CHAPTER 11

FOOD MICROBIOLOGY AND REFRIGERATION

REFRIGERATION'S largest overall application is the prevention or retardation of microbial, physiological, and chemical changes in foods. Even at temperatures near the freezing point, foods may deteriorate through the growth of microorganisms, through changes caused by enzymes, or through chemical reactions. Holding foods at low temperatures merely reduces the rate at which these changes take place. A few spoilage organisms can grow at or below those temperatures at which the freezing of food is initiated.

Refrigeration also plays a major role in maintaining a safe food supply. Overall, the leading factor causing food-borne illness is improper food holding temperatures. Another important factor is improperly sanitized equipment. Engineering directly impacts the safety and stability of the food supply in design of cleanable equipment and facilities, as well as maintenance of environmental conditions that inhibit microbial growth. This chapter briefly discusses the microbiology of foods and the impact of design decisions on the production of safe and wholesome foods. Methods of applying refrigeration to specific foods may be found in other chapters.

BASIC MICROBIOLOGY

Microorganisms play several roles in a food production facility. They can contribute to food spoilage, producing off-odors and flavors, or altering product texture or appearance through slime production and pigment formation. Certain organisms cause disease; others are beneficial and are required to produce foods such as cheese, wine, and sauerkraut through fermentation.

Microorganisms fall into four categories: bacteria, yeasts, molds, and viruses. Bacteria are the most common food-borne pathogens. Bacterial growth rates, under optimum conditions, are generally faster than those of yeasts and molds, making bacteria a prime cause of spoilage, especially in refrigerated, moist foods. Bacteria have many shapes, including spheres (cocci), rods, or spirals, that are usually between 0.3 and 5 to 10 μm in size. Bacteria are capable of growth in a wide range of environments. Some bacteria, notably *Clostridium* and *Bacillus* spp., form endospores (i.e., resting states with extensive temperature, desiccation, and chemical resistance).

Yeasts and molds become important in situations that restrict the growth of bacteria, such as in acidic or dry products. Yeasts can cause gas formation in juices and slime formation on fermented products. Mildew (black mold) on humid surfaces and mold formation on spoiled foods are also common.

Viruses are obligate, intracellular parasites that are specific to an individual host. Human viruses, such as Hepatitis A, cannot multiply outside the human body. Design features must include facilities for good employee handwashing and sanitation practices to minimize potential for product contamination. Bacterial viruses (phages), however, may contribute to starter culture failure in bacterial fermentations if proper isolation, ventilation, and sanitation procedures are not followed. The use of commercial concentrated cultures, selected for phage resistance, has greatly reduced this problem.

Sources of Microorganisms

Bacteria, yeasts, and molds are widely distributed in water, soil, air, plant materials, and the skin and intestinal tracts of humans and animals. Practically all unprocessed foods will be contaminated with a variety of spoilage and, sometimes, pathogenic microorganisms. Food processing environments that contain residual food material will naturally select for the microorganisms that are most likely to spoil the particular product.

Microbial Growth

Changes in microbial populations follow a generalized growth curve (Figure 1). An initial lag phase occurs as organisms start to grow and adapt to new environmental conditions. The lag phase is very important because the maximum extension of shelf life and length of production runs are directly related to the length of the lag phase. Once adaptation has occurred, the culture enters into the maximum (logarithmic) growth rate, and control of microbial growth is not possible without major sanitation or other drastic measures. Numbers can double as fast as every 20 to 30 min under optimum conditions.

Toxin production and spore maturation, if possible, usually occur at the end of the exponential phase as the culture enters a stationary phase. At this time, essential nutrients are depleted and/or inhibitory by-products are accumulated. Eventually culture viability declines; the rate depends on the organism, the medium, and other environmental characteristics. Although refrigeration prolongs generation time and reduces enzyme activity and toxin production, in most cases, refrigeration will not restore lost product quality or safety.

CRITICAL MICROBIAL GROWTH REQUIREMENTS

Factors that influence microbial growth can be divided into two categories: (1) intrinsic factors that are a function of the food itself

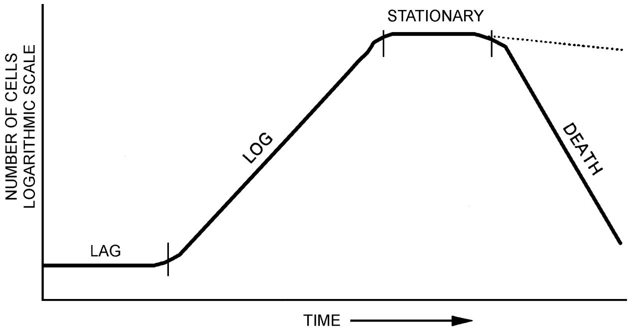

Fig. 1 Typical Microbial Growth Curve

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and (2) extrinsic factors that are a function of the environment in which a food is held.

Intrinsic Factors

Intrinsic factors affecting microbial growth include nutrients, inhibitors, biological characteristics, water activity, pH, and presence of competing microorganisms in a food. Although engineering practices have little impact on these parameters, an understanding of how intrinsic factors influence growth is useful in predicting the types of microorganisms that may be present.

Nutrients. Like other living organisms, microorganisms require food to grow. Carbon and energy sources are usually supplied in the form of sugars and starches. Nitrogen requirements are met by the presence of protein. Vitamins and minerals are also necessary. Lactic acid bacteria have rather exacting nutritional requirements, but many aerobic spore formers have tremendous enzymatic capabilities that allow growth on a wide variety of substrates. Cleanable systems facilitate the removal of residual food material and deprive microorganisms of the nutrients required for growth, thus preventing a buildup of organisms in the environment.

Inhibitors. Either naturally occurring or added as preservatives, inhibitors may be present in food. Preservatives are not substitutes for hygienic practices and, with time, microorganisms may develop resistance. A cleanable processing system is still essential in preventing the development of a resistant population.

Competing Microorganisms. The presence of one type of microorganism affects other organisms in foods. Some organisms produce inhibiting compounds or grow faster; others are better able to use the available nutrients in a food matrix.

Water Activity. All life-forms require water for growth. Water activity (a_w) refers to the availability of water within a food system and is defined at a given temperature as

$$a_w = \frac{\text{Vapor pressure of solution (food)}}{\text{Vapor pressure of solute (water)}}$$

The minimum water activities for growth of a variety of microorganisms, along with representative foods, are listed in Table 1. These a_w minima are also factors in environmental humidity control discussed in the section on Extrinsic Factors.

When food is enclosed in airtight packaging or in a chamber with limited air circulation, an equilibrium a_w is achieved that is equal to the a_w of the food. In these situations, the a_w of the food determines which organism will be capable of growth. If the same foods are exposed to reduced environmental relative humidity, such as meat carcasses hanging in a controlled aging room or vegetables displayed in an open case, surface dehydration acts as an inhibitor to microbial growth. Likewise, if a dry product, such as bread, is exposed to a moist environment, mold growth may occur on the surface as moisture is absorbed. Environmental relative humidity thus has a significant impact on product shelf life.

Table 1 Approximate Minimum Water Activity for Growth of Microorganisms

Organism	a_w	Foods
Pseudomonads	0.98	Fresh fruits, vegetables, meats
Salmonella spp., *E. coli*	0.95	Many processed foods
Listeria monocytogenes	0.93	
Bacillus cereus	0.92	Salted butter
Staphylococcus aureus	0.86	Fermented sausage
Molds	0.84	Soft, moist pet food
	0.80	Pancake syrup, jam
	0.70	Corn syrup
Xerotrophic molds	0.65	Caramels
Osmophilic yeasts	0.62	
Limit of microbial growth	0.60	Wheat flour
	0.40	Nonfat dry milk

pH. For most microorganisms, optimal growth occurs at neutral pH, or 7.0. Few organisms grow under alkaline conditions, while some organisms, such as yeasts, molds, and lactic acid bacteria, are acid tolerant. Figure 2 depicts pH values of a variety of foods and limiting pH values for microorganisms.

Biological Diversity

Most bacteria prefer specific environmental habitats, but others are more broadly adaptable because of enzyme systems they posess. Some grow well at low temperatures with or without oxygen (e.g., *Listeria monocytogenes*) or survive high temperatures as well (e.g., *Bacillus cercus*) and still grow at body temperature and cause illness.

Extrinsic Factors

Extrinsic factors that influence the growth of microorganisms include temperature, environmental relative humidity, and oxygen levels. Refrigeration and ventilation systems play a major role in controlling these factors.

Temperature. Microorganisms are capable of growth over a wide range of temperatures. Minimum growth temperatures for a variety of spoilage and pathogenic bacteria of significance in foods are summarized in Table 2. Previously, 45°F was thought to be sufficient to control the growth of pathogenic organisms. However, the emergence of psychrotrophic pathogens, such as *Listeria monocytogenes*, has demonstrated the need for lower temperatures. In the United States, 41°F is now recognized as the upper limit for safe

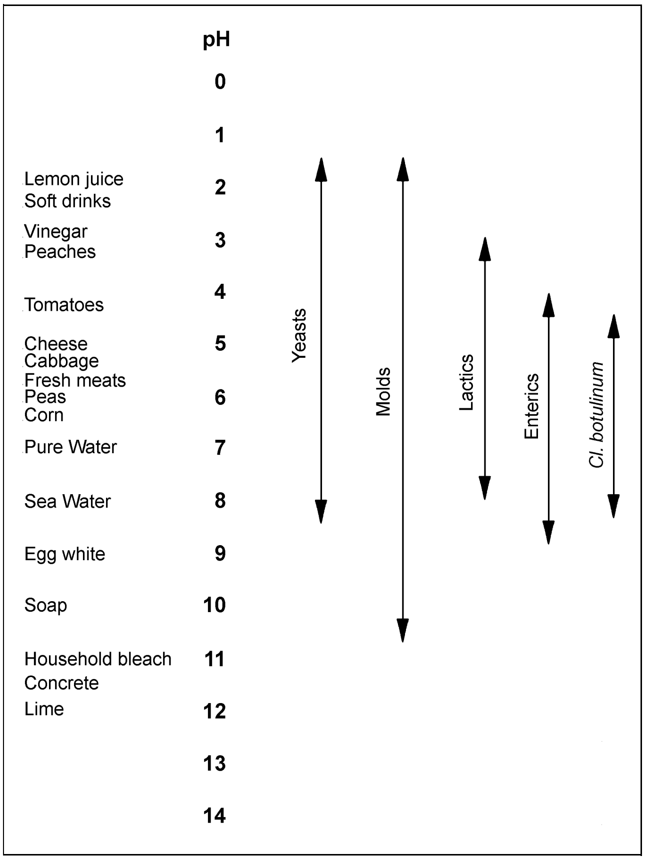

Fig. 2 pH Ranges for Microbial Growth and Representative Examples

Table 2 Minimum Growth Temperatures for Some Bacteria in Foods

Organism	Possible Significance	Approximate Minimum Growth Temperature, °F
Staphylococcus aureus	Food-borne disease	50
Salmonella spp.	Food-borne disease	42
Clostridium botulinum,	Food-borne disease	
proteolytic		50
nonproteolytic		38
Lactobacillus and *Leuconostoc*	Spoilage of fresh and cured meats	32
Listeria monocytogenes	Food-borne disease	34
Acinetobacter spp.	Spoilage of precooked foods	31
Pseudomonads	Spoilage of raw fish, meats, poultry, and dairy products	31

refrigeration temperature, while 34°F or lower may be more appropriate. Foods that will support the growth of pathogenic microorganisms should not be held between 41 and 140°F for more than 2 h.

Temperature is used to categorize microorganisms. Those capable of growth above 113°F, with optimum growth at 130 to 150°F, are **thermophiles**. Thermophilic growth can be extremely rapid, with generation times of 10 to 20 min. Thermophiles can become a problem in blanchers and other equipment that maintain food at elevated temperatures for extended periods. These organisms die or do not grow at refrigeration temperatures.

Mesophiles are organisms that grow best between 68 and 113°F. Most pathogens are in this group, with optimum growth temperatures around 98.6°F (i.e., body temperature). Mesophiles also include a number of spoilage organisms. Growth of mesophiles is quite rapid, with typical generation times of 20 to 30 min. Because mesophiles grow so rapidly, perishable foods must be cooled as fast as possible to prevent spoilage or potential unsafe conditions. Also, slower cooling rates cause mesophiles to adapt and grow at lower temperatures. With mild temperature abuse, prolific growth leading to spoilage or a potential health hazard can occur.

Psychrotrophs are organisms capable of growth at 41°F; some are able to grow at temperatures as low as 23°F and are a primary cause of spoilage of perishable foods. Psychrotrophic growth is slow in comparison to mesophilic and thermophilic growth, with maximum growth rates of 1 to 2 h or longer. However, control of psychrotrophic growth is a major requirement in products with extended shelf life. Because many psychrotrophs have optimum temperatures in the mesophilic range, what may seem to be an insignificant increase in temperature can have a major impact on the growth rate of spoilage organisms. Growth is roughly twice as fast with each 5°F increase in temperature. In practice, shelf life of fresh meat is maximized at 29°F and is reduced 50% by holding at 36°F. Meat freezes at 28°F.

For all the critical growth factors, the range over which growth can occur is characteristic for a given organism. The range for growth is narrower than that for survival. For example, the maximum temperature for growth is slightly above the optimum, and death usually occurs just slightly above the maximum. This is not the case at the lower end of the temperature range. Survival of psychrotrophic and most mesophilic microorganisms is enhanced by storage at low temperatures. Freezing microorganisms is not an effective lethal process. Some organisms, notably gram-negative bacteria, are damaged by freezing and may die slowly, but others are extremely resistant. In fact, freezing is used as an effective means of preserving microbial cultures at extremely low temperatures (e.g., –110°F).

Environmental Relative Humidity. Water, previously discussed as an essential intrinsic growth factor, is also a major extrinsic factor. Environmental water acts as a vector for transmission of microorganisms from one location to another through foot traffic or aerosols. Refrigeration drain pans and drip coils have been identified as significant contributors of *L. monocytogenes* contamination in food processing environments. Aerosols have also transmitted the agent that causes Legionnaires' disease. High relative humidity in cold rooms is a particular problem and leads to black mold buildup on walls and ceilings as well as growth of organisms in drains and other reservoirs of water. Condensation that forms on ceilings supports microbial growth and can subsequently drip onto product contact surfaces. Inadequately drained equipment collects stagnant water and supports microbial growth that is easily transported throughout a production facility when people walk through puddles. It is extremely important to control environmental relative humidity in food production environments. Control measures are discussed further in the section on Regulations and Standards.

Oxygen. Microorganisms are frequently classified by their oxygen requirement. Strictly aerobic microorganisms, such as molds and pseudomonads, require oxygen for growth. Conversely, strict anaerobes, such as *Clostridium* spp., cannot grow in the presence of oxygen. Facultatively anaerobic microorganisms (e.g., coliforms) grow with or without oxygen present, and microaerophiles, such as lactobacilli, grow best in conditions with reduced oxygen levels. Controlled atmospheric chambers for fruit storage use lower oxygen levels to prolong storage life by retarding growth of spoilage organisms in addition to influencing ripening processes. Vacuum packing of foods also uses this extrinsic growth factor by inhibiting the growth of strict aerobes, such as molds and pseudomonads.

DESIGN FOR CONTROL OF MICROORGANISMS

Microorganisms can be controlled by one of three mechanisms: prevention of contamination, prevention of growth, or destruction of the organisms themselves. Design of refrigeration and ventilation systems can impact all these areas.

Prevention of Contamination

To prevent the entry of microorganisms into food production areas, ventilation systems must provide adequately clean air. Because bacteria are generally transported through air on dust particles, 95% filters (as defined in ASHRAE *Standard* 52.1, Gravimetric and Dust-Spot Procedures for Testing Air-Cleaning Devices Used in General Ventilation for Removing Particulate Matter) are sufficient to remove most microorganisms. High-efficiency particulate air (HEPA) filters provide sterile air and are used for cleanrooms.

Air-filtering materials must remain dry. Wet filters in ventilation systems support microbial growth, and organisms are transported throughout the production facility in the air. All ventilation systems must also be protected from water and the formation of condensate to prevent mold growth. This may require increased airflow or dehumidification systems. Positive pressure in the production environment prevents the entry of airborne contamination from sources other than ventilation ducts. Air intakes for production areas must not be positioned toward areas that are prone to contamination, such as puddles on roofs, nesting sites for birds, and so forth. Microbial contamination from airborne sources has been recorded, but quantitative assessments are not available to specify design parameters.

Refrigeration drip pans are a significant source of *L. monocytogenes* contamination. Condensation drip pans should be plumbed directly to drain to prevent contamination of floors and subsequent transport of organisms throughout a production facility. Drip pans must be easily accessible to allow scheduled cleaning, thus preventing organism growth. Air defrost should be avoided in critical areas. Continuous glycol-sprayed evaporative coils offer advantages, because glycol has been found to trap and kill microorganisms.

Being hygroscopic, glycol depresses the dew point of the air, providing a drier environment.

Traffic flow through a production facility should be planned to minimize contact between raw and cooked products. This is mandated through USDA regulations in plants that cook meat products. Straight-line flow of a raw product from one end of a facility to the other prevents cross-contamination. Walls separating raw product from cooked (or dirty from clean), with positive pressure in the cooked area, should be considered, as this provides the best means of protection. Provide adequate storage facilities to allow separate storage of raw ingredients from processed products, especially in facilities that handle meat products, which are a significant source of *Salmonella*. Raw meat must not be stored with cooked meats and/or vegetables or dairy products.

Prevention of Growth

Water control is one of the most effective and most frequently overlooked means of inhibiting microbial growth. All ventilation systems, piping, equipment, and floors must be designed to drain completely. Residual standing water supports rapid microbial growth, and foot and forklift traffic transports organisms from puddles throughout the production facility.

Condensation on ceilings and chilled pipes also supports microbial growth and may drip onto product contact surfaces that are not adequately protected. Efforts to prevent condensation are essential to prevent contamination. Insulation of pipe and/or dehumidifying systems may be necessary, particularly in chilled rooms. Increased airflow may also be useful in removing residual moisture. Maintaining of 70% rh prevents the growth of all but the most resistant microorganisms; less than 60% rh prevents all microbial growth on facility surfaces (see Table 1).

Sanitation procedures use much water and leave the facility and surfaces wet. Adequate dehumidification should be provided for removal of moisture during and after sanitation.

Limiting relative humidity is not always possible. For example, aging meat carcasses requires relative humidities of 90 to 95% to prevent excessive drying of the tissue. In these cases, a temperature of 29°F, just above product freezing point, should be used to inhibit microbial deterioration. Temperatures below 41°F inhibit the most common organisms that cause food-borne illness; however, 34°F is required to inhibit *L. monocytogenes*. Airflow, relative humidity, and temperature must be finely balanced to achieve maximum shelf life with limited deterioration of quality.

Freezing is also an effective means of microbial control. Limited death may occur during freezing, especially during slow freezing of gram-negative bacteria. However, freezing is not a reliable means of inactivating microorganisms. Since no microbial growth occurs in frozen foods, as long as a product remains well below its freezing point, microbial safety issues are nonexistent. Frozen foods must be stored below 0°F for legal and quality reasons.

Destruction of Organisms

High temperature is an effective means of inactivating microorganisms and is used extensively in blanching, pasteurization, and canning. Moist heat is far more effective than dry heat. High temperatures (170°F) may also be used for sanitation when chemicals are not used. Although hot water sanitation is effective against vegetative forms of bacteria, spores are not affected.

In addition to heat, high pressure, pulsed electric fields, high-energy white light, irradiation, ultraviolet light, hydrogen peroxide, ozone, and sanitation chemicals are effective in destroying microorganisms.

THE ROLE OF HACCP

Many of the procedures for the control of microorganisms are managed by the Hazard Analysis and Critical Control Point (HACCP) system of food safety. Developed in the food industry since the 1960s, HACCP is now accepted by food manufacturers and regulators. It is a preventive system that builds safety control features into the food product's design and the process by which it is produced. The HACCP system is used to manage physical and chemical hazards as well as biological hazards. The approach to HACCP is described in the seven principles developed by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF 1998):

1. Conduct a hazard analysis and identify control measures.
2. Identify critical control points.
3. Establish critical limits.
4. Establish monitoring procedures.
5. Establish corrective actions.
6. Establish verification procedures.
7. Establish record keeping and documentation procedures.

Each food manufacturing site should have a HACCP team to develop and implement its HACCP plan. The team is multidisciplinary, with members experienced in plant operations, product development, food microbiology, etc. Because of their knowledge of the manufacturing facility and the process equipment, engineers are important members of the HACCP team. They help identify the potential hazards and respective control measures, implement the HACCP plan, and verify its effectiveness.

SANITATION

Cleaning and sanitation are key elements that incorporate all three strategies for control of microorganisms. The cleaning phase controls microbial growth by removing the residual food material required for proliferation. Sanitizing kills most of the bacteria that remain on surfaces. This prevents subsequent contamination of food being produced. Most microbial issues that occur in food processing environments are caused by unclean equipment, sometimes due to design. Therefore, equipment and facilities designed with cleaning and sanitizing in mind maximize the effectiveness of control.

Products that are frozen prior to packaging are particularly vulnerable to contamination. Many freezing tunnels in food processing facilities are difficult or impossible to clean because of limited access and poor drainage. Although freezing temperatures control microbial growth, proliferation of organisms does occur during downtime, such as on weekends. The following points should be considered during design phases to minimize potential problems:

- Provide good access for the cleaning crew to facilitate cleaning and adequate lighting (50 foot candles) to allow inspection of all surfaces.
- Eliminate inaccessible parts and features that permit product accumulation.
- Design equipment that is easy to dismantle using few tools, especially for areas that are difficult to clean. Design air-handling ducts for ease of cleaning. Provide removable spools or access doors. Washable fabric ducts designed and approved for such use are another option.
- Use smooth and nonporous construction materials to prevent product accumulation. Materials must tolerate common cleaning and sanitizing chemicals listed in Table 3. Consult sanitation personnel to determine chemicals likely to be used. Give special attention to insulation materials, many of which are porous. Insulation must be protected from water to avoid saturation and resultant microbial growth. An effective method is a well-sealed PVC or stainless steel cover. Avoid using fiberglass batts in food processing plants.
- All equipment must drain completely.
- Consult references and regulations on sanitary design principles.

Table 3 Common Cleaning and Sanitizing Chemicals

Cleaning Compounds	Sanitizers
Caustic	Chlorine
Chlorinated alkaline detergents	Iodophors
Acid cleaners	Quaternary ammonium compounds
	Acid sanitizers

Innovation is needed in the area of drying after cleaning is complete. Providing adequately sloped surfaces and sufficient drains to handle water is important. Dehumidification systems and/or increased airflow in new and existing systems could greatly reduce problems associated with water, especially in the cleaning of chilled production environments.

Standard water washing procedures are not appropriate for certain food production facilities such as dry mix, chocolate, peanut butter, or flour milling operations. Refrigeration or ventilation systems for plants of this type must be made to facilitate dry cleaning, reduce condensation, and restrict water to a very confined area if it is absolutely necessary.

REGULATIONS AND STANDARDS

Facilities and equipment should be designed and installed for minimizing microbial growth and maximizing the ease of sanitation. Care should be taken to employ material that can withstand moisture and chemicals.

The food industry has developed several equipment installation standards. In the United States, examples are International Association for Food Protection (IAMFES) 3-A Dairy standards, Baking Industry Sanitation Standards Committee (BISSC, Chicago, IL) bakery standards, and a select group of U.S. Department of Agriculture (USDA) standards for the meat industry. The USDA enforces the Federal Meat Inspection Act and Federal Poultry Inspection Act and requires approval of building and equipment plans.

Chapter VII, Section 701(A) of the Federal Food, Drug and Cosmetic Act as amended establishes current Good Manufacturing Practices (GMPs) in manufacturing, processing, packaging, or holding human food. These GMPs are listed in Section 21 of the Code of Federal Regulations (21 CFR), Part 110.

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