, after a previous microwave-assisted digestion of samples with HNO_3 and H_2O_2 [25]. The applied dilution factor was 10, with LOD of 0.5 μg/L and RSD of 2% for 10 independent determinations in the same sample.

Inductively coupled plasma optical emission spectrometry

ICP-OES is a powerful multi-element technique for the determination of trace elements in foods and offers a very wide linear dynamic range of about five orders of magnitude. Usually, the LODs obtained by ICP-OES are comparable with those obtained by FAAS and higher than those found by GFAAS by about one or two orders of magnitude.

In a study of the mineral profile of infant food, Ca, Cu, Fe, Mn, Ni, Se and Zn were determined by ICP-OES [29]. The wavelengths selected were 393.366, 324.752, 259.939, 257.61, 231.604, 196.026 and 213.857 nm for Ca, Cu, Fe, Mn, Ni, Se and Zn, respectively. LODs obtained are shown in Table 32.5. The accuracy of the method was calculated by RSD, and the values obtained were less than 2% for Mn and Zn, less than 3% for Ca and Fe, less than 5% for Cu, less than 7% for Ni and less than 12% for Se.

In other multi-element studies of baby food samples, ICP-OES was used to determine Ca, Cu, Mg, Fe, Zn, K

and Na in meat and vegetable jars [27] or to determine Ca, Fe, Mg, Mn, Na, K and Zn in meat and fish jars [31]. In both cases, microwave digestion with nitric acid and hydrogen peroxide for sample preparation were used. The limit of quantification (LOQ) data were 5.2, 0.5, 0.1, 0.048, 1.2, 1.1 and 0.5 mg/kg for Ca, Fe, Mg, Mn, K, Na and Zn, respectively.

In short it can be concluded that ICP-OES is a very good technique for evaluating the mineral profile of baby foods, because it allows a large number of elements to be determined simultaneously in a short period of time. It is a robust and sensitive technique with good LODs and the cost of analysis is not excessive despite the high consumption of gas required for plasma stabilization and analyte measurement. ICP-OES is a good choice when the aim is to determine elements at ppm levels in baby foods. Conversely, if the objective is to measure elements at ultratrace levels, the sensitivity of this technique is not sufficiently high and a pre-concentration step will be mandatory.

Inductively coupled plasma mass spectrometry

ICP-MS involves the detection of ions extracted from the sample nebulized inside a high-temperature argon plasma. ICP-MS provides multi-element data with LODs lower than those of FAAS and ICP-OES. Some of the specific advantages of ICP-MS include extremely good sensitivity and selectivity, wide linear dynamic range and very fast acquisition of quantitative information about the elements present in the dissolved samples.

In a multi-element study of infant cereal and puréed infant food, Cu, Mn, Zn, Se, Cr, Ni and Co were determined by ICP-MS [12]. LODs obtained with this method can be seen in Table 32.5. The recovery percentages obtained varied were between 80 and 120%. In this study samples from different countries (Spain, UK, China and USA) were compared.

ICP-MS was also used to determine Al, Ca, Cd, Co, Cr, Cu, Fe, K, Mn, Mo, Na, Ni, Pb and Zn in 74 infant food products for babies aged 4–18 months [28]. The accuracy of the method was verified by analysis of a certified material NIST 1577b Bovine Liver. The concentrations found were within 95–105% of the certified values. To assess possible contamination during sample preparation, blanks were prepared using the same procedure employed for samples.

32.3 Mineral composition of baby foods

The daily mineral intake of children can be evaluated by direct determination of the mineral profile of baby foods (Figure 32.3) or by indirect strategies based on both the estimation of food consumed by babies and the mineral composition of basic foods consumed in a country. One of the most common strategies employed to evaluate the mineral intake of baby foods is based on the Feeding Infants and Toddlers Study (FITS). This provides an estimate of the elemental composition of baby foods by surveying parents about the food intake of their children and by accessing data collected in different food databases. Devaney *et al*. [32] and Heird *et al*. [33] reported data for Ca, P, Mg, Fe, Zn, Na and K for infants and toddlers aged 4–24 months. These studies consisted of three separate telephone interviews: a household interview, a 24-hour dietary recall and a second-day 24-hour dietary recall for a random subsample of respondents with supplementary questions on growth, development and feeding patterns and a second dietary recall for a random subset of samples. Data obtained with the survey were entered in the Software of Intake Distribution Estimation (Iowa State University). This program provides estimates of the percentiles of usual nutrient intake distributions, as well as estimates of the proportion above or below defined cut-off values. Six years later, Butte *et al*. [34] followed the same procedure to estimate the intake of Ca, P, Mg, Fe, Zn, Na and K in children aged 0–4 years.

32.3.1 Essential element content in baby foods

As indicated in the introduction, mineral elements play an important role in the normal development of the child. Essential elements are involved in numerous biochemical reactions in the body, affecting physiological, neuromuscular and cardiac functions. They also participate in the development and maintenance of bones and muscles. In this section we will comment in detail on the influence of each of the commonly analysed elements in baby foods.

Potassium

Potassium is the major cation of intracellular fluid and an almost constant component of lean body tissues, and maintains body water and acid balance. It plays a role in

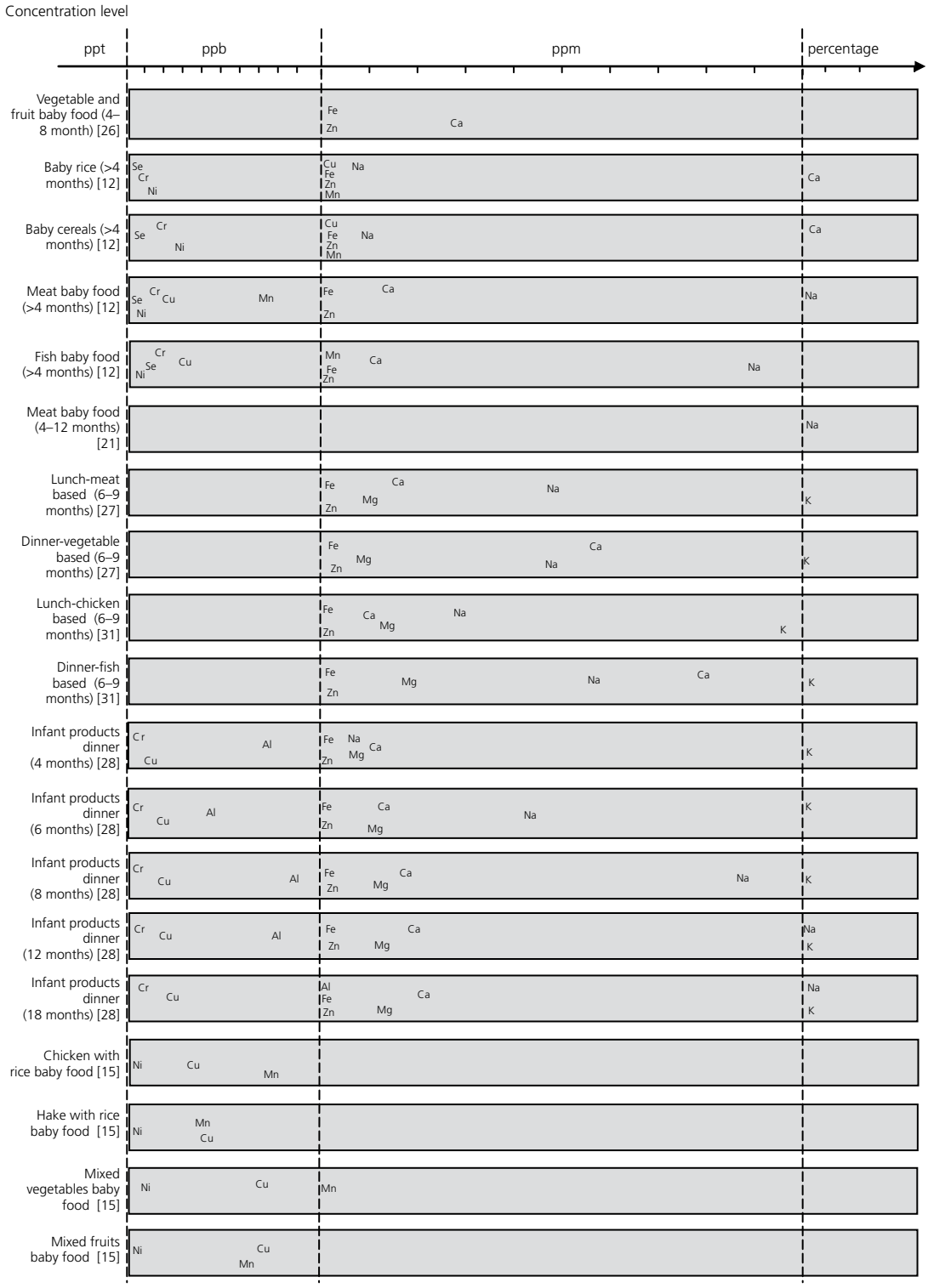


Figure 32.3 Concentrations of mineral elements in baby food samples.

transmission of nerve impulses to muscles, in muscle contraction and in the maintenance of normal blood pressure [35]. About 85% of potassium is absorbed by the human organism. Potassium is a non-problematic element in infant feeding. It is usually present in baby food in adequate quantities at recommended intake values.

Sodium

Sodium is an essential nutrient that is involved in numerous physiological functions, such as maintenance of extracellular osmolarity, controlling the volume of body water compartments and the maintenance of normal blood pressure [36]. The intake of calcium and potassium can affect sodium needs. The major adverse effect of increased sodium chloride intake in babies concerns the increase in blood pressure, a risk factor for cardiovascular and renal diseases [37]. Sodium is one of the most important elements to be controlled in baby diets, because abuse of this element can cause various health problems. Because of this, it is very important to control sodium in commercially available baby foods, and it is recommended to not add salt to baby food. In the literature, values of sodium agree well with the recommended values, but in two studies of meat-based infant food the content of sodium was higher than the recommended values [21, 23].

Calcium

Calcium is required for the correct development and maintenance of the skeleton. It plays a vital role in neuromuscular and cardiac functions. It is stored in the teeth and bones where it provides structure and strength. In the first 2 years of life, infants need 120 mg of calcium daily to allow for normal growth. With human milk, absorption of 120 mg of calcium requires a mean intake of 240 mg and the recommended intake is 300 mg [38]. Sodium intake can affect calcium requirements; 2.3 g of sodium removes about 40 mg of calcium. The average concentration of calcium in breast milk for infants aged 7–12 months is 210 mg/L. This provides a contribution of 126 mg/day from breast milk that is added to the 140 mg/day from complementary foods, giving an AI of 270 mg/day [9]. Following these recommendations, several studies in the literature indicate that the amount of calcium is lower than recommended, for an average consumption of 400 mg of baby food [12, 28]. On the other hand, Zand *et al.* [27, 31] found levels of calcium in vegetable- and fish-based infant food to be higher than those in foods based on meat.

Magnesium

Magnesium is involved in over 300 biochemical reactions in the body. It helps to maintain normal muscle and nerve function, maintains a regular heart rhythm, supports a healthy immune system, and keeps bones strong. Magnesium also helps to regulate blood sugar levels, promotes normal blood pressure, and is known to be involved in energy metabolism and protein synthesis [39]. Magnesium in breast milk or infant formula is absorbed at levels of 50–90% [40, 41]. As for potassium, there is no problem with magnesium because infants can access the recommended amounts in available foods.

Iron

Iron requirements are higher during the periods of rapid growth in early childhood and adolescence. During the first year of life, children increase their weight threefold and should bend body iron content. At 4 months of life there is an increased iron requirement of 0.78 mg/day due to rapid growth and the iron reserves could diminished to pathological levels if needs are not covered with dietary iron [36]. The presence of other nutrients, such as vitamin C and citric, lactic or malic acids, can increase the absorption from vegetables consumed at the same time. In contrast, calcium and zinc can inhibit the absorption of iron [9]. Commercial baby food products are regularly fortified with iron and ascorbic acid. These fortifications must be carefully controlled because a high intake of iron can affect the absorption of other nutrients, such as calcium or zinc. Iron is the most problematic element in terms of bioavailability. In all cases reported in the literature, the amount of Fe in baby food is lower than the recommended intake [12, 23, 25–28, 31].

Zinc

Zinc is essential for growth and development of young children. Dietary intake of iron in some supplements can decrease zinc absorption, which is of particular concern during lactation. The bioavailability of zinc is also inhibited by phytate, which is present in large amounts in cereals and legumes [42]. Absorption of zinc is higher from breast milk than from infant formula based on cow milk or soy; these formulas have a much higher content of zinc that breast milk, which compensates for the low

absorption efficiency. Zinc is also problematic in terms of bioavailability, so it is necessary to control the amount of zinc in infant foods.

Manganese

Manganese is an essential element involved in the formation of bone and the metabolism of carbohydrates, cholesterol and amino acids. Manganese is present in many common foods. Therefore it is extremely rare to have a deficiency of this element in humans [43]. In infant foods based on meat, fish or fruit, manganese levels are lower than the AI or RDA, but in vegetable-based infant food Mn values agree with the recommended values [12, 15].

Copper

Copper is essential for life. It is required for infant growth, bone strength, iron transport, and cholesterol and glucose metabolism [44]. Very high levels of zinc and iron, generally taken as supplements, can affect the absorption of Cu in infants. Copper deficiency can affect the central nervous system and the immune and cardiovascular systems, notably in infants [45]. In general, the values of Cu in complementary foods are adequate to meet the recommended intake, although in some cases values of Cu are lower than the recommended values, for example in dinner products for babies aged 12–18 months analysed by Melø *et al*. [28], meat- and fish-based baby foods from Spain [12] or commercial baby foods containing chicken with rice and hake with rice from Spain [15]. However, it must be taken into account that milk also provides small amounts of Cu.

Selenium

Selenium has been implicated in the protection of body tissues against oxidative stress, the maintenance of defences against infection, and the modulation of growth and development. Cow milk-based formula provides less than one-third of the selenium content of human milk. Estimates of selenium intake by 2-month-old infants were 7.8 μg/day from cow-milk compared with 22.4 μg/day from human milk [7]. Normal content of selenium in baby foods is 50–120 μg/kg depending on type of baby food. High levels of Se have been reposted in fish-based foods [12].

Molybdenum

Molybdenum is absorbed very efficiently by passive transport. Legumes are major contributors of molybdenum in the diet, as are grain products and nuts. Animal foods, fruits and vegetables are low in molybdenum [7]. Molybdenum intakes in Asian countries are higher due to the predominantly vegetarian diet in these countries. Between weaning and 3 years of age, molybdenum intakes appear to be higher but decline thereafter [11].

Chromium

Chromium is important in the metabolism of fats and carbohydrates. Several studies have indicated the beneficial effects of chromium on circulating glucose, insulin and lipids in humans. Chromium deficiency may be seen as impaired glucose tolerance and can be found in infants with protein-calorie malnutrition. Supplementation of chromium could be recommended but Cr absorption is low and its excretion rate is high. Additionally, toxicity of this element is not common [46]. A study on the mineral and trace elements in commercial infant food [28] for infants aged 4–18 months found high values of Cr compared with the AI and RDA. The same results were found in a study of essential elements in infant foods from Spain, the UK, China and the USA [12].

From literature data summarized in Figure 32.3 it can be seen that K followed by Ca and Na are present at percentage levels in baby foods analysed worldwide, with Mg, Fe, Zn, Cu, Mn and Al at ppm levels and Ni, Cr and Se at ppb levels. However, depending on the type of food analysed and the country of origin, the concentrations of the mentioned elements vary. On the other hand, it is important to note that highly toxic elements like As, Pb, Cd or Hg have not been reported in baby foods.

Abbreviations

AI: Adequate intake
AOAC: Association of Official Analytical Chemists
EAR: Estimated average requirement
ESPGHAN: European Society of Paediatric Gastroenterology, Hepatology and Nutrition
FAAS: Flame atomic absorption spectrometry
FAO: Food and Agriculture Organization
FITS: Feeding Infants and Toddlers Study
GFAAS: Graphite furnace atomic absorption spectrometry
HGAAS: Hydride generation atomic absorption spectrometry
ICP-MS: Inductively coupled plasma mass spectrometry
ICP-OES: Inductively coupled plasma optical emission spectrometry

IRMM: Institute for Reference Materials and Measurements
LOD: Limit of detection
LOQ: Limit of quantification
MW: Microwave
NBS: National Bureau of Standards
NIST: National Institute of Standards and Technology
RDA: Recommended daily allowance
RDI: Recommended daily intake
RM: Reference materials
RNI: Recommended nutrient intake
RSD: Relative standard deviation
SRM: Standard reference materials
UL: Tolerable upper intake level
UNICEF: United Nations Children's Fund
WHO: World Health Organization

References

1 ESPGHAN Committee on Nutrition. Complementary feeding: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2008;**46**:99–110.
2 Foote KD, Marriott LD. Weaning of infants. *Arch Dis Child* 2003;**88**:488–492.
3 World Health Organization. *Infant and Young Child Feeding. Model Chapter for Textbooks for Medical Students and Allied Health Professionals*. WHO, Geneva, 2009.
4 World Health Organization. *Guiding Principles for Complementary Feeding of the Breastfed Child*. WHO, Geneva, 2003.
5 World Health Organization. *Strengthening Action to Improve Feeding of Infants and Young Children 6–23 Months of Age in Nutrition and Child Health Programmes*. WHO, Geneva, 2008.
6 Food and Nutrition Board, Institute of Medicine. *Dietary Reference Intake (DRIs): Recommended Intakes for Individuals Elements*. National Academies Press, Washington, DC, 2004.
7 National Health and Medical Research Council. Nutrient reference values for Australia and New Zealand. National Health and Medical Reserch Council, Canberra, Australia, 2005.
8 Scientific Committe on Food. Opinion of the Scientific Committee on Food on the revisión of reference values for nutrition labelling. SCF/CS/NUT/GEN/18 Final, European Commission, 2003.
9 FAO/WHO. *Vitamin and Mineral Requirements in Human Nutrition: Report of a Joint FAO/WHO Expert Consultation*, 2nd edn. Bangkok, Thailand, 2004.
10 Food and Nutrition Board. *Dietary Reference Intake: The Essential Guide to Nutrient Requirements*. National Academies Press, Washington, DC, 2006.
11 World Health Organization. *Trace-Elements in Human Nutrition and Health*. WHO, Geneva, 1996.
12 Carbonell-Barrachina AA, Ramirez-Gandolfo A, Wu X *et al*. Essential and toxic elements in infant foods from Spain, UK, China and USA. *J Environ Monit* 2012;**14**:2447–2455.
13 Mir-Marqués A, González-Masó A, Cervera ML, de la Guardia M. Mineral profile of Spanish commercial baby food. Unpublished report.
14 Ozbek N, Akman S. A slurry sampling method for the determination of iron and zinc in baby food by flame atomic absorption spectrometry. *Food Addit Contam* 2012;**29**:208–216.
15 Viñas P, Pardo-Martínez M, Hernández-Córdoba M. Determination of copper, cobalt, nickel, and manganese in baby food slurries using electrothermal atomic absorption spectrometry. *J Agric Food Chem* 2000;**48**:5789–5794.
16 Viñas P, Pardo-Martínez M, Hernández-Córdoba M. Rapid determination of selenium, lead and cadmium in baby food samples using electrothermal atomic absorption spectrometry and slurry atomization. *Anal Chim Acta* 2000;**412**:121–130.
17 Fernandez DR, Vanderjagt DJ, Williams M *et al*. Fatty acid, amino acid, and trace mineral analyses of five weaning foods from Jos, Nigeria. *Plant Food Hum Nutr* 2002;**57**:257–274.
18 Thomson BM, Vannoort RW, Haslemore RM. Dietary exposure and trends of exposure to nutrient elements iodine, iron, selenium and sodium from the 2003–4 New Zealand Total Diet Survey. *Br J Nutr* 2008;**99**:614–625.
19 Burló F, Ramírez-Gandolfo A, Signes-Pastor A, Haris P, Carbonell-Barrachina A. Arsenic contents in Spanish infant rice, pureed, infant foods and rice. *J Food Sci* 2012;**71**:T15–T19.
20 Ybañez N, Cervera ML, Montoro R, de la Guardia M. Comparison of dry mineralization and microwave-oven digestión for the determination of arsenic in mussel products by platform in furnace Zeeman-effect atomic-absorption spectrometry. *J Anal Atom Spectrom* 1991;**6**:379–384.
21 Okesli V, González-Bermúdez CA, Vidal-Guevara ML, Dalmau J, Ros G. Alimentación complementaria: ¿es igual el perfil nutricional de un pure casero que el de un tarrito commercial? *Acta Pediátr España* 2011;**69**:235–240.
22 Association of Official Analytical Chemists. *Official Methods of Analysis*, 15th edn. AOAC, Rockville, MD, 1990, pp. 931–945.
23 Van den Boom S, Kimber AC, Morgan JB. Nutritional composition of home-prepared baby meals in Madrid. Comparison with comercial products in Spain and home-made meals in England. *Acta Paediatr* 1997;**86**:57–62.
24 Association of Official Analytical Chemists. *Official Methods of Analysis*, 15th edn. AOAC, Rockville, MD, 1990, pp. 806–807.
25 Bosscher D, Van Cauwenbergh R, Robberecht H, Van Caillie-Bertrand M, Deelstra H. Daily dietary iron, zinc and copper intake of infants in Belgium. *Eur Food Res Technol* 2002;**215**:275–278.
26 Bosscher D, Van Cauwenbergh R, Van der Auwera J, Robberecht H, Deelstra H. Calcium, iron and zinc availability from weaning meals. *Acta Paediatr* 2002;**91**:761–768.
27 Zand N, Chowdhry BZ, Zotor FB, Wray DS, Amuna P, Pullen FS. Essential and trace elements content of commercial infant foods in the UK. *Food Chem* 2011;**128**:123–128.

28 Melø R, Gellein K, Evje L, Syversen T. Minerals and trace elements in commercial infant food. *Food Chem Toxicol* 2008;**46**:3339–3342.
29 Pandelova M, Lopez WL, Michalke B, Schramm K. Ca, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn contents in baby foods from the EU market: comparison of assessed infant intakes with the present safety limits for minerals and trace elements. *J Food Compos Anal* 2012;**27**:120–127.
30 Plessi M, Bertelli D, Monzani A. Determination of aluminum and zinc in infants formulas and infant foods. *J Food Compos Anal* 1997;**10**:36–42.
31 Zand N, Chowdhry BZ, Wray DS, Pullen FS, Snowden MJ. Elemental content of commercial 'ready to-feed' poultry and fish based infant foods in the UK. *Food Chem* 2012;**135**: 2796–2801.
32 Devaney B, Ziegler P, Psc S, Karwe V, Barr S. Nutrient intake of infants and toddlers. *J Am Diet Assoc* 2004;**104**: S14–S21.
33 Heird W, Ziegler P, Reidy K, Briefel R. Current electrolyte intakes of infants and toddlers. *J Am Diet Assoc* 2006;**106**: S43–S51.
34 Butte NF, Fox MK, Briefel RR *et al*. Nutrient intakes of US infants, toddlers, and preschoolers meet or exceed dietary reference intakes. *J Am Diet Assoc* 2010;**110**:S27–S37.
35 Food and Agriculture Organization. Alimentation, nutrition et agricultura, Alimentación, nutrición y agricultura. Available at www.fao.org/docrep/x2650T/x2650T00.htm.
36 Gil Hernández A, Uauy Dagach R, Dalmau Serra J. Bases for adequate complementary feeding in infants and young children [in Spanish]. *An Pediatr (Barc)* 2006;**65**:481–495.
37 Graudal NA, Galloe AM, Garred P. Effects of sodium restriction on blood pressure, renin, aldosterone, catecholamines, cholesterols and triglyceride: a meta-analysis. *JAMA* 1998;**279**:1383–1391.
38 FAO/WHO. *Human Vitamin and Mineral Requirements. Report of a Joint FAO/WHO Expert Consultation*. Bangkok, Thailand, 2002.
39 Office of Dietary Supplements. Strengthening knowledge and understanding of dietary supplements: Magnesium. Available at http://ods.od.nih.gov/factsheets/Magnesium-Consumer/.
40 Lonnerdal B. Magnesium nutrition of infants. *Magnesium* 1995;**8**:99–105.
41 Lonnerdal B. Effects of milk and milk components on calcium, magnesium and trace element absorption during infancy. *Physiol Rev* 1997;**77**:643–669.
42 Brown KH, Wuehler SE, Peerson JM. The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency. *Food Nutr Bull* 2001;**22**:113–125.
43 World Health Organization. *Guidelines for Drinking-water Quality: Manganese in Drinking-water*. Available at www.who.int/water_sanitation_health/dwq/chemicals/manganese.pdf.
44 Scientific Committe on Food. *Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Copper*. SCF/CS/NUT/UPPLEV/57 Final, European Commission, 2003.
45 Turnlund JR. Copper. In: Shils ME, Olson JA, Shike M, Ross AC (eds) *Modern Nutrition in Health and Disease*, 9th edn. Williams & Williams, Baltimore, 1999, pp. 241–252.
46 Food and Nutrition Board, Institute of Medicine. Chromium in diet: MedlinePlus Medical Encyclopedia, US National Library of Medicine, 2011.

CHAPTER 33
Human milk

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Abstract: Milk is the sole food for infants in the first months of life and human milk is taken as the reference for preparing infant formulas that can substitute human milk if necessary. It is therefore essential to determine human milk composition, and particularly trace elements, to provide data on which to base nutrient recommendations during infancy. Trace element composition changes with stage of lactation, sampling procedure, maternal diet, type of lactation (programmed or on demand) and individual factors that give a wide and varying range of data in some human milk components which change over time. This chapter presents a review of the scientific data published in recent years from different countries, and topics essential for understanding the differences between human milk, cows' milk and infant formula (e.g. speciation and bioavailability of trace elements) are examined.

Keywords: human milk, trace elements, speciation, bioavailability

33.1 Chemical composition of human milk

Milk is the first product secreted by the mammary gland at the end of pregnancy in mammals, and its composition is highly dependent on the biological species. Human milk composition changes with stage of lactation, hour of the day, moment of sampling during feeding session, sampling procedure, maternal diet, type of lactation (programmed or on demand) and individual factors. Because of all these factors, it is important to identify the sampling conditions in order to deduce significant conclusions from the results obtained [1]. The first product secreted by the human mammary gland is the colostrum, followed by transitional milk and later mature milk [2]. Some researchers [3] do not agree with the use of the terms 'colostrum', 'transitional milk' and 'mature milk', but we will use these terms here because they are the most commonly used in the scientific literature. Colostrum is a thick yellowish (due to β-carotene) liquid produced by the mammary gland between the first and fourth days postpartum. Even if the neonate does not ingest the colostrum, it is produced during the first 3 days postpartum. The composition of colostrum changes even within the same feeding stage, the lipid content increasing and the aqueous phase and glucose content decreasing. Its high content of antibodies protects the neonate from bacteria and viruses at the moment of birth and during the first days of life. Proteins, liposoluble vitamins and minerals are in higher concentrations than in transitional and mature milk. Although the total lipids in colostrum are in lower concentration (2%) than in transitional milk (2.9%) and mature milk (3.6%), the percentage of phospholipids decreases (35%, 31% and 27% in colostrum, transitional and mature milk, respectively) [1, 4].

The composition of transitional milk varies with day of lactation but more slowly than with colostrum. During this lactation stage (from 7–10 days to second week postpartum) the levels of lactose, lipids and hydrosoluble vitamins increase, but total protein, liposoluble vitamins and immunoglobulin levels decrease.

Mature milk (from the third week postpartum) is the mammary product used to compare with milks produced by other mammals. In this product, water is the principal constituent; its secretion is regulated by the synthesis of lactose. The aqueous phase of milk is called milk whey and can be obtained by ultracentrifugation [5]. Whey contains water, sugars, proteins, IgA (immunoglobulin A) and ions.

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33.1.1 Fat

Fat (3–5%) is the most variable constituent in breast milk, depending on individual, gestational age [6, 7], the left and right breast, within days, between days [8], and volume of milk nursed in the previous feeding session [1] and also during the current session [9]. Maternal intake and dietary habits influence the lipid composition of breast milk [10] but not total lipid content. The fat phase in milk can be obtained by chemical extraction with different solvents [11] or by centrifugation [5, 12], but total isolation of fat is not easy because some of the lipids are bound to proteins. The lipids in breast milk form fat globules (1–10 μm) [13] emulsified in an aqueous phase. The inner core of fat globules is constituted by non-polar lipids and surrounded by bipolar compounds that avoid the coalescence of fat globules by acting as stabilizers [14]. The protective membrane of the fat globules is formed by phospholipid complexes. Other phospholipids are dispersed in the non-fat milk phase. Human milk fat contains monoglycerides, diglycerides, triglycerides, free fatty acids, glucolipids, phospholipids, sterols and sterol esters. The fat phase also contains liposoluble vitamins (vitamins A, D and K), alkyl glyceryl esters and glyceryl ester diesters.

33.1.2 Proteins

Milk proteins (0.9% in human milk) are classified into whey proteins, caseins and mucins. The dominant protein in breast milk is lactoferrin (20–25%), a protein with a structure that makes it difficult to digest [15]. Nevertheless, some fragments of lactoferrin may still be absorbed by the small intestine through the mucosa [16]. This milk whey protein can inhibit bacterial growth, particularly by binding to iron and making it inaccessible to pathogens which require iron to grow [17]. Lactoferrin has a special relationship with lysozyme [18], an active enzyme present in higher concentration in breast milk than in bovine milk (3000-fold higher). In addition to lactoferrin, α-lactalbumin is one of the main proteins that harbours tumour-selective capabilities. HAMLET (human α-lactalbumin made lethal to tumour cells) is formed by binding of α-lactalbumin to oleic acid, resulting in the release of its Ca^{2+} ion and a folding change [19]. It induces apoptosis in tumour cells but leaves normal differentiated cells unaffected [20]. Bile salt-stimulated lipase (BSSL) comprises only 1–2% of total milk proteins, but this is substantial for an active enzyme. In the gut lumen, it hydrolyses milk fat, vitamin A esters, cholesterol esters and lysophospholipids [21].

Casein is a group of proteins (α-, β- and κ-casein) with an isoelectric point of 4.6. Casein forms micelles with Ca^{2+} and PO_4^{3-}. Its amino acid composition and physicochemical properties differ from cows' casein. The characteristic white colour of milk is due to these micelles. β-Casein has multiple phosphorylated amino acids along its backbone, and when digested smaller casein phosphopeptides are formed facilitating calcium absorption. κ-Casein in breast milk is heavily glycosylated and is an inhibitor of bacterial adhesion to human gastric mucosa (e.g. *Helicobacter pylori*) [22]. Studies have found that κ-casein, as well as sIgA, can inhibit *H. pylori* adhesion to human gastric mucosa, and lactoferrin can inhibit *H. pylori* growth [21]. This indicates that proteins in milk work together in a synergistic manner to protect against pathogens.

Mucins specifically surround milk fat globules, forming a chemical barrier: milk fat globule membrane proteins. They make up only about 1–2% of all breast milk proteins, but are essential in fat globule stabilization. No infant formula contains these milk fat globule membrane proteins, because they are usually lost or discarded in dairy processing [23]. Human milk mucins can bind to rotaviruses and inhibit viral replication [24]. Membrane mucins on milk fat globules inhibit binding of S-fimbriated *Escherichia coli* to buccal epithelia [25], and reduce the incidence of diarrhoea [26].

In breast milk, immunoglobulins such as IgA, IgM and IgG are present but in lower concentrations than sIgA (90% of total milk inmunoglobulins). sIgA, as opposed to other types of IgA, is stable against proteolytic enzymes in the infant's gut and binds to bacterial and viral antigens, promoting inhibition of attachment to the mucosal lining [23]. Human milk sIgA concentration decreases rapidly over the first 4 weeks of lactation, and then more gradually from 4 to 12 weeks. Milk sIgA concentration decreases to 50% of the day 3 value by 8 weeks [27].

33.1.3 Vitamins

Water-soluble vitamins

The water-soluble vitamins have no storage tissue and therefore tissue depletion occurs rapidly during a period of low dietary intake. Breast milk concentration of water-soluble vitamins is significantly affected by the diet and consumption of supplements by the mother, but does not change during lactation [28].

Vitamin B_1 (thiamin) is present in human milk as thiamin (30% w/w) and thiamin monophosphate (70% w/w). Its level increases from colostrum to mature milk and is relatively constant in mature breast milk (14 μg/dL) [1, 28], but lower than the level found in cows' milk (40 μg/dL) [1, 29]. Average breast milk levels of thiamin show differences among women from diverse geographic origins, from 1.1 μg/dL in India to 11.6 μg/dL in Cameroon [30]. Concentration of vitamin B_1 in breast milk is strongly dependent on maternal intake and status. Maternal supplementation rapidly improves the vitamin concentration in milk [31, 32].

Vitamin B_2 or riboflavin is found in human milk as flavin adenine dinucleotide (54%), riboflavin (39%), and small amounts of other flavins [31]. Average levels in mature breast milk (30 μg/dL) [1, 29] are lower than in cows' milk (175 μg/dL) [28, 29]. In mothers whose diet has been supplemented with riboflavin, the level increases in breast milk in the early lactation stage; however, 1–6 weeks postpartum these differences are not significant [1].

Vitamin B_6 is the generic name of three pyridines: pyridoxine (also called pyridoxal), pyridoxal and pyridoxamine, which are interconvertible. The predominant form of vitamin B_6 in breast milk is pyridoxal (75%), with smaller amounts of pyridoxal phosphate (9%), pyridoxamine and pyridoxine [31]. Vitamin B_6 levels in breast milk are highly dependant on the vitamin intake of the mother during pregnancy, the bioavailability of the vitamin source and the stage of lactation [28]. In mature breast milk, levels of vitamin B_6 are 10–18 μg/dL [1, 28]; values are lower than those found in cows' milk (50–64 μg/dL) [1, 29].

Vitamin B_{12} or cobalamins comprise methylcobalamin (important in folate metabolism) and 5-deoxyadenosylcobalamin (important in carbohydrate and lipid metabolism) [28]. The dominant form of vitamin B_{12} in human milk is methylcobalamin. Colostrum levels of vitamin B_{12} are higher than in mature milk, but supplementation of the mother's diet does not have significant influence on the breast milk; nevertheless, some studies recommend supplementation during pregnancy [31].

Over half the folate in bovine milk is present as the polyglutamate of methyltetrahydrofolate, while in human milk the monoglutamate of this folate predominates. Bovine milk contains considerably more folate than human milk, but folate polyglutamates have approximately half the biological activity of monoglutamates; therefore, human milk is a source of folate with high availability [28]. The content of folate in breast milk increases with lactation time even if maternal reserves start to be depleted. Reported values show a wide range. In mature breast milk folate varies from 0.1 to 13 μg/dL. Supplementation with 5-methyltetrahydrofolate or folic acid does not affect concentrations of milk folate; it only increases the amount of unmetabolized folic acid in milk [31].

Niacin in human milk is known to be a component of nicotinamide, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), among others. Sakurai *et al*. [33] estimated the niacin concentration in human milk as the concentration of unbound nicotinamide, and found values (12.5–53.3 μg/dL) lower than the values reported by other authors for nicotinamide (51–309 μg/dL) [1, 28, 34]. The use of maternal supplements can raise low breast milk levels [1, 28].

The mean concentration of vitamin C (ascorbic acid) in breast milk from well-nourished mothers varies from 0.1 to 7 mg/dL [1, 33–35]. The levels of vitamin C do not change significantly with lactation time. Supplementation of vitamin C in malnourished women has been shown to increase levels in milk, but there is a regulatory mechanism limiting transfer to breast milk [1, 28].

Pantothenic acid is the functional component of coenzyme A. Variation with lactation time does not seem to be significant [1, 34]. The mean values reported in mature milk vary from 147 to 360 μg/dL [1, 33, 34].

Biotin is an essential cofactor for enzymes. No symptomatic biotin deficiencies have been reported in breast-feeding infants, but they have been reported in infants fed with an amino acid formula and hypoallergenic rice [36]; nevertheless, there are few studies of the biotin content in human milk. Biotin concentrations vary significantly during the different hours of the day, and increase 5- to 30-fold during the lactation period [1, 36]. Levels of biotin in cows' milk (2.8–4 μg/dL) are higher than those in human milk (0.5–1.1 μg/dL) [1, 29].

Fat-soluble vitamins

The fat-soluble vitamins in breast milk decline rapidly during early lactation. The mechanisms underlying this phenomenon are not well understood, but they might involve selective mechanisms related to mammary uptake or secretion into the milk.

Vitamin A is a generic term for a group of retinoids with similar biological activity, including retinol, retinal and retinoic acid. The diet provides retinoids, usually as retinyl esters and some carotenoids with provitamin A activity. Quantitatively, and in terms of provitamin A activity, the most important of these is β-carotene. The concentration of β-carotene in human milk is in the range 10–220 pg/dL, depending on the number of days postpartum and seems to reflect maternal dietary intake [28]. Vitamin A decreases with progression of lactation in most mammals investigated so far, while total lipids increase at the same time.

There are eight naturally occurring compounds with vitamin E activity, α-tocopherol being the most active isomer in breast milk. Vitamin E content is higher in colostrum (1.5 mg/dL) than in late milk (0.9 mg/dL transitional and 0.25 mg/dL mature milk) and is lower in cows' milk (0.07 mg/dL) [1]. The α-tocopherol content is higher in early milk than in mature milk, but the highest content of β- tocopherol and κ-tocopherol is found in mature milk [28].

Vitamin D is present in the aqueous and fat breast milk fractions, but the hidrosoluble fraction is constituted by inactive metabolites. The vitamin D levels in breast milk are dependent on diet and sunlight exposure, and are always higher in colostrum than in late milk [1]. Vitamin D is present in human milk mainly as ergocalciferol (about 0.3 μg/L), cholecalciferol (0.04 μg/L) and 25-hydroxyvitamin D (about 0.16 μg/L). The hydroxylated form offers the highest biological activity. Human milk contains only a low concentration of vitamin D, which decreases with lactation time [34].

Vitamin K [37] (generic term for a group of 2-methyl-1,4-naphthoquinone derivatives) in breast milk occurs mainly as phylloquinone (vitamin K_1). Human milk contains a low concentration of vitamin K. The levels in mature milk [28] can vary from 1 to 10 μg/L, lower than levels found in cows' milk (40 μg/L) [29].

33.1.4 Minerals

The minerals in milk can be classified into major or minor components as a function of their concentrations. The main major components in milk are K, Na, Cl, Ca, Mg and P. All these components in cows' milk are in higher concentrations than in breast milk [1, 29, 38]. The sum of the monovalent ion concentration (K, Na and Cl) is more or less inversely proportional to the lactose concentration [38]. The Na/K ratio is physiologically important, and the higher levels of K and lower levels of Na in breast milk make this product beneficial from a physiological point of view for the newborn. Na, K and Cl are present as free ions [39]. Cl was not considered important but its deficiency has been related to a syndrome described in babies being fed with formulas with low Cl content [1]. Bauer *et al.* [7] have shown that the Na and Cl concentrations in breast milk are lower in preterm mothers (31–33 weeks gestational age) than term mothers (about 40 weeks gestational age), whereas there was no significant difference in K concentrations.

The main polyvalent ions in milk are Ca, Mg, citrate, phosphate and sulfate. Studies on divalent ion distribution in milk [39] reported that Ca is present in the aqueous phase and associated with casein, whereas Mg was found mainly in the aqueous phase, but significant amounts were also associated with lipid phase and the membranous compartment [1]. The concentrations are higher in cows' milk than in human milk [38], except for sulfur. Many studies have been performed on Ca supplementation in pregnant and lactating mothers with the objective of increasing the content of Ca in breast milk. The results show that Ca in the diet is not correlated with Ca in breast milk, and does not prevent bone loss in the mother during the breast-feeding period, although it helps in the recovery of bones after weaning [40]. Nevertheless, Maru *et al.* [41] found significant differences for Ca in colostrum between two populations in Ethiopia with different diets. Citrate and phosphate are in the aqueous phase bound mainly to Ca and Mg ions [39].

The minor mineral components in milk can be classified [42] as essential (Fe, Cu, Cr, Zn, Mn, Mo, Ni, Co, Se, I, F) or toxic (Hg). Other elements are not definitively classified because they have not been proven to be essential for humans (As, B, Br, Cd, Li, Pb, Si, Sn, V) or their role in metabolism is not well understood. The interindividual variations in microelement concentrations in breast milk are high compared with those for the macroelements, except for Cu and Se. The mammary gland is capable of regulating concentrations of essential elements such as Cu, Fe and Zn in milk to protect the newborn infant against deficiency and excess of these elements [43].

Fe is one of the most studied trace elements not only because it is an oxygen carrier but also because it plays an important role in a number of physiological functions, including psychomotor development and behaviour in children and in immune functions. If we consider that

the infant in the first year of life needs 8–10 mg/day of exogenous Fe, the range of reported levels of Fe in human milk (10–168 μg/dL) [34, 44–50] might not be enough to reach that level. Nevertheless, there is no anaemia or other symptoms of Fe deficiency in breast-fed babies from healthy mothers [1]. There is no relationship between the mother's Fe status and Fe concentration in breast milk, or between the mother's diet [44] or supplementation and Fe content in breast milk [51]. Fe content in cows' milk varies between 6 and 70 μg/dL [1, 5, 29, 51–53]. Fe values are the highest in infant formula (290–630 μg/dL) [54] and depend on fortification levels.

Cu is a constituent of many metalloenzymes involved in the synthesis of connective tissue, melanin and catecholamine [51]. Its deficiency can cause anaemia, bone and connective tissue abnormalities, and impaired immune function [43]. Reported levels of Cu are between 3 and 98 μg/dL [34, 35, 45–47, 49, 50, 55–61] in breast milk, within the range 55–107 μg/dL [61] in infant formulas and between 0.4 and 36 μg/dL [29, 51–53, 62, 63] in cows' milk. Maternal intake of Cu does not seem to influence milk Cu concentrations [64].

Zinc is a cofactor for numerous enzymes playing a wide variety of biochemical roles *in vivo* and its deficiency or excess could affect children's development. No significant correlations between maternal dietary Zn intake and milk Zn concentrations have been found [44, 65]. Reported values of Zn in breast milk vary from 0.03 to 8.3 mg/dL [34, 44–47, 49, 50, 55, 57–60, 66], from 0.2 to 0.8 mg/mL in cows' milk [5, 29, 51–53, 63], and from 0.3 to 1 mg/mL in infant formulas [67].

Mn is an essential trace element known to activate many enzymes involved in metabolic processes (protein and fat metabolism), neurological and immune system processes as well as for sugar regulation [68]. While deficiency of Mn in humans seems not to be a major problem, Mn toxicity is known to occur in humans exposed to high environmental concentrations especially in the neonatal period [69]. Reported Mn values in breast milk in recent years vary from 2×10^{-4} to 3 μg/dL [45, 47, 50, 56, 59, 60, 68] in agreement with the values obtained previously by Lönnerdal *et al*. [70]. Kim *et al*. [71] report higher levels in preterm breast milk (10–12 μg/dL) and in cows' milk (0.7–5.5 μg/dL) [29, 53, 64, 72].

Molybdenum acts as a cofactor of several enzymes [51], such as xanthine oxidase, sulfite oxidase and aldehyde oxidase, and its role is strongly associated with the levels of Cu, Fe, and Zn [73]. The range of Mo levels reported in breast milk is 1×10^{-4} to 2.6 μg/dL [47, 56, 59, 74], and 0.3–6 μg/dL [29, 51, 52, 73] in cows' milk.

Regarding Co, it is varies from 1×10^{-4} to 0.2 μg/dL [47, 50, 56, 60] in breast milk, whereas Smith *et al*. [75] reported 0.04 μg/dL in cows' milk. Abdulrazzaq *et al*. [56] found no difference in Co values in the breast milk of women at different postpartum periods. Co available for humans is part of the active site of vitamin B_{12}. However no reports have distinguished between Co bound to vitamin B_{12} and free Co in milk [51].

Selenium is essential for humans. Deficiencies are associated with an increased risk of cancer, cardiomegaly, osteoarthropathy, encephalopathy, transient hypothyroidism, neural tube defects, and infections, and with reduced resistance to environmental pollutants. However, excess Se can be toxic. Initial symptoms of poisoning include alopecia, dystrophic fingernail changes, gastrointestinal symptoms and memory difficulties [76]. Se concentration in milk is directly affected by levels in the food chain; low selenium in soil results in a low dietary intake, increasing the risk of developing selenium deficiency. Neonates and children are at greater risk of inadequate intakes of Se because they have greater Se requirements due to their rapid rate of growth. Se concentrations of 0.02–6.1 μg/mL [34, 44, 47, 53, 56, 60, 77, 78] have been reported in breast milk from term mothers and 1–2.1 μg/mL from preterm mothers [44]. In cows' milk, the reported values vary from 0.7 to 4.8 μg/mL [52, 63, 77, 79–82].

Iodine is an essential trace element in small amounts for humans because it plays an important role in the synthesis of thyroid hormones. These hormones control cellular metabolism, growth, development of body structures, and neuronal function and development [83, 84]. In the past, iodine deficiency occurred frequently in regions where the soil had low iodine content. Public health policies to prevent iodine deficiency, such as salt iodination programmes, were developed successfully [85]. Iodine content in breast milk depends on maternal iodine intake. The reported values in recent years are 2–45 μg/dL [34, 44, 86, 87]. Higher levels can be found in cows' milk, but these can be related to the use of fortified silages and prophylactic uses to prevent some cow diseases [86].

Fluorine deficiency in humans has been well established. There is an inverse relationship between F intake and dental caries, but chronic exposure to F in large amounts interferes with bone formation and can

cause fluorosis [88]. F supplementation does not have a large influence on F transferred to milk [89]; nevertheless, direct correlation between F in drinking water and F content in breast milk has been found [90, 91]. Esala *et al*. [90] reported a mean value of F of 0.7 μg/mL in breast milk from mothers living in a place with low F content in drinking waters (0.2 μg/mL, Helsinki). The authors reported a mean value of 1.1 μg/dL in milk from mothers who drink waters with higher F content (1.7 μg/mL, Elimäki). Opinya *et al*. [91] found a mean value of 3.3 μg/dL of F in breast milk in an area with higher levels of F in drinking water (9 μg/mL, Kenya). More recent data have been reported in cows' milk [52, 92]. The mean values are higher than those found in breast milk but the range is very wide, from less than 1.3 to 29 μg/dL.

Al is neurotoxic for humans, but is not considered a significant toxic in healthy individuals. The skin, lungs and intestinal tract act as effective barriers to Al uptake and its absorption is very low [51]; nevertheless, in preterm infants with an immature gastrointestinal tract these barriers are not as effective and Al may accumulate in the tissues with severe consequences. Levels of Al in milk are correlated with dietary intake and environmental exposure, and thus the reported ranges in breast and cow milks vary widely: 0.01–493 μg/dL for breast milk [47, 50, 56] and 0.3–168 μg/dL for cows' milk [53, 93, 94].

Tradidionally, As is considered to be a toxic element. It is involved in the development of cancer, skin lesions, diabetes mellitus, chronic bronchitis, cardiovascular disease and peripheral neuropathy, as well as haematologic, liver, and kidney effects in humans [95]. Nevertheless, despite its potential toxicity, evidence is accumulating that arsenic may be an essential element for human physiology [96]. Arseniosis occurs in areas where high environmental levels occur naturally (volcanism) or with high industrial and mining contamination. The main As source for human intake is drinking water. There is some evidence for the relatively low transfer of ingested As to human [97–99] and cows' milk [100]. The reported values for As in breast milk vary from 1×10^{-4} to 0.8 μg/dL [47, 56, 60, 97, 99] and from less than 0.09 to 2.7 μg/dL [100, 101] for cows' milk.

Cadmium is classified as a human carcinogen by the International Agency for Research on Cancer. It is stored in the kidneys and the liver, and its presence decreases Ca absorption and increases the risk of osteoporosis [102]. There is a direct link between heavy metal atmospheric deposits and the distribution of Cd in the food chain [103]. Absorption from cigarette smoke (25–50%) [104] is higher than gastrointestinal absorption (5–8%) [102]; thus, the high content in some breast milk samples [46, 105, 106] can reflect atmospheric contamination and/or active or passive mother smokers. Cd content in breast milk is usually very low (0.004–0.1 μg/dL) [47, 50, 56, 59, 107, 108]. In cows' milk it is highly area dependent (<0.009–4.7 μg/dL) [51, 53, 93, 108]. Rivero *et al*. [108] reported that Cd in breast and cows' milk is associated with the fat fraction.

As in the case of Cd, lead is highly toxic and has effects on intellectual and behavioural development. Both elements are poorly absorbed by mammals and are concentrated in the bones; thus, Pb levels in milk are generally low: 0.007–1.9 μg/dL in breast milk [47, 56, 59, 60, 105–108] and 0.06–1.9 μg/dL in cows' milk [53, 108]. Therefore, milk is not considered an important source of exposure [51]. Some authors report higher levels in breast [46, 50] and cows' [93] milk in contaminated areas. Rivero *et al*. [108] found a different distribution of Pb, associated with the fat fraction in breast milk and with the casein fraction in cows' milk.

Hg is neurotoxic and its alkyl derivatives are the most toxic chemical forms. There are some reported cases of environmental contamination that have led to high Hg contents in breast milk, for example Minamata, Japan in 1977 [109] and Iraq in 1960 [110]. In general, the levels reported in recent years are low (0.001–0.6 μg/dL) [56, 106, 108, 111, 112]. There is very little information on normal levels in cows' milk but they are generally low (<0.4 μg/dL).

33.2 Mineral and trace element concentrations in human milk from different countries

This section aims to provide updated information on concentrations of mineral and trace elements in human milk from different countries. The study published by WHO and IAEA in 1989 [113] is the most commonly used source of background data for establishing the adequate daily intakes of infants for many elements. However the diet and the environmental and social conditions of women worldwide have changed greatly, and updated information is necessary in order to understand how these changes have affected mineral and trace element content in breast milk. The data presented in Table 33.1 correspond to mineral and trace elements in

Table 33.1 Mineral and trace elements in mature human milk from different countries.

Element	Concentration	Origin	Year	Reference
Ca (mg/dL)	26–35	Sweden	2012	[47]
	0.25–0.35	Egypt	2012	[55]
	18–25	Germany	2011	[7]
	26–40	China (Mongolia)	2011	[34]
	6.0–9.8	Nigeria	2011	[46]
	22–26	China	2010	[45]
	20–34	Brazil	2010	[50]
	16–30	Japan	2005	[115]
	27–31	Canada	1999	[114]
Cl (mg/dL)	35–42	Germany	2011	[7]
	68–110	China (Mongolia)	2011	[34]
	25–54	Japan	2005	[115]
K (mg/dL)	59–67	Sweden	2012	[47]
	38–52	Germany	2011	[7]
	36–65	China (Mongolia)	2011	[34]
	43–71	Brazil	2010	[50]
	33–54	Japan	2005	[115]
Mg (mg/dL)	2.3–3.3	Sweden	2012	[47]
	1.2–1.4	Niger	2012	[49]
	0.08–0.12	Egypt	2012	[55]
	2.7–3.9	Germany	2011	[7]
	2.7–4.7	China (Mongolia)	2011	[34]
	2.6–3.9	China	2010	[45]
	1.9–3.0	Brazil	2010	[50]
	1.6–3.5	Japan	2005	[115]
	2.5–3.1	Italy	2000	[59]
Na (mg/dL)	14–29	Sweden	2012	[47]
	21–31	Germany	2011	[7]
	8.2–19	China (Mongolia)	2011	[34]
	9.1–89	Brazil	2010	[50]
	4–18	Japan	2005	[115]
P (mg/dL)	15–20	Sweden	2012	[47]
	14–19	China (Mongolia)	2011	[34]
S (mg/dL)	14–18	Sweden	2012	[47]
Ag (μg/dL)	0.002–0.07	Sweden	2012	[47]
	$0–5 \times 10^{-3}$	United Arab Emirates	2008	[56]
As (μg/dL)	0.04–0.46	Sweden	2012	[47]
	1×10^{-4}–0.03	United Arab Emirates	2008	[56]
	0.5–0.7	Portugal	2008	[60]
	0.03–1.9	Bangladesh	2008	[99]
	0.08–0.8	Argentina	1998	[97]
Al (μg/dL)	2.1–439.3	Sweden	2012	[47]
	18.2–25.3	Brazil	2010	[50]
	0.01–2.4	United Arab Emirates	2008	[56]
B (μg/dL)	1.4–3.4	Sweden	2012	[47]
	5×10^{-4}–0.14	United Arab Emirates	2008	[56]

(Continued)

Table 33.1 (Continued)

Element	Concentration	Origin	Year	Reference
Ba (μg/dL)	1.0–1.4	Sweden	2012	[47]
	0.5–1.1	Brazil	2010	[50]
	6×10^{-4}–9×10^{-3}	United Arab Emirates	2008	[56]
	1.2–2.2	Italy	2000	[59]
Be (μg/dL)	$<5\times10^{-4}$–0.002	Sweden	2012	[47]
	1×10^{-4}–5×10^{-3}	United Arab Emirates	2008	[56]
Bi (μg/dL)	0–2×10^{-3}	United Arab Emirates	2008	[56]
	9.7×10^{-3}–10×10^{-3}	Italy	2000	[59]
Cd (μg/dL)	<0.01–0.1	China	2013	[107]
	0.004–0.01	Sweden	2012	[47]
	0.06–0.3	Egypt	2012	[105]
	0.09–0.3	Iran	2012	[106]
	76.1–119.5	Niger	2011	[46]
	<0.04	Brazil	2010	[50]
	0–0.01	United Arab Emirates	2008	[56]
	0.04–0.07	Spain	2001	[108]
	0.06–0.1	Italy	2000	[59]
Co (μg/dL)	0.002–0.04	Sweden	2012	[47]
	0.01–0.2	Brazil	2010	[50]
	1×10^{-4}–4×10^{-3}	United Arab Emirates	2008	[56]
	0.05–0.09	Portugal	2008	[60]
Cr (μg/dL)	0.003–0.16	Sweden	2012	[47]
	74–118	Niger	2011	[46]
	1.5–4.7	Brazil	2010	[50]
	0–0.25	United Arab Emirates	2008	[56]
	<0.01–1.9	Japan	2008	[74]
	2.2–13	Japan	2005	[115]
Cs (μg/dL)	0.1–1.1	Sweden	2012	[47]
Cu (μg/dL)	66–77	Niger	2013	[49]
	40–55	Sweden	2012	[47]
	5–15	Egypt	2012	[55]
	20–30	Turkey	2012	[58]
	17–57	China (Mongolia)	2011	[34]
	68–98	Niger	2011	[46]
	42–52	China	2010	[45]
	20–67	Brazil	2010	[50]
	25–47	Iran	2010	[57]
	0–0.8	Brazil	2009	[116]
	3.3–164	United Arab Emirates	2008	[56]
	36–64	Portugal	2008	[60]
	11–61	Japan	2005	[115]
	24–48	Spain	2001	[61]
	49–62	Italy	2000	[59]
F (μg/dL)	1.1–7.3	Kenya	1991	[91]
	0.2–5.1	Finland	1982	[90]

Table 33.1 (Continued)

Element	Concentration	Origin	Year	Reference
Fe (μg/dL)	65–78	Niger	2013	[49]
	21–47	Sweden	2012	[47]
	21–50	Spain	2012	[48]
	152–168	Egypt	2012	[55]
	30–70	China (Mongolia)	2011	[34]
	77–113	Niger	2011	[46]
	42–60	China	2010	[45]
	10–77	Brazil	2010	[50]
	10–70	Mexico	2009	[44]
	0.2–0.4	Brazil	2009	[116]
	0–195	Japan	2005	[115]
Hg (μg/dL)	0.001–0.6	Saudi Arabia	2013	[112]
	0.04–0.1	Iran	2012	[106]
	0.01–0.09	Iran	2012	[111]
	0–0.02	United Arab Emirates	2008	[56]
	<0.1–15	From mining areas	2008	[118]
	<0.4	Spain	2001	[108]
I (μg/dL)	11–45	Chinese (Mongolia)	2011	[34]
	3.4–5.1	Mexico	2009	[44]
	2.2	New Zealand	2005	[87]
	10.5–11.3	Spain	1999	[86]
La (μg/dL)	1×10^{-4}–1×10^{-3}	United Arab Emirates	2008	[56]
Li (μg/dL)	0.1–0.2	Sweden	2012	[47]
	0.4–0.5	Brazil	2010	[50]
	1×10^{-4}–5×10^{-3}	United Arab Emirates	2008	[56]
	0.06–0.1	Italy	2000	[59]
Mn (μg/dL)	59–69	Niger	2013	[49]
	0.2–0.4	Sweden	2012	[47]
	72–114	Niger	2011	[46]
	0.4–0.6	China	2010	[45]
	0.5–3.2	Brazil	2010	[50]
	2×10^{-4}–0.9	United Arab Emirates	2008	[56]
	0.3–0.7	Portugal	2008	[60]
	1.2–2.0	Italy	2000	[59]
Mo (μg/dL)	0.08–0.6	Sweden	2012	[47]
	1×10^{-4}–0.2	United Arab Emirates	2008	[56]
	<0.01–2.6	Japan	2008	[74]
	0.5–1.1	Italy	2000	[59]
Ni (μg/dL)	<0.009–4.7	Sweden	2012	[47]
	0.5–0.8	Brazil	2010	[50]
	0.02–1.2	United Arab Emirates	2008	[56]
	0.4–0.8	Portugal	2008	[60]

(Continued)

Table 33.1 (Continued)

Element	Concentration	Origin	Year	Reference
Pb (μg/dL)	0.2–0.6	China	2013	[107]
	0.06–0.2	Sweden	2012	[47]
	0.3–1.1	Iran	2012	[106]
	62–112	Niger	2011	[46]
	0.2–0.6	Egypt	2011	[105]
	0.8–8.8	Brazil	2010	[50]
	0–0.06	United Arab Emirates	2008	[56]
	0.007–0.2	Portugal	2008	[60]
	0.1–0.2	Spain	2001	[108]
	0.7–1.9	Italy	2000	[59]
Rb (μg/dL)	61–82	Sweden	2012	[47]
Sb (μg/dL)	0.001–0.007	Sweden	2012	[47]
	3×10^{-4}–0.09	United Arab Emirates	2008	[56]
Se (μg/dL)	62–68	Niger	2013	[49]
	1.0–1.6	Sweden	2012	[47]
	1.1–1.9	China (Mongolia)	2011	[34]
	71–113	Niger	2011	[46]
	1.0–2.1	Mexico	2009	[44]
	0.02–6.1	United Arab Emirates	2008	[56]
	2.4–4.0	Portugal	2008	[60]
	1.7–4.3	Greece	2005	[78]
	0.9–2.1	Japan	2005	[115]
	1.3–1.9	Kuwait	2001	[77]
Sn (μg/dL)	0.03–0.05	Sweden	2012	[47]
Sr (μg/dL)	2.1–4.5	Sweden	2012	[47]
	5.8–11	Italy	2000	[59]
Th (μg/dL)	0.001–0.003	Sweden	2012	[47]
Tl (μg/dL)	7×10^{-3}–0.01	Italy	2000	[59]
U (μg/dL)	0.002–0.08	Sweden	2012	[47]
V (μg/dL)	0.002–0.06	Sweden	2012	[47]
	1.3–17	Brazil	2010	[50]
Zn (mg/dL)	0.13–0.16	Niger	2013	[49]
	0.11–0.14	Uruguay	2013	[117]
	0.2–0.4	Sweden	2012	[47]
	7–11	Egypt	2012	[55]
	0.05–0.09	Turkey	2012	[58]
	0.1–0.3	China (Mongolia)	2011	[34]
	5.7–8.3	Niger	2011	[46]
	0.2–0.3	China	2010	[45]
	0.07–0.5	Brazil	2010	[50]
	0.2–0.4	Iran	2010	[57]
	0.03–0.4	Mexico	2009	[44]
	0.03–6.3	United Arab Emirates	2009	[56]
	0.002–0.004	Brazil	2009	[116]
	0.2–0.4	Portugal	2008	[60]
	0.08–0.4	USA	2007	[65]
	0–0.1	Japan	2005	[115]
	0.03–0.7	Spain	2001	[66]
	0.26–0.36	Italy	2000	[59]

mature breast milk. The comparison and interpretation of data published must be done carefully because there is no homogeneity of sampling and analysis methods.

In mature breast milk in general the Ca levels reported in recent years have been between 16 and 35 mg/dL [7, 45, 47, 50, 114, 115]. The lowest values were reported for Nigerian mothers (6.0–9.8 mg/dL) [46]; similar levels were found in German preterm breast milk [7], and the highest values were from Chinese (Mongolia) mothers (26–40 mg/dL) [34]. For the same Chinese population, Cl levels in breast milk (68–110 mg/dL) [34] are higher than for Japanese mothers (25–54 mg/dL) [115] and for term (35–42 mg/dL) and preterm (10–16 mg/dL) German mothers [7]. K in mature breast milk in the latest data reported varies from 33 to 71 mg/dL [7, 34, 47, 50, 115], and for Mg from 1.6 to 5 mg/dL [7, 34, 45, 50, 59, 115], except for Egyptian mothers whose Mg levels were between 0.08 and 0.12 mg/L [55]. The level of Na in mature breast milk varies significantly between and within different countries, with values from 4 to 89 mg/dL [7, 34, 47, 50, 115]. In a recent study performed in Sweden by Björklund *et al*. [47], the comparatively higher levels in breast milk shown possibly result from an increased salt intake over time. The reported values for P are similar (10–20 mg/dL) from such diverse populations as the Swedish [47], Japanese [115] and Chinese [34].

There are no significative differences among As, Be, Co, F, Ni and Sb levels in mature human milk from different countries. For Cd, Cr and Pb, the reported values for Nigerian breast milk [46] are significantly higher than breast milk from other countries. Japanese and Brazilian breast milk levels of Cr, reported by Yamawaki *et al*. [115] and Nascimento *et al*. [50], respectively, are higher than Swedish [47], Japanese [74] and United Arab Emirates levels [56]. The high correlation of Al with dietary intake and environmental exposure is reflected in the differences found among the different countries.

Cu levels in breast milk from different countries reported in recent years are between 11 and 98 μg/dL [34, 45–47, 49, 60, 115]. Lower levels were found in Egypt [55] and Brazil [116]. In the United Arab Emirates a wider range of Cu was found (3.3–164.4 μg/dL) by Abdulrazzaq *et al*. [56].

The range of Fe in mature breast milk in different countries is 20–78 μg/dL [34, 44, 45, 47–50], with some higher values such as those of Niger (76–113 μg/dL) [46] and Egypt (152–168 μg/dL) [55]. Brazil stands out for its lower values (0.2–0.4 μg/dL) [116]. Yamawaki *et al*. [115] reported a wide range of Fe, between 0 and 195 μg/dL for Japanese mature breast milk. Zn has a similar behaviour to Fe, with levels in most countries between 0.03 and 0.4 mg/dL [34, 44, 45, 47, 49, 50, 57–60, 66, 115, 117], and with higher levels for Niger (5.7–8.3 mg/dL) [46] and Egypt (7–11 mg/dL) [55]. For the United Arab Emirates, the Zn levels reported in mature breast milk vary widely (0.03–6.3 mg/dL) [56].

Hg in human milk is in very low levels in all the data reported for different countries, except in mining areas [118].

Iodine concentrations in human milk are highly dependent on maternal intake [85, 119]. Levels of I in mature breast milk have been reported in the range 10–45 μg/dL [34, 85, 86], but values lower than 5 μg/dL have been reported in countries like Mexico [44] and New Zealand [87].

As in the case of iodine, Se levels vary according to the mother's diet and even within the same country depending on Se in soils. The lowest values of Se reported recently for human mature milk were from Kuwaiti [77], Japanese [115], Mexican [44], Chinese [34] and Swedish [47] mothers (0.9–2.1 μg/dL), slightly higher from Greek [78] and Portuguese [60] mothers (1.7–4.3 μg/dL) and the highest from Nigerian mothers (62–113 μg/dL) [46, 49].

The highest values of Mn in breast milk were found, as in the case of Se, from Nigerian mothers (59–144 μg/dL) [46, 49]. The other levels reported are between 2×10^{-4} and 2 μg/dL [45–47, 59, 60].

All the data reported for Li (1×10^{-4} to 0.5 μg/dL) [46, 47, 50, 59] and Mo (1×10^{-4} to 2.6 μg/dL) [46, 47, 59, 74] in mature breast milk show low levels for these elements.

33.3 Changes in trace element concentrations during lactation

The mammary gland is able to regulate the volume and composition of milk to the newborn's needs, so that trace element concentrations can vary during the lactation period (colostrum, transitional and mature milk). The data published in recent years are shown in Table 33.2. In general, Cu [34, 50, 60, 71, 115], Zn [34, 50, 60, 71, 115] and Ni [50, 60] decrease in a consistent pattern throughout lactation, but not all the results for Fe [34, 50, 71, 115] are so consistent. There is no evidence reported to assess variation during lactation for

Table 33.2 Trace element concentrations during the lactation period (colostrum, transitional and mature milk).

Element	Colostrum	Transitional	Mature	Origin	Reference
Ca (mg/dL)	25–32	24–32	26–40	China (Mongolia) (2011)	[34]
	21–34	—	20–34	Brazil (2010)	[50]
	22–37	26–35	16–30	Japan (2005)	[115]
	26–30	25–29	27–31	Canada (1999)	[114]
Cl (mg/dL)	76–92	68–111	68–110	China (Mongolia) (2011)	[34]
	22–47	11–65	25–54	Japan (2005)	[115]
K (mg/dL)	35–57	32–60	36–65	China (Mongolia) (2011)	[34]
	60–78	—	43–71	Brazil (2010)	[50]
	60–85	54–74	33–54	Japan (2005)	[115]
Mg (mg/dL)	2.9–4.4	2.8–4.4	2.7–4.7	China (Mongolia) (2011)	[34]
	1.9–2.7	—	1.9–3	Brazil (2010)	[50]
	2.7–3.7	2.3–3.5	1.6–3.5	Japan (2005)	[115]
Na (mg/dL)	7.7–14	7.6–25	8.2–19	China (Mongolia) (2011)	[34]
	29–92	—	9.1–89	Brazil (2010)	[50]
	16–50	14–34	4–18	Japan (2005)	[115]
P (mg/dL)	12–16	13–18	14–19	China (Mongolia) (2011)	[34]
	12–20	15–21	10–18	Japan (2005)	[115]
As (μg/dL)	0.6–1	—	0.5–0.7	Portugal (2008)	[60]
Al (μg/dL)	8.1–24	—	18–25	Brazil (2010)	[50]
Ba (μg/dL)	0.03–2.9	—	0.5–1.1	Brazil (2010)	[50]
Cd (μg/dL)	<0.04	—	<0.04	Brazil (2010)	[50]
Co (μg/dL)	0.06–0.2	—	0.01–0.2	Brazil (2010)	[50]
	0.03–0.1	—	0.05–0.09	Portugal (2008)	[60]
Cr (μg/dL)	2.8–5.9	—	1.5–4.7	Brazil (2010)	[50]
	0.7–2.7	0–9.8	2.2–13	Japan (2005)	[115]
Cu (μg/dL)	27–74*	146–152*	25–35*	Korea (2012)	[71]
	0–144	11–107	17–57	China (Mongolia) (2011)	[34]
	30–88	—	20–67	Brazil (2010)	[50]
	28–124	—	36–64	Portugal (2008)	[60]
	22–52	36–56	11–61	Japan (2005)	[115]
	55–63*	35–41*	23–31*	USA (1983)	[35]
Fe (μg/dL)	42–102*	30–66*	33–65*	Spain (2012)	[48]
	36–79	22–60	21–50	Spain (2012)	[48]
	30–70	30–70	30–70	China (Mongolia) (2011)	[34]
	10–74	—	10–77	Brazil (2010)	[50]
	56–164	53–99	0–195	Japan (2005)	[115]
I (μg/dL)	7.8–36	5.5–61	11–45	China (Mongolia) (2011)	[34]
Li (μg/dL)	0.2–0.7	—	0.4–0.5	Brazil (2010)	[50]
Mn (μg/dL)	13–14*	12–13*	9.9–12*	Korea (2012)	[71]
	0.6–5.3	—	0.5–3.2	Brazil (2010)	[50]
	0.3–1.3	—	3.1–6.7	Portugal (2008)	[60]
	0.4–2.0	0–9.1	0.1–2.3	Japan (2005)	[115]
Ni (μg/dL)	0.7–7.1	—	0.5–0.8	Brazil	[50]
	0.03–1.6	—	0.4–0.8	Portugal (2008)	[102]
Pb (μg/dL)	1–13	—	0.8–8.8	Brazil (2010)	[50]
	0.02–0.3	—	0.007–0.2	Portugal (2008)	[60]
Se (μg/dL)	0.9–1.9	1.5–2.3	1.1–1.9	China (Mongolia) (2011)	[34]
	1.1–1.2*	1.1–1.2*	0.9–1.2*	Korea (2012)	[71]
	3.6–10.9	—	2.4–4.0	Portugal (2008)	[60]
	1.8–3.2	1.9–3.5	0.9–2.1	Japan (2005)	[115]

Table 33.2 (Continued)

Element	Colostrum	Transitional	Mature	Origin	Reference
V (μg/dL)	2.5–19	—	1.3–17	Brazil (2010)	[50]
Zn (mg/dL)	0.7–0.8*	0.8–1.0*	0.5–0.8*	Korea (2012)	[71]
	0.1–0.5	0.1–0.5	0.1–0.3	China (Mongolia) (2011)	[34]
	0.3–0.8	—	0.07–0.5	Brazil (2010)	[50]
	0.7–1.7	—	0.2–0.4	Portugal (2008)	[60]
	0.2–0.7	0.2–0.4	0–0.1	Japan (2005)	[115]
	0.4–0.6*	0.1–0.4*	0.1–0.2*	USA (1983)	[35]

*Preterm.

Ca [34, 50, 114, 115], Cl [34, 115], K [34, 50, 115], Mg [34, 50, 115], Na [34, 50, 115], P [34, 115], As [60], Al [50], Ba [50], Cd [50], Co [50, 56, 60], I [34, 119], Li [50], Pb [50, 60], Se [34, 60, 71, 115] and V [50]. The results reported for Mn [50, 60, 71, 115] and Cr [50, 115] are contradictory.

33.4 Problems in the determination of trace elements in human milk

Nowadays the determination of trace elements at very low levels in biological samples is not a problem, but there are other issues to take into account if reliable results are to be obtained. Such analyses, despite the important advances in analytical techniques for trace and ultratrace elements, are still subject to significant errors due to the high risk of contamination in the process of sample preparation and analysis, and the problem of the lack of suitable reference materials to assess the accuracy of the results obtained.

In addition to these possible sources of error, there are some important problems to solve in studies of breast milk before making comparisons of data from different sources. Because of the high variation of breast milk composition collected from the left or the right breast, foremilk or hindmilk, diurnal and seasonal variation, lactation stage, dietary habits and environmental conditions, it is often not possible to perform comparisons of breast milk trace element concentrations across studies. Many excellent studies have been made from the standpoint of analytical analysis, but the use of pooled samples, small populations, different methods of sample collection or no well-defined study population often resulted in data that could not be used to make comparisons and establish a pattern.

33.5 Speciation of trace elements in human milk

The importance of trace elements was acknowledged at the beginning of the twentieth century. Today, it is possible to determine trace elements in foods at very low levels with good precision and accuracy. Nevertheless, in the last two decades scientists have demonstrated that this information is not sufficient. In order to have information about mobility, bioavailability, storage, retention and toxicity of a trace element in a living system, knowledge of the different chemical species of this element present in food is necessary. The International Union of Pure and Applied Chemistry (IUPAC) has defined speciation as 'the specific form of a chemical element defined according to its molecular, complex, electronic or nuclear structure' [120]. Speciation analysis can be considered as the identification and quantification of the different chemical and physical forms of an element in a sample. In the nutrition field it is of great importance to distinguish the different chemical species of a trace element present in foods. In infant nutrition, human milk is the first diet and it is believed that this food covers all nutritional needs of the newborn since it contains trace elements, proteins, enzymes, etc. [121].

To perform speciation analysis, the hyphenation of a powerful separation technique with an element- or molecule-selective detection system is necessary. The

analytical methodologies available for performing speciation analysis are described in Chapter 18. The study of trace element speciation in human milk was developed in the early years of the speciation era, and was probably an important contribution to this field. The state of art of the speciation of the most significant elements follows.

Iodine speciation studies in milk have been limited due to the lack of suitable analytical methodology. The first studies on iodine speciation in human milk were performed by Brätter *et al*. [122], using size-exclusion chromatography (SEC) coupled to inductively coupled plasma mass spectrometry (ICP-MS). They found that 80% of iodine in human milk was present as iodide. Moreover, they observed six different peaks in the molecular range 5–300 kDa. Michalke *et al*. [123] investigated iodine speciation in breast milk using SEC-ICP-MS and a strong anion-exchange SAX-ICP-MS. The SEC fractionation showed iodide as the predominant specie and two more iodine species with retention times pointing to the hormones thyroxine (T_4) and triiodothyronine (T_3). The SAX-ICP-MS showed that iodide is the major species and confirmed the presence of T_4. Fernández-Sánchez and Szpunar [86] compared the iodine species present in different types of milk using SEC-ICP-MS, and found that iodide seems to be the major, and practically the only, species of iodine in cows' and goats' milk, whereas a more complex speciation with at least two different species occurs in human milk and infant formulas. Later, Fernández-Sánchez [124] using ESI-MS/MS to identify the iodine species separated by SEC found iodide, T_3, T_4 and monoiodotyrosine (MIT) in human milk samples.

Manganese levels in human milk are very low (<10 µg/L) and there are few studies on its speciation in human milk. One of the first studies was performed in 1985 by Lönnerdal *et al*. [70]. In this study after the separation of the milk components into whey, fat and caseins by ultracentrifugation, the authors found that the major part of the Mn is in the whey fraction (71%), with the remainder in the casein fraction (11%) and the lipid fraction (18%). This distribution is very different in cows' milk where the higher concentration (67%) is in the casein fraction. In the human milk whey fraction separated by SEC the authors found that the principal Mn species was bound to lactoferrin, while in cows' whey Mn was mainly forming complexes with molecular masses less than 200 kDa. In 1998, Brätter *et al*. [125] using SEC fractionation in human milk confirmed the peak corresponding to Mn–lactoferrin, and another peak that presents the same retention time as Zn–citrate. In 2000, Bocca *et al*. [126] published a multi-element speciation study including data about Mn speciation using SEC fractionation. They found 28% of Mn attributed to the void volume (>2000 kDa) and 30% of Mn corresponding to the 'non-proteic' fraction that contains low-molecular-weight (LMW) compounds. In 2004, Michalke and Schramel [68] performed a study using a prefractionation via ultracentrifugation and obtained three fractions: fat, protein and an LMW supernatant fraction. Later, using SEC-ICP-MS and SAX-ICP-MS, they found that Mn was predominantly LMW bound (90%), and the rest distributed among bigger molecules. Using the standard addition experiments on the LMW fraction, they pointed to Mn–citrate as the most important Mn species in breast milk. Inorganic Mn was observed in low amounts. These results are in agreement with those of Brätter *et al*. since both found a predominant species at the citrate retention time and low concentrations of Mn corresponding to the 78kDa fraction.

Not many groups have worked on selenium speciation in human milk. The first study was from the Brätter group [127]. Using an SEC column and on-line digestion followed by HG-ICP-AES detection, the authors found that Se was associated with LMW compounds. Later, they repeated the study using human milk samples from the seleniferous region of Venezuela [128] using SEC-ICP-MS detection in parallel with Se quantification by instrumental neutron activation analysis (INAA) for quality control. In this case, Se was predominantly associated with high-molecular-weight (HMW) compounds. In another study performed in human milk samples from different countries (Portugal, Germany and Venezuela) [129], the authors found variations in the elution pattern among the samples, and this was related to differences in Se intake. The Michalke group [130–132] have published various papers using pooled human milk samples from days 7–14 after delivery. The defatted milk samples were separated by SEC. Fractions were characterized by capillary zone electrophoresis (CZE) and by capillary isoelectric focusing. Se was determined using electrothermal vaporization (ETV)-ICP-MS. Results show that Se was present in the LMW compounds, such as Se-carrying glutathione (GSeH), Se-cystamine (SeCM), Se-cystine (SeC) and Se-methionine (SeM). These results

are in accordance with the results of Brätter *et al*. [127] published in 1988, but not with the results published in 1995 [129]. This can be explained because the Michalke group used samples from Se-depleted Germany, while Brätter in 1995 and partly in 1998 analysed samples from the seleniferous areas of Venezuela. Later, Michalke [133] studied Se speciation in human milk samples using capillary electrophoresis (CE) coupled to ICP-MS. This confirmed the previous results and showed that the Se species in human milk changes at different lactation stages. On the other hand, in a study on Se in the milk of Kuwaiti and non-Kuwaiti lactanting mothers [77], a decrease in the activity of glutathione peroxidase with lactaction period was observed, and these authors showed that the major chemical form of Se was selenomethionine. More recently, De la Flor St Remy *et al*. [134], using SEC-ICP-MS, studied multi-element distribution patters in premature human milk and found four Se peaks at molecular masses of 350, 64, 19 and <10 kDa. In conclusion, Se speciation in samples from regions with Se deficiency shows that Se is associated with LMW compounds (GSeH, SeCM, SeC and SeM). In samples from Se-rich regions various peaks were observed, one of which was assigned to glutathione peroxidase.

Zinc is an essential trace element, especially for newborns because it is involved with protein synthesis, cell proliferation and enzymatic regulation. Its levels in breast milk are lower than levels present in infant formula. Nevertheless, the first problems of Zn deficiency have been observed in non-breast-fed newborns [135, 136]. This behaviour can be due to differences in Zn speciation in infant formulas and breast milk. The first studies performed by Lönnerdal *et al*. [135] isolated LMW Zn-binding ligands from human milk using SEC separation, and a second dimension by ion-exchange chromatography to identify the Zn ligands. The SEC separation produced two minor Zn peaks with molecular mass above 70,000 Da and a bigger peak with molecular mass 600–650 Da; this peak was identified as citrate. This species, Zn–citrate, probably enhances the availability of Zn in newborns. On the other hand, Zn in cows' milk normally presents levels 10 times higher, but it is bound to casein and therefore not available for infants. Although the use of SEC to perform the separation of Zn ligands has been critized by some authors [137] due to the possibility of cation saturation of charged groups of the stationary phase, citrate was always the predominant Zn ligand in breast milk. Using SEC, other Zn ligands found were albumin and lactoferrin [127, 135]. Michalke *et al*. [138, 139] studied Zn speciation using a simple process of centrifugation for fat separation and generation of LMW and HMW fractions. Both fractions were separated by SEC using the respective columns, and the Zn ligands were identified according to their retention times. Later, they were confirmed using a second dimension isoelectric focusing of the collected fractions and using a citrate-specific determination kit. The Zn species found were lactoferrin, casein, albumin, metallothionein and citrate. Brätter *et al*. [125] and Negretti de Brätter *et al*. [128] studied Zn speciation in samples from Venezuela using SEC separation combined with INAA or ICP-AES detection. They found that the principal species was Zn–citrate. Moreover, three further Zn peaks were observed in non-resolved peaks that were assigned to casein and albumin. Coni *et al*. [140] in a multi-element speciation study used SEC separation and performed the off-line metal determination in the five fractions using ICP-MS as a detector. They observed the presence of Zn in all the fractions, including HMW fractions (casein, lactoferrin, inmunglobulins and albumin) and LMW fractions (free amino acids, citrate, etc.). In a study of several polluted regions in Italy, Bocca *et al*. [126] found an elution pattern with five protein fractions assigned to casein aggregates, inmunoglobulins, albumin, α-lactalbumin and citrates. Similar results were observed by Bermejo *et al*. [66] in a comparative study of infant formulas and breast milk. In this study the most significant result was the presence of Zn in most breast milk fractions, whereas in infant formulas Zn was only detected in fractions of molecular mass below 5000 Da. This difference was also observed by Rivero Martino *et al*. [141]. Recently in 2012, Milacic *et al*. [142] developed a new method using a weak anion-exchange convective interaction media (CIM) diethylaminoethyl (DEAD) monolithic chromatography column for species separation and flame atomic absorption spectroscopy (FAAS) and ICP-MS for Zn detection. Quantitative separation of Zn–citrate complexes with good recoveries and reproducibility was obtained. The chromatographic peaks of Zn-binding ligands were identified by ESI-MS/MS. Results obtained in this study demonstrated that 23% of total Zn was present in the LMW milk fraction and that the principal Zn species is Zn–citrate. In conclusion, all investigations found Zn–citrate to be the predominant Zn species in breast milk, and this can explain the high bioavalability of Zn in breast milk.

The first studies on iron speciation were performed by Fransson and Lönnerdal [143] using ultrafiltration and SEC. They found that a considerable fraction of Fe was bound to LMW compounds in breast milk. Brätter *et al*. [127] found Fe in an HMW fraction (>600 kDa) and in the LMW fraction (9–10 kDa) that eluted with citrate. Suzuki *et al*. [12] in a study on daily changes in components of breast milk according to the number of lactation days, observed Fe in two peaks corresponding to transferrin and citrate according to retention times. Negretti de Brätter *et al*. [128] studied a wide range of proteins and obtained a complicated chromatographic pattern, concluding that columns with a separation range below 250 kDa should be used. The chromatographic pattern was similar to that obtained by Coni *et al*. [140]; they found homogeneity of Fe distribution in protein fractions of infant formulas, whereas for breast milk the Fe concentration was larger in fractions with molecular masses between 10 and 100 kDa. Later, in a study on the binding pattern for different elements in human milk using SEC-ICP-OES [126], the same group obtained five fractions corresponding to casein aggregates, inmunoglobulins, human serum albumin and lactoferrin, α-lactalbumin and a last fraction corresponding to LMW non-proteic compounds. Fe appeared homogeneously spread over all these fractions. Bermejo *et al*. [54] using SEC and electrothermal atomic absorption spectrometry (ETAAS) observed that Fe was present in all the fractions separated by SEC, but fractions corresponding to 3 and 76 kDa present the higher Fe concentrations. Recently, Fernández-Sanchéz *et al*. [144] found that Fe is mainly eluted in the region of IgA for colostrums and transitional milk (>160, 180 kDa), but for mature milk it was eluted mainly in the region of IgE (230 kDa) and IgG (160 kDa). Moreover, a small peak bigger than 60 kDa was observed in colostrums and this peak can be assigned to IgM.

33.6 Bioavailability of trace elements

One further area of knowledge that will complement our understanding of the nutritional role of trace elements in food is the study of the bioavailable fraction of these elements for humans. The bioavailability concept was defined previously in Chapters 3 and 8. Bioavailability involves the release of the element, the chemical process that takes place in the gastrointestinal tract (bioaccessibility), and absorption into the circulatory system [145]. The bioavailability of an element in food depends on gastrointestinal conditions [146], on the chemical form of the element [147–151], as well as on the presence of other components of the diet that can improve or inhibit the absorption process [52, 149, 150, 152]. The whole bioavailability process involves digestion, absorption, transport, utilization and elimination. Therefore, bioavailability studies are unable to include all these aspects, especially the absorption and utilization of the elements, stages that are highly dependent on the physiological conditions of the individual, such as trace element status, age or presence of disease. Estimating the bioavailability of compounds in food can be performed by *in vivo* methods (dosing experimental humans or animals with several concentrations of the target compound) or *in vitro* methods. *In vivo* methods, including those using stable isotopes in humans [153–158], provide the best estimates of bioavailability and are preferred for measuring absorption because using radioisotopes is considered unethical in infants. Because of ethical and practical issues (high cost, time-consuming and large interindividual variations), *in vitro* methods are preferred in initial studies of bioavailability. These are based on the simulation of gastric and intestinal digestion conditions and measurement of the concentration of the trace element after this treatment.

In vitro methods involve conditions (temperature, agitation, pH, and enzyme and chemical composition) similar to those found in the human body during digestion. The simplest approach for *in vitro* methods used to estimate the bioavailability of trace elements is the study of the bioaccessible fraction (see Chapter 8, section 8.4). Lönnerdal and Glazier [159], Bermejo *et al*. [160] and Peña *et al*. [161] used neonatal gastrointestinal conditions to study the bioaccessibility of essential trace elements in human milk, cows' milk and infant formulas. In human milk, the reported solubility values, under neonatal gastrointestinal conditions, varied from 23 to 34% for Fe [160], from 35 to 67% for Zn [160] and from 45 to 78% for Cu [161].

Another approach for bioavailability studies is the use of semi-permeable membranes to simulate the diffusion process during the intestinal stage. With this approach it is possible to estimate the bioavailable fraction (dialysable), the element fraction that can cross the intestinal wall. Dialysability of trace elements in cows' milk was

studied by Pérez *et al*. [152], Muñiz *et al*. [148] and Argyri *et al*. [145], while in infant formulas it was studied by Sarriá and Vaquero [162], and Domínguez *et al*. [147]. Bosscher *et al*. [163] studied the bioavailability of Fe, Zn and Ca in human milk and found dialysability percentages of 7.8–8.4% for Fe, 12–14% for Zn and 12–14% for Ca. A continuous dialysis method was used by Bosscher *et al*. [146] to simulate first-year infant gastrointestinal conditions for Fe, Ca and Zn availability from infant formulas and human milk. Another continuous flow dialysis system was used by Judprasong *et al*. [164] to estimate Ca, Mg, P, Fe and Zn bioavailability in infant formulas.

The *in vitro* methods have been improved by incorporating a cell culture model to simulate the transport and nutrient retention process that occurs in the small intestine. Caco-2 cell monolayers (human colon carcinoma cell line) have been used to simulate morphological properties of mature human enterocytes in bioavailability studies. Glahn *et al*. [165] and Jovaní *et al*. [166] used this method to study Fe bioavailability in infant formulas and Pullakhandam *et al*. [167] in human milk. Pullakhandam *et al*. [167] demonstrated for the first time ferric reductase activity in LMW human milk fractions and also provided evidence for its association with enhanced ferric iron solubility and uptake in Caco-2 cells. Kibangou *et al*. [168] found consistent data that justify some of the differences in Fe bioavailability between breast and cows' milk. They found that β-casein (present in breast milk) increased Fe absorption; the results obtained with Caco-2 cells were consistent with *in vivo* (rat) studies. Finally, Etcheverry *et al*. [169] use a Caco-2 cell culture model to study bioavailability of Ca, Zn and Fe in commercial human milk and found maximum values of 88, 33 and 63% respectively.

33.7 Conclusions

According to WHO/UNICEF, breast-feeding is an unequalled way to provide an ideal food for the healthy growth and development of infants, and human milk is recommended as the sole source of infant nutrition in the first 6 months of life. However, less than 35% of the world's infants are exclusively breast-fed at this age [113]. This is why it is so important to have human milk studies that provide reliable background data for setting adequate daily intakes. A major cause of the diversity in data on trace elements in human milk is the non-harmonized approach for assessing reference values [170]. To compare data from different sources and to establish reference values it is necessary to follow well-defined sampling and analysis procedures and to perform analytical quality assurance of obtained data. Thus, the report published jointly by the WHO and IAEA in 1989 [113] is still largely used as background data. It is therefore necessary to perform studies with large populations, using harmonized sampling and analytical methods.

Bioavailability studies are necessary to establish the nutritional or toxic reference values of trace elements in human milk. *In vitro* methods are useful tools for estimating nutrient bioavailability prior to developing expensive and time-consuming *in vivo* studies. These methods allow us to plan and develop bioavailability and/or bioaccessibility studies that include different physiological conditions, inhibitors and enhancers of absorption, and interactions among metal absorption.

Elemental speciation of trace elements in human milk is of great importance in order to understand the bioavailability of these elements for the newborn. Moreover, this knowledge is necessary in the preparation of infant formulas with similar trace element species. Until now, most speciation studies have been performed with SEC as element detector, but in order to advance the identification of species the use of multidimensional separations and molecular mass spectrometry techniques are necessary. On the other hand, certain studies of quality control have shown that problems during sampling, storage and sample preparation can change the original species present in the sample, and thus more studies on species stability are also recommended.

Abbreviations

BSSL: Bile salt-stimulated lipase
CE: Capillary electrophoresis
CIM: Convective interaction media
CZE: Capillary zone electrophoresis
DEAE: diethylaminoethyl
ESI-MS/MS: Electrospray ionization tandem mass spectrometry
ETAAS: Electrothermal atomic absorption spectrometry
ETV: Electrothermal vaporization
FAAS: Flame atomic absorption spectroscopy
GSeH: Se-carrying glutathione

HAMLET: Human α-lactalbumin made lethal to tumour cells
HMW: High molecular weight
HG-ICP-AES: Hydride generation inductively coupled plasma atomic emission spectroscopy
IAEA: International Atomic Energy Agency
ICP-MS: Inductively coupled plasma mass spectrometry
INAA: Instrumental neutron activation analysis
IUPAC: International Union of Pure and Applied Chemistry
LMW: Low molecular weight
MIT: Monoiodotyrosine
NAD: Nicotinamide adenine dinucleotide
NADP: Nicotinamide adenine dinucleotide phosphate
SAX: Strong anion exchange
SAX-ICP-MS: Strong anion exchange coupled to ICP-MS
SEC: Size-exclusion chromatography
SEC-ICP-MS: Size-exclusion chromatography coupled to ICP-MS
SeCM: Selenocystamine
SeC: Selenocystine
SeM: Selenomethionine
WHO: World Health Organization

References

1 Lawrence RA, Lawrence RM. *Breastfeeding: A Guide for the Medical Profession*, 6th edn. Elsevier, Madrid, 2007.
2 Lönnerdal B, Forsum E, Hambraeus L. A longitudinal study of the protein, nitrogen, and lactose contents of human milk from Swedish well-nourished mothers. *Am J Clin Nutr* 1976;**29**:1127–1133.
3 Neville MC, Morton J, Umenura S. Lactogenesis: the transition from pregnancy to lactogenesis. *Pediatr Clin North Am* 2001;**48**:35–52.
4 Zou X, Guo Z, Huang J *et al*. Human milk fat globules from different stages of lactation: a lipid composition analysis and microstructure characterization. *J Agric Food Chem* 2012;**60**: 7158–7167.
5 Bermejo P, Domínguez R, Bermejo A. Direct determination of Fe and Zn in different components of cow milk by FAAS with a high performance nebulizer. *Talanta* 1997;**45**:325–330.
6 Moltó-Puigmartí C, Castellote AI, Carbonell-Estrany X, López-Sabater MC. Differences in fat content and fatty acid proportions among colostrum, transitional, and mature milk from women delivering very preterm, preterm, and term infants. *Clin Nutr* 2011;**30**:116–123.
7 Bauer J, Gerss J. Longitudinal analysis of macronutrients and minerals in human milk produced by mothers of preterm infants. *Clin Nutr* 2011;**30**:215–220.
8 Sala-Vila A, Castellote AI, Rodriguez-Palmero M. Lipid composition in human breast milk from Granada (Spain): changes during lactation. *Nutrition* 2005;**21**:467–473.
9 Charpak N, Ruiz JG. Breast milk composition in a cohort of pre-term infants' mothers followed in an ambulatory programme in Colombia. *Acta Paediatr* 2007;**96**:1755–1759.
10 Roya K, Bagher H, Ramin I *et al*. A study on lipid content and fatty acid of breast milk and its association with mother's diet composition. *Journal of Research in Medical Sciences: the Official Journal of Isfahan University of Medical Sciences* 2012;**17**:824–827.
11 Lammi-Keefe CJ, Jesen RG. Lipids in human milk: a review. *J Pediatr Gastroenterol Nutr* 1984;**3**:172–198.
12 Suzuki KT, Tamagawa H, Hirano S, Kobayashi E, Takahashi K. Changes in element concentration and distribution in breast-milk fractions of a healthy lactating mother. *Biol Trace Elem Res* 1991;**28**:109–121.
13 Jensen RG, Ferris AM, Lammi-Keefe CJ. Lipids in human milk and infant formulas. *Annu Rev Nutr* 1992;**12**:417–441.
14 Jensen RG, Bitman J, Carlson SE, Couch SC, Hamosh M, Newburg DS. Human milk lipids. In: Jensen RG (ed.) *Handbook of Milk Composition*. Academic Press, San Diego, CA, 1995, pp. 495–541.
15 Davidson LA, Lönnerdal B. Persistence of human milk proteins in the breast-fed infant. *Acta Paediatr Scand* 1987;**76**: 733–740.
16 Suzuki YA, Shin K, Lönnerdal B. Molecular cloning and functional expression of a human intestinal lactoferrin receptor. *Biochemistry* 2001;**40**:15771–15779.
17 Arnold RR, Brewer M, Gauthier JJ. Bactericidal activity of human lactoferrin: sensitivity of a variety of microorganisms. *Infect Immun* 1980;**28**:893–898.
18 Ellison RT, Giehl TJ. Killing of Gram-negative bacteria by lactoferrin and lysozyme. *J Clin Invest* 1991;**88**:1080–1091.
19 Shashi S, Avinash S, Joshi YM, Dnyanesh L, Vilasrao K. Human milk: excellent anticancer alternative. *Int J Res Pharm Chem* 2012;**2**:575–584.
20 Mossberg AK, Wullt B, Gustafsson L, Maasson W, Ljunggren E, Svanborg C. Bladder cancers respond to intravesical instillation of HAMLET (human α-lactalbumin made lethal to tumor cells). *Int J Cancer* 2007;**121**:1352–1359.
21 Miehlke S, Reddy R, Osato MS, Ward PP, Conneely OM, Graham DY. Direct activity of recombinant human lactoferrin against *Helicobacter pylori*. *J Clin Microbiol* 1996;**34**: 2593–2594.
22 Strömqvist M, Falk P, Bergström S *et al*. Human milk κ-casein and inhibition of *Helicobacter pylori* adhesion to human gastric mucosa. *J Pediatr Gastroenterol Nutr* 1995;**21**:288–296.
23 Lönnerdal B. Bioactive proteins in breast milk. *J Paediatr Child Health* 2013;**49**(Suppl. 1):1–7.
24 Yolken RH, Peterson JA, Vonderfecht SL, Fouts ET, Midthun K, Newburg DS. Human milk mucin inhibits rotavirus replication and prevents experimental gastroenteritis. *J Clin Invest* 1992;**90**:1984–1991.

25 Schroten H, Hanisch FG, Plogmann R *et al*. Inhibition of adhesion of S-fimbriated *Escherichia coli* to buccal epithelial cells by human milk fat globule membrane components: a novel aspect of the protective function of mucins in the nonimmunoglobulin fraction. *Infect Immun* 1992;**60**:2893–2899.
26 Zavaleta N, Kvistgaard AS, Graverholt G *et al*. Efficacy of an MFGM-enriched complementary food in diarrhea, anemia, and micronutrient status in infants. *J Pediatr Gastroenterol Nutr* 2011;**53**:561–568.
27 Kawano A, Emori Y. Changes in maternal secretory immunoglobulin A levels in human milk during 12 weeks after parturition. *Am J Hum Biol* 2013;**25**:399–403.
28 Powers HJ. Vitamin requirements for term infants: considerations for infant formulae. *Nutr Res Rev* 1997;**10**:1–33.
29 Schlimme E, Buchheim W. *La Leche y Sus Componentes. Propiedades Químicas y Físicas*, 2nd edn. Acribia S.A., Zaragoza, 2002.
30 Hampela D, Yorka ER, Allen LH. Ultra-performance liquid chromatography tandem mass-spectrometry (UPLC–MS/MS) for the rapid, simultaneous analysis of thiamin, riboflavin, flavin adenine dinucleotide, nicotinamide and pyridoxal in human milk. *J Chromatogr B* 2012;**903**:7–13.
31 Allen LH. B vitamins in breast milk: relative importance of maternal status and intake, and effects on infant status and function. *Adv Nutr* 2012;**3**:362–369.
32 Ortega1 RM, Martínez RM, Andrés P, Marin-Arias, López-Sobaler AM. Thiamin status during the third trimester of pregnancy and its influence on thiamine concentrations in transition and mature breast milk. *Br J Nutr* 2004;**92**:129–135.
33 Sakurai T, Furukawa M, Asoh M, Kanno T, Kojima T, Yonekubo A. Fat-soluble and water-soluble vitamin contents of breast milk from Japanese women. *J Nutr Sci Vitaminol* 2005;**51**:239–247.
34 Shi YD, Sun GQ, Zhang ZG *et al*. The chemical composition of human milk from Inner Mongolia of China. *Food Chem* 2011;**127**:1193–1198.
35 Moran JR, Vaughan R, Stroop S, Coy S, Johnston H, Greene HL. Concentrations and total dairy output of micronutrients in breast milk of mothers delivering preterm: a longitudinal study. *J Pediatr Gastroenterol Nutr* 1983;**2**:629–634.
36 Mock DM, Mock NI, Stratton BS. Concentrations of biotin metabolites in human milk. *J Pediatr* 1997;**131**:456–458.
37 Greer FR. Vitamin K the basic. What's new? *Early Hum Dev* 2010;**86**:543–457.
38 Atkinson S, Alston-Mills B, Lönnerdal B, Neville MC. Major minerals and ionic constituents of human milk and bovine milk. In: Jensen RG (ed.) *Handbook of Milk Composition*. Academic Press, San Diego, CA, 1995, pp. 593–620.
39 Neville MC, Zhang P, Allen JC. Ionic interactions in milk. In: Jensen RG (ed.) *Handbook of Milk Composition*. Academic Press, San Diego, CA, 1995, pp. 577–591.
40 Kalkwarf HJ, Specker BL, Bianchi DC, Ranz J, Ho M. The effect of calcium supplementation on bone density during lactation and after weaning. *N Engl J Med* 1997;**337**:523–528.
41 Maru M, Birhanu T, Tessema DA. Calcium, magnesium, iron, zinc and copper, compositions of human milk from populations with cereal and 'enset' based diets. *Ethiopian J Health Sci* 2013;**23**:90–97.
42 Escanero JF. Minerales: Elementos traza. In: Cocho JA, Escanero JF, González de Buitrago JM (eds) *Elementos Traza: Aspectos Bioquímicos, Analíticos y Clínicos*. SEQ (Sociedad Española de Bioquímica Clínica y Patología Molecular), Barcelona, 1998.
43 Lönnerdal B. Trace element transport in the mammary gland. *Annu Rev Nutr* 2007;**27**:165–177.
44 Hannan MA, Faraji B, Tanguma J, Longoria N, Rodriguez RC. Maternal milk concentration of zinc, iron, selenium, and iodine and its relationship to dietary intakes. *Biol Trace Elem Res* 2009;**127**:6–15.
45 Luo Y, Zhang B, Chen M *et al*. Rapid and simultaneous determination of essential minerals and trace elements in human milk by improved flame atomic absorption spectroscopy (FAAS) with microwave digestion. *J Agric Food Chem* 2010;**58**:9396–9400.
46 Adesiyan AA, Akiibinu MO, Olisekodiaka MJ, Onuegbu AJ, Adeyeye AD. Concentrations of some biochemical parameters in breast milk of a population of Nigerian nursing mothers using hormonal contraceptives. *Pakistan J Nutr* 2011;**10**:249–253.
47 Björklund KL, Vahter M, Palm B, Grander M, Lignell S, Berglund M. Metals and trace element concentrations in breast milk of first time healthy mothers: a biological monitoring study. *Environ Health* 2012;**11**:92. doi: 10.1186/1476-069X-11-92.
48 Fernandez-Sanchez ML, de la Flor St Remy RR, Iglesias HG *et al*. Iron content and its speciation in human milk from mothers of preterm and full-term infants at early stages of lactation: a comparison with commercial infant milk formulas. *Microchem J* 2012;**105**:108–114.
49 Rahamon SK, Arinola GO, Akiibinu MO. Total antioxidant potential and essential trace metals in the breast milk and plasma of Nigerian human immunodeficiency virus-infected lactating mothers. *J Res Med Sci* 2013;**18**:27–30.
50 Nascimento RS, Froes RES, Silva NOC *et al*. Comparison between ordinary least squares regression and weighted least squares regression in the calibration of metals present in human milk determined by ICP-OES. *Talanta* 2010;**80**:1102–1109.
51 Casey CC, Smith A, Zhang P. Microminerals in human and animal milks. In: Jensen RG (ed.) *Handbook of Milk Composition*. Academic Press, San Diego, 1995, pp. 622–673.
52 Zohoori V, Seal CJ, Moynihan PJ, Steen IN, Maguire A. The impact of fluoridated milks on the availability of trace elements in milk. *J Sci Food Agric* 2009;**89**:595–602.
53 Sola-Larrañaga C, Navarro-Blasco I. Chemometric analysis of minerals and trace elements in raw cow milk from the community of Navarra, Spain. *Food Chem* 2009;**112**:189–196.

54 Bermejo P, Peña E, Domínguez R, Bermejo A, Fraga JM, Cocho JA. Speciation of iron in breast milk and infant formula whey by size exclusion chromatography-high performace liquid chromatography and electrothermal atomic absorption spectrometry. *Talanta* 2000;**50**:1211–1222.
55 El-Farrash RA, Ismail EAR, Nada AS. Cord blood iron profile and breast milk micronutrients in maternal iron deficiency anemia. *Pediatr Blood Cancer* 2012;**58**:233–238.
56 Abdulrazzaq YM, Osman N, Nagelkerke N, Kosanovic M, Adem A. Trace element composition of plasma and breast milk of well-nourished women. *J Environ Sci Health A* 2008;**43**:329–334.
57 Khaghani S, Ezzatpanah H, Mazhari N, Givianrad MH, Mirmiranpour H, Sadrabadi FS. Zinc and copper concentrations in human milk and infant formulas. *Iranian J Pediatr* 2010;**20**:53–57.
58 Örun E, Yalcin SS, Aykut O, Orhan G, Morgil GK. Zinc and copper concentrations in breast milk at the second month of lactation. *Indian Pediatr* 2012;**49**:133–135.
59 Coni E, Bocca B, Galoppi B, Alimonti A, Caroli S. Identification of chemical species of some trace and minor elements in mature breast milk. *Microchem J* 2000;**67**:187–194.
60 Almeida A, Lopes C, Silva A, Barrado E. Trace elements in human milk: correlation with blood levels, inter-element correlations and changes in concentration during the first month of lactation. *J Trace Elem Med Biol* 2008;**22**:196–205.
61 Bermejo P, Peña E, Fompedriña D *et al*. Copper fractionation by SEC-HPLC and ETAAS: study of breast milk and infant formulae whey used in lactation of full-term newborn infants. *Analyst* 2001;**126**:571–575.
62 Bermejo-Barrera P, Domínguez-González R, Bermejo-Barrera A. Direct copper determination in whole milk, non-fat milk and whey milk by electrothermal atomic absorption spectrometry. *Fresenius J Anal Chem* 1997;**357**:457–461.
63 Pechová A, Pavlata L, Dvořák R, Lokajová E. Contents of Zn, Cu, Mn and Se in milk in relation to their concentrations in blood, milk yield and stage of lactation in dairy cattle. *Acta Vet Brno* 2008;**77**:523–531.
64 Lönnerdal B. Regulation of mineral and trace elements in human milk: exogenous and endogenous factors. *Nutr Rev* 2000;**58**:223–229.
65 O'Brien CE, Krebs NF, Westcott JL, Dong F. Relationships among plasma zinc, plasma prolactin, milk transfer, and milk zinc in lactating women. *J Hum Lact* 2007;**23**:179–183.
66 Bermejo P, Peña E, Fompedriña D *et al*. Speciation of Zn in low molecular weight proteins of breast milk and infant formulas by size exclusion chromatography/flame atomic absorption spectrometry. *J AOAC Int* 2001;**84**:847–852.
67 Domínguez R, Peña E, Bermejo A, Cocho JA, Fraga JM, Bermejo P. Fe, Cu and Zn distribution in different components of commercial infant formulas. *Eur Food Res Technol* 2005;**221**:529–537.
68 Michalke B, Schramel P. Manganese speciation in human milk using size exclusion chromatography combined with strong anion exchange chromatography and inductively coupled plasma mass spectrometry detection. *J Anal Atom Spectrom* 2004;**19**:121–128.
69 Erikson KM, Thompson K, Aschner J, Aschner M. Manganese neurotoxicity: a focus on the neonate. *Pharmacol Ther* 2007;**113**:369–377.
70 Lönnerdal B, Keen CL, Hurley LS. Manganese binding proteins in human and cow's milk. *Am J Clin Nutr* 1985;**41**:550–559.
71 Kim SY, Park JH, Kim EA, Lee-Kim YC. Longitudinal study on trace mineral compositions (selenium, zinc, copper, manganese) in Korean human preterm milk. *J Korean Med Sci* 2012;**27**:532–536.
72 Anderson RR. Comparison of trace elements in milk of four species. *J Dairy Sci* 1992;**75**:3450–3055.
73 Yigmatepe E, Yaman M. Determination of molybdenum in biological samples by flame atomic spectrometry after preconcentration on activated carbon. *Monatsh Chem* 2011;**142**:131–136.
74 Yoshida M, Takada A, Hirose J, Endo M, Fukuwatari T, Shibata K. Molybdenum and chromium concentrations in breast milk from Japanese women. *Biosci Biotechnol Biochem* 2008;**72**:2247–2250.
75 Smith RM. Cobalt. In: Mertz W (ed.) *Trace Elements in Human and Animal Nutrition*, 5th edn. Academic Press, San Diego, CA, 1987, pp. 143–183.
76 Aldosary BM, Sutter ME, Schwartz M, Morgan BW. Case series of selenium toxicity from a nutritional supplement. *Clin Toxicol* 2012;**50**:57–64.
77 Al-Awadi FM, Srikumar TS. Determination of selenium concentration and its chemical forms in the milk of Kuwaiti and non-Kuwaiti lactating mothers. *J Trace Elem Exp Med* 2001;**14**:57–67.
78 Theodorolea S, Thomaidis NS, Piperaki E. Determination of selenium in human milk by electrothermal atomic absorption spectrometry and chemical modification. *Anal Chim Acta* 2005;**547**:132–137.
79 Zachara BA, Pilecki A. Daily selenium intake by breast-fed infants and the selenium concentration in the milk of lactating women in Western Poland. *Med Sci Monitor* 2001;**7**:1002–1004.
80 Muñiz-Naveiro O, Domínguez-González R, Bermejo-Barrera A, Cocho JA, Fraga JM, Bermejo-Barrera P. Determination of total selenium and selenium distribution in the milk phases in commercial cow's milk by HG-AAS. *Anal Bioanal Chem* 2005;**381**:1145–1151.
81 Muñiz-Naveiro O, Domínguez-González R, Bermejo-Barrera A *et al*. Selenium content and distribution in cow's milk supplemented with two dietary selenium sources. *J Agric Food Chem* 2005;**53**:9817–9822.
82 Muñiz-Naveiro O, Domínguez-González R, Bermejo-Barrera A, Bermejo-Barrera P, Cocho JA, Fraga JM. Selenium speciation in cow milk obtained after supplementation with different selenium forms to the cow feed using liquid chromatography coupled with hydride generation-atomic fluorescence spectrometry. *Talanta* 2007;**71**:1587–1593.

83 Underwood EJ. *Trace Elements in Human and Animal Nutrition*, 4th edn. Academic Press, New York, 1977.

84 Zimmermann MB, Jooste PL, Pandav CS. Iodine-deficiency disorders. *Lancet* 2008;**372**:1251–1262.

85 Dorea JG. Iodine nutrition and breast feeding. *J Trace Elem Med Biol* 2002;**16**:207–220.

86 Fernández-Sánchez L, Szpunar J. Speciation analysis for iodine in milk by size-exclusion chromatography with inductively coupled plasma mass spectrometric detection (SEC-ICP MS). *J Anal Atom Spectrom* 1999;**14**:1697–1702.

87 Skeaff SA, Ferguson EL, McKenzie JE, Valeix P, Gibson RS, Thomson CD. Are breast-fed infants and toddlers in New Zealand at risk of iodine deficiency? *Nutrition* 2005;**21**: 325–331.

88 Gupta R, Kumar AN, Bandhu S, Gupta S. Skeletal fluorosis mimicking seronegative arthritis. *Scand J Rheumatol* 2007;**36**: 154–155.

89 Ekstrand J, Spak CJ, Falch J, Afseth J, Ulvestad H. Distribution of fluoride to human breast milk following intake of high doses of fluoride. *Caries Res* 1984;**18**:93–95.

90 Esala S, Vuori E, Helle A. Effect of maternal fluorine intake on breast milk fluorine content. *Br J Nutr* 1982;**48**: 201–204.

91 Opinya GN, Bwibo N, Valderhaug J, Birkeland JM, Lokken P. Intake of fluoride and excretion in mothers' milk in a high fluoride (9 ppm) area in Kenya. *Eur J Clin Nutr* 1991; **45**:37–41.

92 Ozbek N, Akman S. Determination of fluorine in milk samples via calcium-monofluoride by electrothermal molecular absorption spectrometry. *Food Chem* 2013;**138**:650–654.

93 Kazi TG, Jalbani N, Baig JA *et al*. Assessment of toxic metals in raw and processed milk samples using electrothermal atomic absorption spectrophotometer. *Food Chem Toxicol* 2009;**47**:2163–2169.

94 Al-Ashmawy MAM. Prevalence and public health significance of aluminium residues in milk and some dairy products. *J Food Sci* 2011;**76**:T73–T76.

95 International Agency for Research on Cancer. Some drinking-water disinfectants and contaminants including arsenic. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Vol. 84. IARC, Lyon, 2004.

96 Gropper S, Smith J, Groff J. *Advanced Nutrition and Human Metabolism*, 5th edn. Wadsworth Cengage Learning, Belmont, CA, 2009.

97 Concha G, Vogler G, Nermell B, Vahter M. Low-level arsenic excretion in breast milk of native Andean women exposed to high levels of arsenic in the drinking water. *Int Arch Occup Environ Health* 1998;**71**:42–46.

98 Watanabe C, Inaoka T, Matsui T, Ishigaki K, Murayama N, Ohtsuka R. Effects of arsenic on younger generations. *J Environ Sci Health A* 2003;**38**:129–139.

99 Fängström B, Moore S, Nermell B *et al*. Breast-feeding protects against arsenic exposure in Bangladeshi infants. *Environ Health Perspect* 2008;**116**:963–969.

100 Sigrist M, Beldomenico H, Repetti MR. Evaluation of the influence of arsenical livestock drinking waters on total arsenic levels in cow's raw milk from Argentinean dairy farms. *Food Chem* 2010;**121**:487–491.

101 Rosas I, Belmont R, Armienta A, Baez A. Arsenic concentrations in water, soil, milk and forage in Comarca Lagunera, Mexico. *Water Air Soil Pollut* 1999;**112**:133–149.

102 Nawrot TS, Staessen JA, Roels HA *et al*. Cadmium exposure in the population: from health risks to strategies of prevention. *BioMetals* 2010;**23**:769–782.

103 Vidovic M, Sadibasic A, Cupic S, Lausevic M. Cd and Zn in atmospheric deposit, soil, wheat, and milk. *Environ Res* 2005;**97**:26–31.

104 Nordberg GF, Nogawa K, Nordberg M, Friberg LT. Cadmium. In: Nordberg GF, Nogawa K, Nordberg M, Friberg LT (eds) *Handbook on the Toxicology of Metals*, 3rd edn. Elsevier, Amsterdam, 2007, pp. 446–485.

105 Moussa WM. Determination of lead and cadmium in human milk and measure of some of its composition. *Aust J Basic Appl Sci* 2011;**5**:236–240.

106 Goudarzi MA, Parsaei P, Nayebpour F, Rahimi E. Determination of mercury, cadmium and lead in human milk in Iran. *Toxicol Ind Health* 2013;**29**:820–823.

107 Liu KS, Hao JH, Xu YQ *et al*. Breast milk lead and cadmium levels in suburban areas of Nanjing, China. *Chinese Med Sci J* 2013;**28**:7–15.

108 Rivero FA, Fernández ML, Sanz Medel A. The potential of double focusing-ICP-MS for studying elemental distribution patterns in whole milk, skimmed milk and milk whey of different milks. *Anal Chim Acta* 2001;**442**: 191–200.

109 Fujita M, Takabatake E. Mercury levels in human maternal and neonatal blood, hair and milk. *Bull Environ Contam Toxicol* 1977;**18**:205–209.

110 Bakir F, Damluji SF, Amin-Zaki L *et al*. Metthylmercury poisoning in Iraq. *Science* 1973;**181**:230–241.

111 Dahmardeh Behrooz R, Esmaili-Sari A, Peer FE, Amini M. Mercury concentration in the breast milk of Iranian women. *Biol Trace Elem Res* 2012;**147**:36–43.

112 Al-Saleh I, Abduljabbar M, Al-Rouqi R, Elkhatib R, Alshabbaheen A, Shinwari N. Mercury (Hg) exposure in breast-fed infants and their mothers and the evidence of oxidative stress. *Biol Trace Elem Res* 2013;**153**:145–154.

113 World Health Organization. *Minor and Trace Elements in Breast Milk: Report of a Joint WHO/IAEA Collaborative Study*. WHO, Geneva, 1989.

114 Friel JK, Andrews WL, Jackson SE *et al*. Elemental composition of human milk from mothers of premature and full-term infants during the first 3 months of lactation. *Biol Trace Elem Res* 1999;**67**:225–247.

115 Yamawaki N, Yamada M, Kan-no T, Kojima T, Kaneko T, Yonekubo A. Macronutrient, mineral and trace element composition of breast milk from Japanese women. *J Trace Elem Med Biol* 2005;**19**:171–181.

116 Mello-Neto J, Rondo P, Oshiiwa M, Morgano M, Zacari C, Domingues S. The influence of maternal factors on the concentration of vitamin A in mature breast milk. *Clin Nutr* 2009;**28**:178–181.

117 Severi C, Hambidge M, Krebs N, Alonso R, Atalah E. Zinc in plasma and breast milk in adolescents and adults in pregnancy and pospartum; a cohort study in Uruguay. *Nutr Hosp* 2013;**28**:223–228.

118 Bose-O'Reilly S, Lettmeier B, Roider G, Siebert U, Drasch G. Mercury in breast milk: a health hazard for infants in gold mining areas? *Int J Hyg Environ Health* 2008;**211**:615–623.

119 Semba RD, Delange F. Iodine in human milk: perspectives for infant health. *Nutr Rev* 2001;**59**:269–278.

120 Quevauviller, P. Method validation for speciation analysis. *Trends Anal Chem* 2000;**19**:67–68.

121 Jensen RG (ed.) *Handbook of Milk Composition*. Academic Press, San Diego, CA, 1995.

122 Brätter P, Blasco IN, Negretti de Brätter VE, Raab A. Maternal selenium status influences the concentration and binding form of iodine in human milk. In: Centeno J, Collery P, Vernet G, Finkelman R, Gibb H, Etienne J (eds) *Metal Ions in Biology and Medicine*, Vol. **6**. John Libbey Eurotext, Montrouge, France, 2000, pp. 751–754.

123 Michalke B. *Elementsspezieanalytik in Bio-medizinischen und Umweltproben*. Habilitations-schrift an der technischen Universität Graz, 1998.

124 Fernandez Sánchez L. *Especiación de iodo en leche mediante HPLC-ICP-MS*. PhD thesis, University of Santiago de Compostela, Spain, 2004.

125 Brätter P, Negretti de Brätter VE, Raab A, Blasco IN. Speciation as an analytical aid in trace element research in infant nutrition. *Analyst* 1998;**123**:821–826.

126 Bocca B, Alimonti A, Coni E *et al*. Determination of the total content and binding pattern of elements in human milk by high performance liquid chromatography-inductively coupled plasma atomic emission spectrometry. *Talanta* 2000;**53**: 295–303.

127 Brätter P, Gerker B, Rösick U, Tomiak A. Speciation of trace element in human milk and milk formulas. In: Brätter P, Schramel P (eds) *Trace Element Analytical Chemistry in Biology and Medicine. Proceedings of the Fifth International Workshop*. Walter de Gruyter, Berlin, 1988, pp. 145–156.

128 Negretti de Brätter VE, Recknagel S, Gawlik D. Speciation of Se, Fe and Zn in human milk whey: the use of instrumental neutron activation analysis (INAA) to corroborate element profiles measured with inductively coupled plasma atomic emission spectrometry (ICP-AES). *Fresenius J Anal Chem* 1995;**353**:137–142.

129 Brätter P, Negretti de Brätter VE, Recknagel S, Brunetto R. Maternal selenium status influences the concentration and binding pattern of zinc in human milk. *J Trace Elem Med Biol* 1997;**4**:203–209.

130 Michalke B. Capillary electrophoretic methods for a clear identification of selenoamino acids in complex matrices such as human milk. *J Chromatogr A* 1995;**716**:323–329.

131 Michalke B, Schramel P. Selenium speciation in human milk with special respect to quality control. *Biol Trace Elem Res* 1997;**59**:45–56.

132 Michalke B, Schramel P. Application of capillary zone electrophoresis-inductively coupled plasma mass spectrometry and capillary isoelectric focusing-inductively coupled plasma mass spectrometry for selenium speciation. *J Chromatogr A* 1998;**807**:71–80.

133 Michalke B. CE-ICP-MS: advantages and improvements in selenium speciation. *Spectroscopy* 2000;**15**:32–34.

134 De la Flor St Remy RR, Fernández-Sánchez ML, Lòpez-Sastre JB, Sanz-Medel A. Multielement distribution patters in premature human milk whey and pre-term formula milk whey by size exclusion chromatography coupled to inductively coupled plasma mass spectrometry with octopole reaction cell. *J Anal Atom Spectrom* 2004;**19**:1104–1110.

135 Lönnerdal B, Stanislowski AG, Hurley LS. Isolation of a low molecular weight zinc binding ligand from human milk. *J Inorg Biochem* 1980;**12**:71–78.

136 Lönnerdal B, Hoffman B, Hurley LS. Zinc and copper binding proteins in human milk. *Am J Clin Nutr* 1982;**36**: 1170–1176.

137 Arnaud J, Andre D, Bouillet MC, Kia D, Favier A. Problems associated with the use of exclusion-diffusion chromatography for identification of zinc ligands in human milk. *J Trace Elem Electrolytes Health Dis* 1992;**6**:81–90.

138 Michalke B, Munch DC, Schramel P. Contribution to zinc-speciation in human breast milk: fractionation of organic compounds by HPLC and subsequent Zn-determination by DCP-AES. *J Trace Elem Electrolytes Health Dis* 1991;**5**:251–258.

139 Michalke B, Muench DC, Schramel P. Advanced quality control for zinc speciation investigations in human breast milk. *Fresenius J Anal Chem* 1992;**344**:306–310.

140 Coni E, Alimonti A, Bocca A *et al*. Speciation of trace elements in human milk by high performance liquid chromatography combined with inductively coupled plasma-mass spectrometry. *Trace Elem Electrolytes* 1996;**13**:26–32.

141 Rivero Martino A, Fernández-Sánchez ML, Sanz Medel A. Multi-elemental fractionation in milk whey by size exclusion chromatography coupled on line to ICP-MS. *J Anal Atom Spectrom* 2002;**17**:1271–1277.

142 Milacic R, Ajlec D, Zuliani T, Zigon D, Scancar J. Determination of Zn-citrate in human milk by CIM monolithic chromatography with atomic and mass spectrometry detection. *Talanta* 2012;**101**:203–210.

143 Fransson GB, Lönnerdal B. Iron in human milk. *J Pediatr* 1980;**96**:380–384.

144 Fernández-Sánchez ML, de la Flor St Remy RR, González Iglesias H *et al*. Iron content and its speciation in human milk from mothers of preterm and full-term infants at early stages of lactation: a comparison with commercial infant milk formulas. *Microchem J* 2012;**105**:108–114.

145 Argyri K, Birba A, Miller DD, Komaitis M, Kapsokefalou M. Predicting relative concentrations of bioavailable iron in foods using *in vitro* digestion: new developments. *Food Chem* 2009;**113**:602–607.

146 Bosscher D, Lu Z, Van Cauwenbergh R, Van Caille-Bertrand M, Robberecht H, Deelstra H. A method for *in vitro* determination of calcium, iron and zinc availability from first-age infant formula and human milk. *Int J Food Sci Nutr* 2001;**52**:173–182.

147 Domínguez R, Barreiro T, Sousa E *et al*. Study of the effect of different iron salts used to fortify infant formulas on the bioavailability of trace elements using ICP-OES. *Int Dairy J* 2004;**14**:1081–1087.

148 Muñiz O, Domínguez R, Bermejo A, Bermejo P, Cocho J, Fraga J. Study of the bioavailability of selenium in cow's milk after a supplementation of cow feed with different forms of selenium. *Anal Bioanal Chem* 2006;**385**:189–196.

149 Perales S, Barberá R, Lagarda MJ, Farré R. Availability of iron from milk-based formulas and fruit juices containing milk and cereals estimated by *in vitro* methods (solubility, dialysability) and uptake and transport by Caco-2 cells. *Food Chem* 2007;**102**:1296–1303.

150 Robberecht H, Van Cauwenbergh R, Van Vlaslaer V, Hermans N. Dietary silicon intake in Belgium: sources, availability from foods, and human serum levels. *Sci Total Environ* 2009;**407**:4777–4782.

151 Zhu L, Glahn RP, Nelson D, Miller DD. Comparing soluble ferric pyrophosphate to common iron salts and chelates as sources of bioavailable iron in a Caco-2 cell culture model. *J Agric Food Chem* 2009;**57**:5014–5019.

152 Pérez A, Lorenzo L, Cabrera C, López MC. Influence of enrichment with vitamins and minerals on the bioavailability of iron in cow's milk. *J Dairy Res* 2002;**69**:473–481.

153 Ziegler EE, Serfass RE, Nelson SE *et al*. Effect of low zinc intake on absorption and excretion of zinc by infants studied with Zn70 as extrinsic tag. *J Nutr* 1989;**119**:1647–1653.

154 Fomon SJ, Serfass RE, Nelson SE, Rogers RR, Frantz JA. Less than 80% of absorbed iron is promptly incorporated into erythrocytes of infants. *J Nutr* 2000;**130**:45–52.

155 Fomon SJ, Serfass RE, Nelson SE, Rogers RR, Frantz JA. Time course and time effect of dietary iron level on iron incorporation into erythrocytes by infants. *J Nutr* 2000;**130**:541–545.

156 Fairweather-Tait S, Fox T, Wharf SG, Eagles J. The bioavailability of iron in different weaning foods and the enhancing effect of a fruit drink containing ascorbic acid. *Pediatr Res* 1995;**37**:389–394.

157 Davidsson L, Galán P, Kastenmayer P *et al*. Iron bioavailability studied in infants: the influence of phytic acid and ascorbic acid in infant formulas based on soy isolate. *Pediatr Res* 1994;**36**:816–822.

158 Abrams SA, Wen J, Stuff JE. Absorption of calcium, zinc, and iron from breast milk by five-to seven-month-old infants. *Pediatr Res* 1996;**39**:384–390.

159 Lönnerdal B, Glazier C. An approach to assessing trace element bioavailability from milk *in vitro*. *Biol Trace Elem Res* 1989;**19**:57–59.

160 Bermejo P, Peña EM, Domínguez R, Bermejo A, Cocho JA, Fraga JM. Iron and zinc in hydrolysed fractions of human milk and infant formulas using an *in vitro* method. *Food Chem* 2002;**77**:361–369.

161 Peña E, Domínguez R, Bermejo A, Cocho JA, Fraga JM, Bermejo P. Enzymolysis approach to compare Cu availability from human milk and infant formulas. *J Agric Food Chem* 2004;**52**:4887–4892.

162 Sarriá B, Vaquero MP. Zinc and iron bioavailability in a powder or in-bottle-sterilized infant formula estimated by *in vitro* and in suckling rats. *J Nutr Biochem* 2001;**12**:266–273.

163 Bosscher D, Van Caille-Bertrand M, Robberecht H, Van Dyck K, Van Cauwenbergh R, Deelstra H. *In vitro* availability of calcium, iron and zinc from first-age infant formulae and human milk. *J Pediatr Gastroenterol Nutr* 2001;**32**:54–58.

164 Judprasong K, Ornthai M, Siripinyanond A, Shiowatana J. A continuous-flow dialysis system with inductively coupled plasma optical emission spectrometry for *in vitro* estimation of bioavailability. *J Anal Atom Spectrom* 2005;**20**:1191–1196.

165 Glahn RP, Lai C, Hsu J, Thompson JF, Guo M, Van Campen DR. Decreased citrate improves iron bioavailability from infant formula: application of an *in vitro* digestion/caco-2 cell culture model. *J Nutr* 1998;**128**:257–264.

166 Jovaní M, Barberá R, Farré R, Martín de Aguilera E. Calcium, iron, and zinc uptake from digests of infant formulas by Caco-2 cells. *J Agric Food Chem* 2001;**49**:3480–3485.

167 Pullakhandam R, Nair M, Kasula S, Kilari S, Thippande T. Ferric reductase activity of low molecular weight human milk fraction is associated with enhanced iron solubility and uptake in Caco-2 cells. *Biochem Biophys Res Commun* 2008;**374**:369–372.

168 Kibangou I, Bouhallab S, Henry G *et al*. Milk proteins and iron absorption: contrasting effects of different caseinophosphopeptides. *Pediatr Res* 2005;**58**:731–734.

169 Etcheverry P, Wallingford JC, Miller DD, Glahn RP. Calcium, zinc, and iron bioavailabilities from a commercial human milk fortifier: a comparison study. *J Dairy Sci* 2004;**87**:3629–3637.

170 Caroli S, Alimonti A, Coni E, Petrucci F, Senofonte O, Violante N. The assessment of reference values for elements in human biological tissues and fluids: a systematic review. *Crit Rev Anal Chem* 1994;**24**:363–398.

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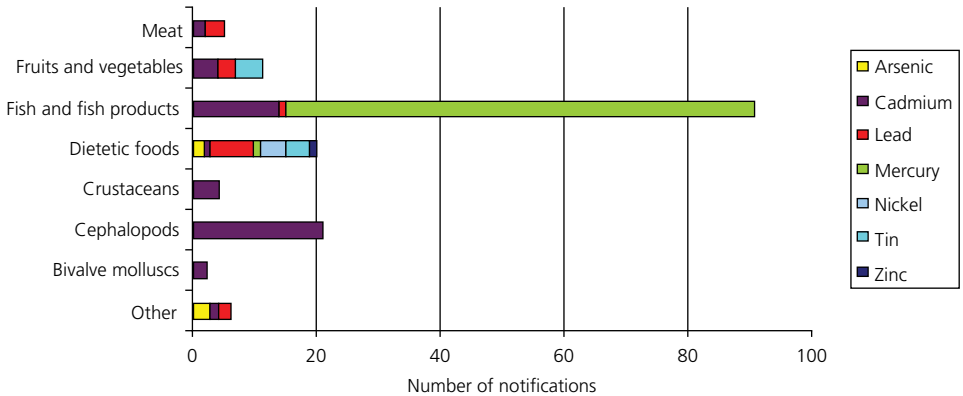


Figure 4.6 RASFF heavy metals in food notifications during the period 2008–2012.

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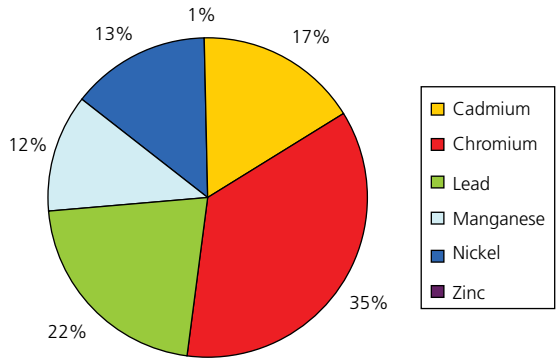


Figure 4.7 RASFF notifications on heavy metals in food contact materials during the period 2008–2012.

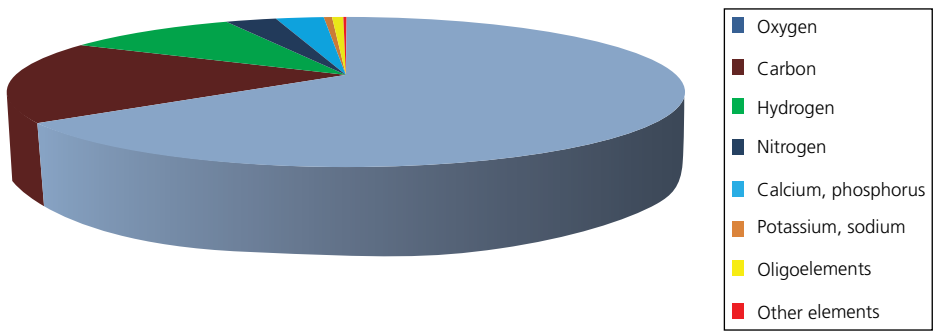


Figure 5.1 Elements in the human body (mass percent).

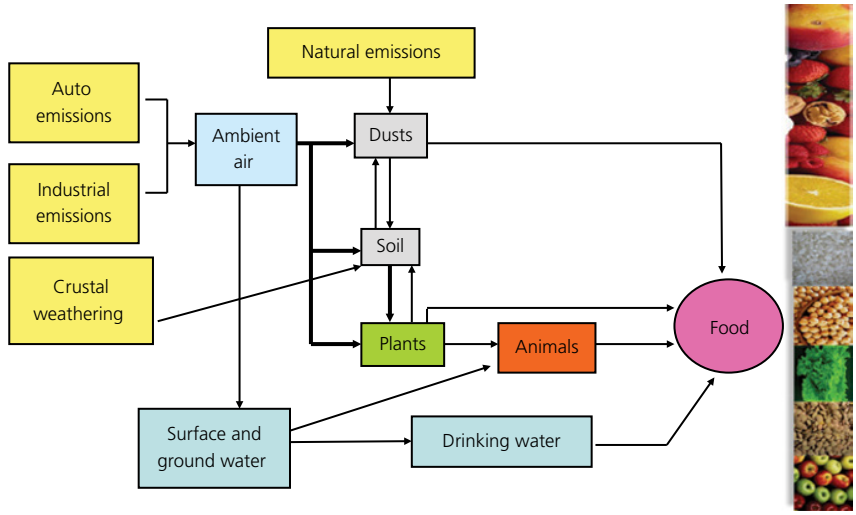


Figure 6.1 Pathways of metals from the environment to food.

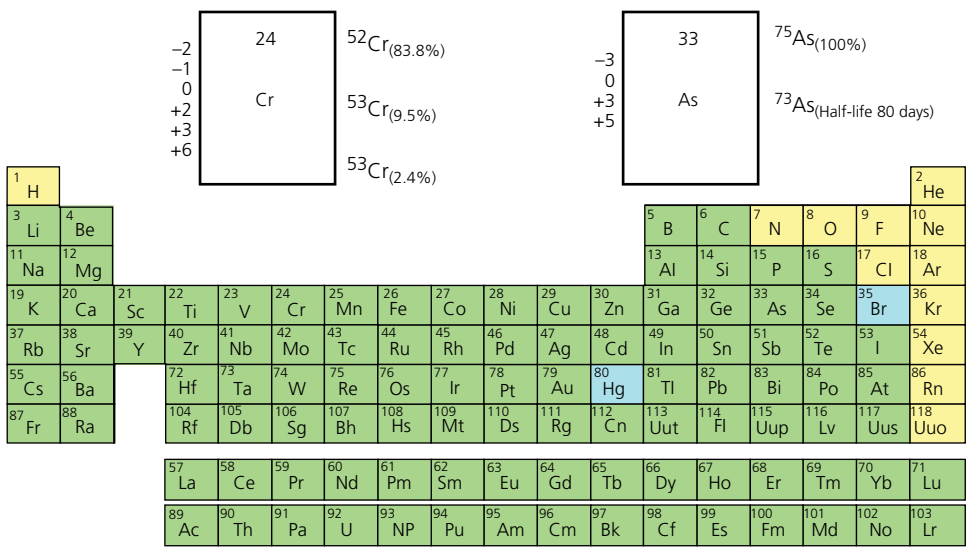
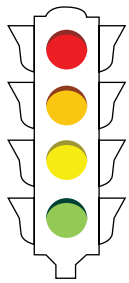


Figure 9.1 Periodic table of the elements. *Inset*: stable isotopes and possible oxidation states of Cr and As as examples of the complexity of mineral element behaviour.



AsH_3 (30 min 80–160 mg/m^3 lethal; 800 mg/m^3 instantly lethal)

As (III)
As (V)
} LOEL for As: 0.017 mg/kg daily

DMA (category B2 carcinogen)

Arsenosugars → DMA

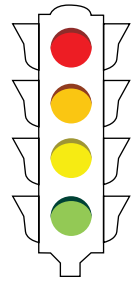
MMA, Arsenobetaine, Arsenocholine

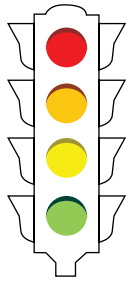
MeHg (Lethal 0.1–0.5 mg/kg daily)

PhHg (PhHg compounds) → MeHg

Hg(II) (Lethal 25–60 mg/kg in kidneys or liver)

Hg(0) toxic by inhalation





Cr(VI) (0.14 µg/kg daily)

Cr(III)

Cu(I) Used in pesticides

Cu(II)

Pb (organic, tetraethyllead)
(Lethal 1470 µg/kg)

Pb (inorganic) (Lethal 500 mg absorbed)

Trimethyltin (3.0 mg/kg)

Triethyltin (70 mg in 8 days)

Tributyltin (EPA toxicity class II)

$NaSn_2F_5$ (LOD rats: 590 mg/kg)
$SnCl_2$ (LOD rats: 700 mg/kg)

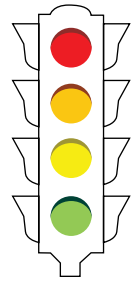


Figure 9.3 Toxicity of different species of mineral elements with regard to food safety.

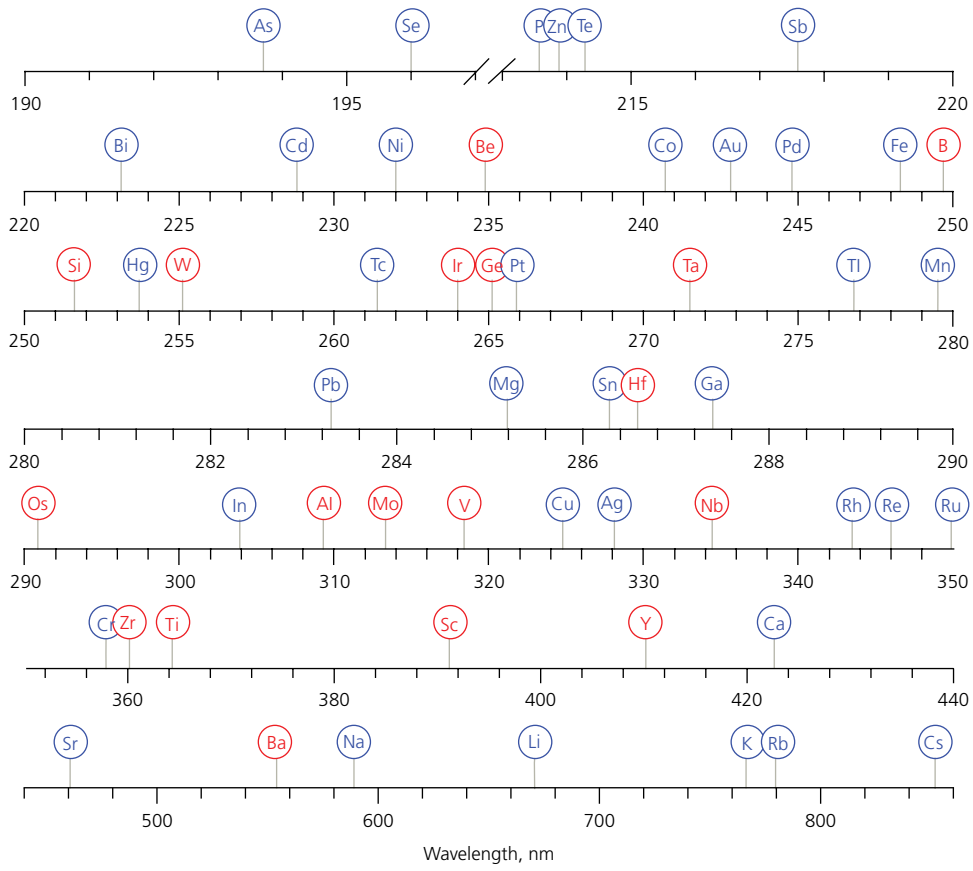


Figure 10.5 Wavelengths used for FAAS purposes with indication of the flame recommended: N_2O–C_2H_2 (red); air–C_2H_2 (blue).

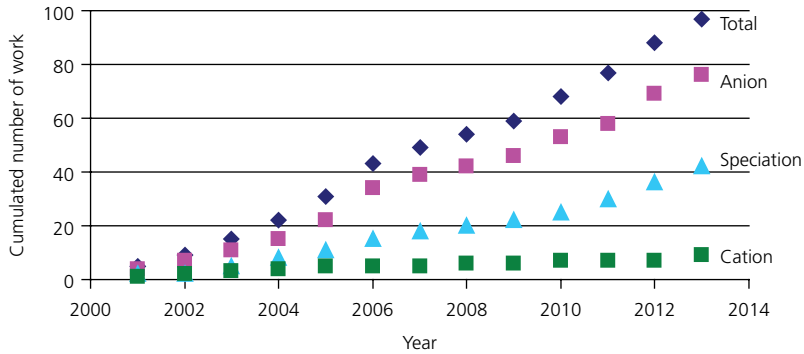


Figure 16.2 Evolution of the scientific literature on ion chromatographic applications in food analysis published during the twenty-first century.

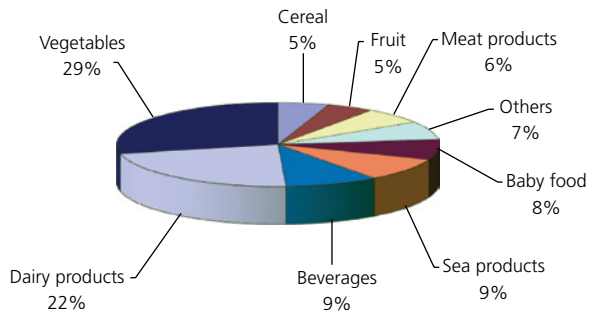


Figure 16.3 Distribution of published papers on determination of mineral components in food by means of ion chromatography as a function of the matrix considered for the period 2001–2013.

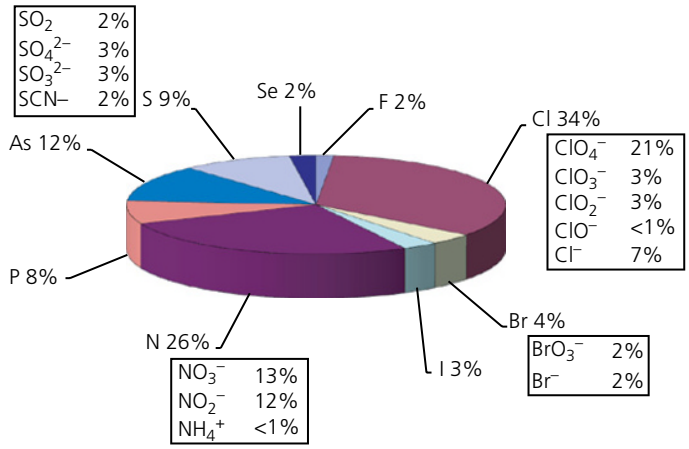


Figure 16.4 Distribution of anionic species determination in food samples by mean of ion chromatography.

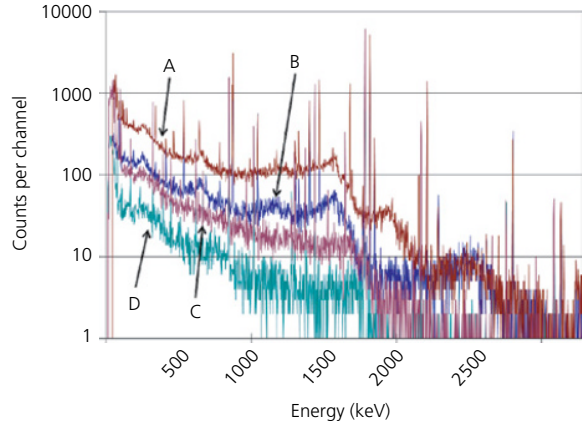


Figure 17.5 Comparison of four spectra for a coal sample irradiated for short-lived NAA for four different irradiation and counting modes. A, thermal normal; B, thermal Compton; C, epithermal normal; D, epithermal Compton.

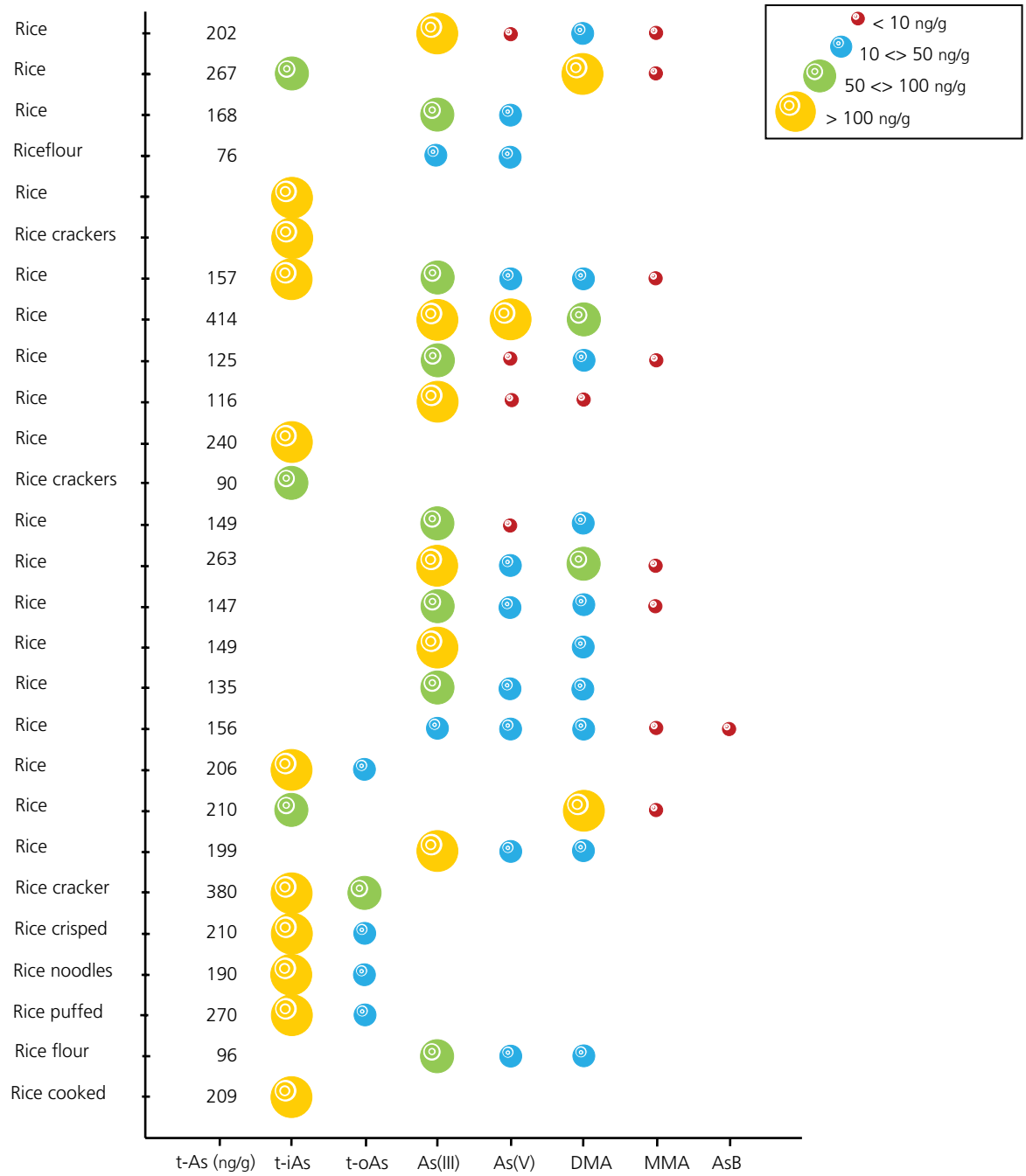


Figure 22.5 Speciation of As in cereals and pulses.

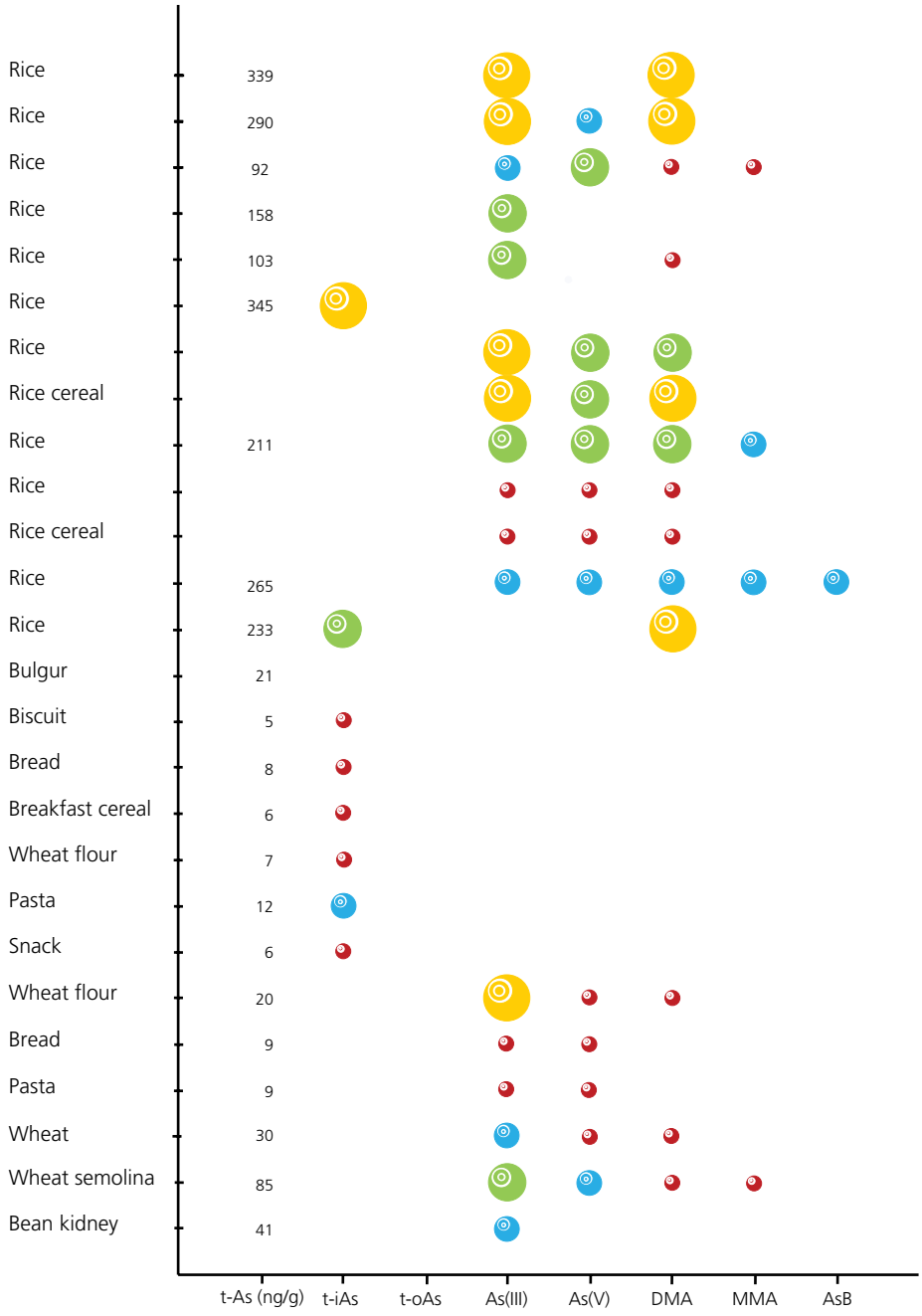


Figure 22.5 (Continued)

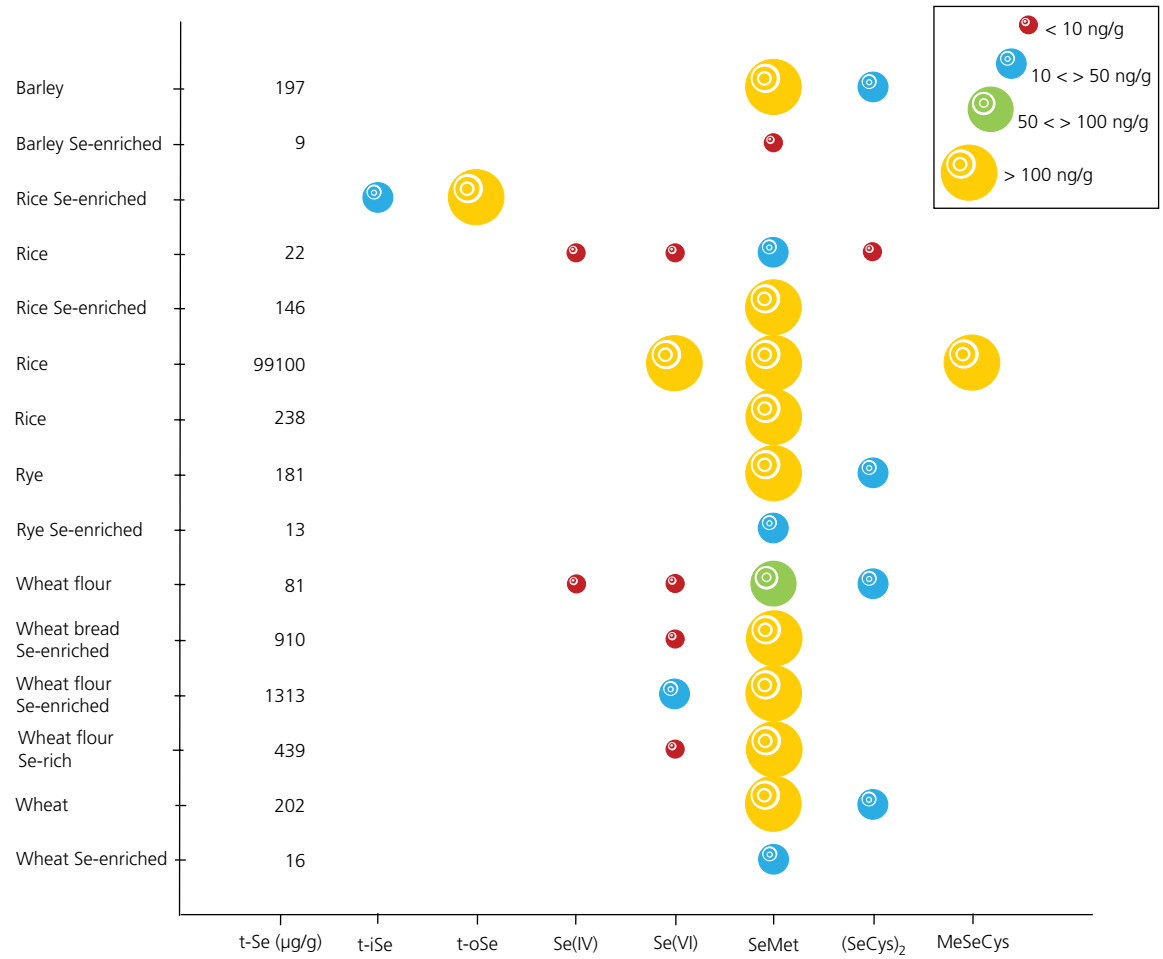


Figure 22.6 Speciation of Se in cereals and pulses.

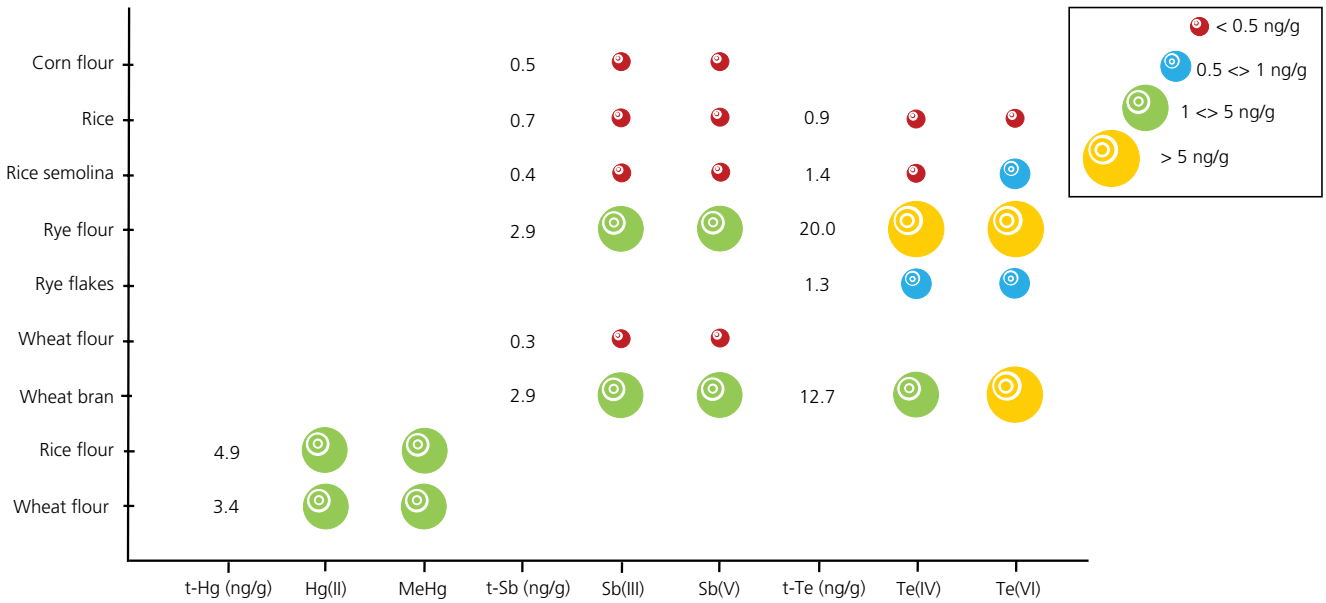


Figure 22.7 Speciation of Hg, Sb and Te in cereals and pulses.

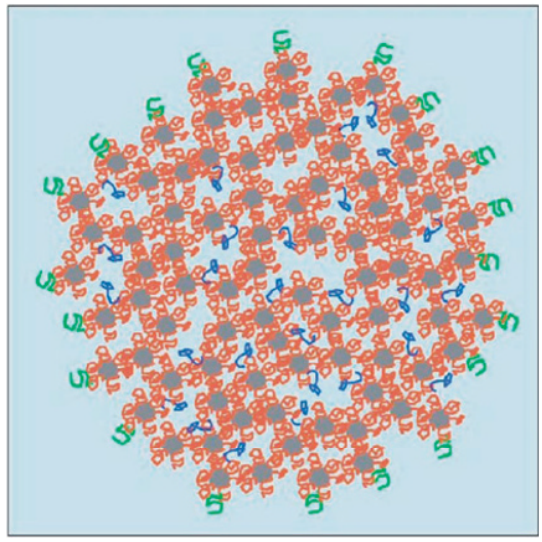


Figure 28.2 Schematic structure of the casein micelle incorporating calcium phosphate nanoclusters (grey) with their attached caseins (red) and κ-casein (green) located on the surface. The 'hydrophobically bound' mobile β-casein is shown in blue, within the water channels inside the micelle. For clarity, the relative sizes of the individual components are not to scale. From Dalgleish [62]. Reproduced with permission of The Royal Society of Chemistry.

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