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Food Safety Engineering

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Food Safety Engineering

Springer

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Preface

Food safety engineering is an emerging discipline, which brings food engineering, microbiology, and food science disciplines together for safe food production at all stages of foods, from farm to table, by preventing foodborne diseases. While doing this, the quality of the foods must be maintained, which is an important guiding principle of food engineering in addition to providing affordable food. This is becoming a more and more significant issue as the world's population is expected to increase by 2 billion in the next 30 years, from 7.7 billion currently to 9.7 billion in 2050, and can go up about 11 billion by 2100 according to a recent United Nations report. Furthermore, food safety is still a concern even in the developed countries. For example, according to the US Centers for Disease Control and Prevention (CDC), each year, approximately 1 in 6 Americans gets sick, including 128,000 hospitalizations and 3000 deaths from foodborne diseases.

Enough safe foods with good quality to this growing world's population cannot be achieved just by only increasing the size of farmland or growing crops or animals by more efficient methods on the farm. We need to develop new processing, handling, storage, and transportation methods to provide good quality and safe foods. Again, according to a recent study by the Food and Agriculture Organization (FAO) of the United Nations, roughly one-third of food produced for human consumption is lost or wasted globally, which totals about 1.3 billion tons per year. This might be due to the lack of resources and technologies needed as well as the lack of education. Therefore, we must not only enhance the technologies at the affordable levels even for the underdeveloped and developing countries but also educate the public at the same time.

Improved technologies can provide better quality and safer foods. Enhancements of the technologies must start at the farm level – not only how to grow agricultural products in more effective ways, including genetically modified plants and animals, but also when and how to harvest them without reducing the quality. Furthermore, storage and transportation technologies are significant areas for improvements so that the quality and safety are ensured. The next enhancements must be done on the unit operation levels during the further processing of the foods by developing more

effective processing technologies to keep quality and safety while keeping the cost less. For these, novel methods are needed above and beyond the conventional methods. These must also include smart packaging and monitoring technologies, which not only keep the quality and safety but also provide alerts if quality or safety changes due to the failures or abuses during transportation and storage of the foods before the consumption. Preventive practices such as Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Points (HACCP) are essential to ensure the food safety. Also, when and if any outbreak happens due to the consumption of contaminated food products, traceability and recall strategies must be established to prevent further damages.

To educate and train the workforce for all things mentioned above, educational tools are essential. Therefore, this book has been designed to provide not only foundational knowledge required for food safety but also conventional processing methods and shed light for developments on food decontamination technologies as well as aseptic and post-packaging technologies. Therefore, the content of this book is divided into six parts. Part I provides an "overview of food microbiology" to cover background for intrinsic and extrinsic factors affecting microbial survival and growth in food systems, foodborne pathogens, microbial toxins, conventional and novel rapid methods for detection and enumeration of microorganisms, and interactions of foodborne pathogens with foods. Part II addresses "preventive practices" such as Good Manufacturing Practices (GMP), Sanitation Standard Operating Procedures (SSOP), Hazard Analysis and Critical Control Points (HACCP), Hazard Analysis and Risk-Based Preventive Controls (HARPC), as well as food traceability and recall strategies. Part III covers plant layout, equipment design, maintenance, and cleaning. Part IV includes mathematical modeling for microbial growth and inactivation by thermal and nonthermal processes. Part V focuses on conventional and novel preventive controls for food safety including conventional and advanced thermal processing technologies, irradiation, light-based technologies, high hydrostatic pressure, pulsed electric fields, ultrasonic processes, nonthermal plasma technology, as well as "hurdle technologies" for combined food processing technologies. Finally, Part VI covers aseptic processing and post-packaging technologies to prevent post- or cross-contaminations. Each chapter has been contributed by well-known experts in either academic institutions or the food industry.

In conclusion, we hope that this book will serve as a go-to reference for someone in the food and related industries to learn all aspects of food safety under one cover. It can also be a valuable textbook in the programs at universities and colleges all around the world.

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Part I
Overview of Food Microbiology

Chapter 1
Intrinsic and Extrinsic Factors Affecting Microbial Growth in Food Systems

Catherine Rolfe and Hossein Daryaei

1.1 Introduction

The ability of microorganisms to grow and sustain within a food product is determined based on the food composition and the environment, applied processing conditions, and the storage conditions of the food product throughout its shelf-life. Intrinsic factors are defined as the characteristics inherent to the food matrix. Whereas, extrinsic factors are the properties of environmental surroundings especially during processing and storage. From the initial individual ingredients to the final product during storage, various changes occur in the food matrix and environmental conditions potentially contributing towards product development.

To withstand these changes, each microorganism has a minimum and maximum threshold, along with optimum conditions for growth for each of the intrinsic and extrinsic parameters. These values, which are not absolute, are specific to each microorganism and vary amongst different species and strains. For a microorganism to establish within a food environment, it must be able to acquire the necessary nutrients needed for energy production and cellular biosynthesis. Suitable temperatures and atmospheric conditions are also essential. Understanding the metabolic requirements for each targeted microorganism reduces the chances of them potentially becoming problematic within a product. A combination of multiple factors can be used as a method to inactivate or inhibit the growth of spoilage and pathogenic microorganisms.

Through utilizing intrinsic and extrinsic factors, whether naturally occurring or artificially induced, actions can be taken to increase the overall quality and safety of a product. The adjustment of these parameters can aid in preventing/minimizing the

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establishment and growth of microbial populations or may be used to extend the shelf-life of foods. Factors which are innate to the food product itself are discussed below.

1.2 Intrinsic Factors

Factors which are inherent to foods are described as *intrinsic factors*. These are the physical, chemical, and biological properties within the food matrix which can be naturally or artificially occurring. Examples of such parameters include food composition, pH levels, moisture content, water activity, oxidation-reduction (redox) potential, antimicrobial components, and biological structures. Each one of these are discussed below in-depth.

1.2.1 Compositions of Foods

For growth and maintenance of metabolic processes within a food matrix, microorganisms require certain nutritional components. The five key components being: water, source of energy (carbohydrates, proteins, and lipids), source of nitrogen (amino acids and nucleotides), minerals, vitamins, and related growth factors (Jay et al. 2005). Although the amounts and types of nutrients varies depending on the microorganism, all microorganisms require the uptake of these nutrients from their immediate environment.

Water is not necessarily considered a nutrient, however, all microorganisms require water in the available form as a transport medium for metabolic reactions which aid in the synthesis of cell biomass and energy (Ray and Bhunia 2008). Details on the importance of water present in a food system will be discussed further in Sect. 1.2.3.

The amount of a certain nutrient within a food environment is dependent on the type of food product. For example, meat products have sufficient amounts of proteins, lipids, vitamins, and minerals but levels of carbohydrates are limited. Whereas plant products typically contain high quantities of various carbohydrates and limiting amounts of protein, minerals, and vitamins. Dairy products are commonly associated with abundant concentrations of all nutritional components (Ray and Bhunia 2008).

The nutritional requirements of Gram-positive bacteria (*Listeria*, *Staphylococcus,* and *Clostridium* spp., etc.) are the greatest followed by yeasts and then Gram-negative bacteria (*Escherichia coli*, *Salmonella,* and *Campylobacter* spp., etc.). Molds have the lowest nutrient requirements. Gram-positive bacteria contain a thick cell wall with multiple layers of peptidoglycan and lack an outer membrane. Gram-negative bacteria have a thin cell wall made from a single peptidoglycan layer, and also have an outer membrane containing lipopolysaccharides (LPS). The

differences observed between Gram-positive and Gram-negative bacteria can be attributed to the ability of Gram-negative bacteria and molds to synthesize a portion of their own nutrients, whereas Gram-positive bacteria need to acquire much of their necessary nutrients from the environment (Jay et al. 2005).

As an energy source, microorganisms are able to use simple carbohydrates (e.g., glucose, sucrose, and lactose), alcohols, and amino acids. Some microorganisms are able to use complex carbohydrates (e.g., starches and cellulose), large proteins (e.g., casein), and lipids. The ability to use these complexes as an energy source is achieved through production of extracellular enzymes, which can hydrolyze the compounds to a simpler form outside of the cell, allowing for their transport into the cell. This action is most commonly observed in molds. In addition, intracellular enzymes capable of converting large compounds into simpler forms can be released during cell death and lysis. This allows for a mixed population within the same food environment. Microorganisms which are not able to produce their own extracellular enzymes can utilize the product from reactions of lysed intracellular enzymes (Jay et al. 2005; Ray and Bhunia 2008).

Nitrogen source is required by microorganisms for the synthesis of amino acids, proteins, DNA, and RNA. As a nitrogen source, amino acids are most frequently utilized. Other compounds such as nucleotides, peptides, and proteins can also be metabolized (Jay et al. 2005).

Small amounts of elements such as phosphorus, calcium, magnesium, iron, sulfur, manganese, and potassium are required for cellular metabolism (Ray and Bhunia 2008). Generally, these elements are readily available within a food system and easily acquired. B vitamins are also a requirement for microorganisms. Although present at sufficient quantitates within most food systems, some microorganisms are self-sufficient for synthesizing B vitamins. Gram-positive bacteria tend to require B vitamins in their environment, whereas Gram-negative bacteria and molds are able to synthesize them through their own processes (Jay et al. 2005).

1.2.2 pH

The pH level within a food system can greatly affect which microorganisms are able to grow and persevere. Many bacteria grow in the pH range of 4.5–9.0, and many yeasts and molds grow in the pH range of 2.0–10.0. Optimum growth of most microorganisms is observed around neutral pH levels (e.g., 6.6–7.5), with a small number being able to grow below a pH of 4.0 (Jay et al. 2005). Fermentation or the addition of acids can decrease the pH levels and, in turn, cause growth restrictions and/or death of various microbial cells (Levine and Fellers 1940). Bacteria tend to have a narrower range of pH levels compared to yeasts and molds, especially pathogenic foodborne bacteria. In addition, Gram-negative bacteria are shown to be more sensitive to extreme pH levels compared to Gram-positive bacteria (Mendonca et al. 1994; Ray and Bhunia 2008). The pH growth ranges of

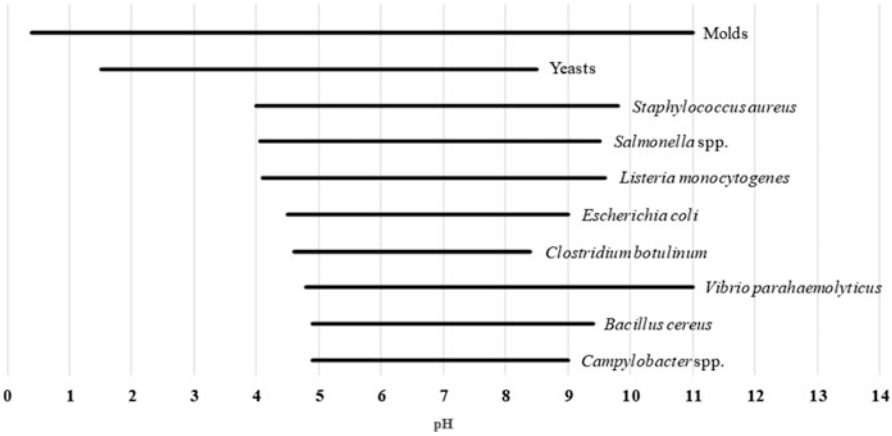

Fig. 1.1 The pH growth ranges for foodborne microorganisms. (Adapted from Jay et al. 2005)

microorganisms within food systems (Fig. 1.1) is not an exact measurement, as many factors play a role in the survival of cells in relation to pH levels.

Table 1.1 presents the pH range for the growth of selected bacteria in different food products. *Lactobacillus* spp., for example, can grow at a pH level as low as 2.8. This is associated with the production of yogurt through fermentation of milk by various lactic acid bacteria, resulting in an acidic environment, which many pathogenic microorganisms cannot endure. Starter cultures are frequently adapted to this condition in order to maintain their levels throughout shelf-life. Similarly, spoilage by yeasts and mold is commonly observed in fruits. This is contributed to the growth of these microorganisms at levels below that of other spoilage and pathogenic microorganisms.

Food products can be grouped into high-acid (pH below 4.6) and low-acid (pH above 4.6) foods. This distinction is based on the ability of the foodborne pathogen *Clostridium botulinum* to grow at the lower limit of pH 4.6. High-acid foods are associated with products such as fruits, fruit juices, salad dressings, and fermented foods. Low-acid foods include most vegetables, meats, fish, breads, and milk. Even though a product is considered low-acid, the majority of low-acid foods are still below pH 7.0. The acidic conditions within food systems can be naturally occurring (most commonly due to weak acids), produced during fermentation, or added during processing. Certain foods can demonstrate a buffering capacity and resist a decrease in pH, such as milk and meat products. In dairy products, the phosphate, citrate, organic acids, caseins, and whey proteins that are present contribute towards resisting pH changes (Salaün et al. 2005). Similarly, in meat products, higher protein content correlates with an increase in buffering capacity. The pH of foods with a lower buffering capacity readily changes with small changes in acidic or alkaline conditions. Whereas, foods which have a higher buffering capacity require greater quantities of acid/alkaline to reach a target pH (Sebranek 2004). During spoilage, carbohydrate-rich foods tend to undergo acid hydrolysis causing

Table 1.1 The pH range for the growth of selected bacteria and examples of associated food products

Bacteria	pH range[a]	Associated food products
Lactobacillus spp.	2.8–7.0	Dairy products, vacuum- or modified atmosphere-packaged meat and poultry products, alcoholic beverages, soft drinks, canned and acidified or fermented fruit and vegetable products
Staphylococcus aureus	4.0–9.8	Raw meat and poultry, ham, seafood, unpasteurized milk and dairy products, egg products, salads, cakes, pastries
Salmonella spp.	4.0–9.5	Raw meat and poultry, eggs, unpasteurized milk
Listeria monocytogenes	4.1–9.6	Unpasteurized milk, soft cheeses made from unpasteurized milk, raw meat and poultry, deli meats, pâté, fermented sausages, raw fruits and vegetables, seafood
Yersinia enterocolitica	4.2–9.0	Unpasteurized or inadequately pasteurized milk, non-ripened/non-fermented dairy products, raw or undercooked meats (especially pork) and poultry, seafood, vegetables, miscellaneous prepared foods, including salads
Escherichia coli O157:H7	4.4–9.0	Raw or undercooked ground beef, unpasteurized milk and beverages
Shigella spp.	4.8–9.3	Raw vegetables, salads
Clostridium botulinum Group I (proteolytic: type A and some of types B and F)	4.6–8.5	Home-preserved foods (e.g., canned vegetables and cured meats)
Vibrio parahaemolyticus	4.8–11.0	Raw or undercooked seafood, including fish and shellfish
Bacillus cereus	4.9–9.3	Meat and meat products, pasteurized liquid egg products, starchy foods (e.g., rice, potatoes, and pasta), ready-to-eat vegetables, milk and dairy products, sauces, puddings, soups, casseroles, pastries, salads
Clostridium botulinum Group II (nonproteolytic: type E and some of types B and F)	5.0–8.5	Minimally heated, chilled foods, vacuum-packaged smoked fish
Clostridium perfringens	5.0–8.3	Meat and poultry products
Pseudomonas spp.	5.0–9.0	Fresh vegetables, refrigerated meat and poultry products, fish, eggs, milk and dairy products

[a]The pH range can be narrower depending on the strain/species and other factors such as the type of acid/acidulant, a_w, and temperature.
Adapted from Jay et al. (2005), Wareing et al. (2010b, c).

the pH level to lower. Alternatively, protein-rich foods tend to demonstrate an increase in pH level, raising concern for the growth of foodborne pathogens (Ray and Bhunia 2008; Wareing et al. 2010a).

When the pH decreases below the ideal pH range, microbial growth stops and loss in viability occurs. The extent to which the cells lose viability is determined by the degree of pH drop. Weak acids cause a greater loss in viability, primarily those with higher dissociation constants (pKa). Undissociated molecules are lipophilic, and when entering the cell cause an increase in H^+ and lowers the pH of the cytoplasm (Ray and Bhunia 2008; Miller et al. 2009). Alvarez-Ordóñez et al. (2010) investigated the growth of *Salmonella* Typhimurium under acidic conditions using different types of acids and found the growth to be most affected as follows:

$$\text{Acetic acid} > \text{Lactic acid} > \text{Citric acid} > \text{Hydrochloric acid}$$

Their study demonstrated the inhibitory properties of weak acids (acetic, lactic, and citric) compared to those of strong acids (hydrochloric), due to their lack of dissociation and ability to enter the cell membranes. The intracellular pH needs to be maintained above a critical pH point. If not, membrane transport functions and essential biochemical pathways are destroyed, intracellular proteins are irreversibly denatured, and ultimately results in cell death. The following responses are associated with maintaining the intracellular pH in the presence of undissociated molecules: homeostatic response, acid tolerance response, and synthesis of acid shock protein (Montville and Matthews 2007). Details on these responses are described in Sect. 1.4.1.

1.2.3 Water Activity

The influence of water on shelf-life and preservation of food products has been utilized for many centuries. Traditional methods of drying (desiccation) have been observed in early records, however, the origin of this technique is unclear. In desiccation (dehydration), moisture within the product is removed or bound and thus the food is preserved. Methods such as heating or freeze drying, and the addition of humectants (salts, sugars) can be used to lower the water activity through removal or binding of water, respectively. Without the availability of water, potentially harmful microorganisms are not able to survive and proliferate. The water requirements of microorganisms are described in reference to the water activity (a_w) in the food system. Water activity is defined as the ratio of the water vapor pressure (i.e. the pressure that vaporized water molecules produce) of food substrate to the vapor pressure of pure water at the same temperature (Eq. 1.1):

$$a_w = p/p_o \tag{1.1}$$

where p is the vapor pressure of the solution and p_o is the vapor pressure of the pure solvent (generally water). This equation is also in relation to equilibrium relative humidity (ERH) (Eq. 1.2) (Jay et al. 2005):

$$a_w = ERH/100 \tag{1.2}$$

For the theoretical analysis of a_w in single-component solutions, the solution using thermodynamic approach is applicable, and a_w can be calculated using the following equation (Kozak et al. 1968; Miyawaki et al. 1997):

$$a_w = \gamma_w X_w = \gamma_w (1 - X_s) \tag{1.3}$$

where γ_w is the activity coefficient of water, X_w is the molar fraction of water, and X_s is the molar fraction of solute.

The term "water activity" is commonly confused with moisture or water content, which is a volumetric analysis for total amount of water present. For example, a certain product may contain a higher amount of water content and be considered safe, whereas another product with lower water content is susceptible to microbial growth. The product with higher water content is seen as "wetter", but the water is chemically bound to other components within the food matrix making it unavailable (lowering a_w). The bound water cannot be removed by normal drying and only freezes at very low temperatures. Water has a dominating effect on the mobility of hydrophilic food components due to its plasticizing action.

The water activity of pure water is 1.00 and the majority of fresh produce have an a_w above 0.99 (Jay et al. 2005) while the majority of low-moisture foods have an a_w of <0.60 and shelf-stable foods with an a_w between 0.60 and 0.85 (Beuchat et al. 2013).

Water activity can then be used as a tool to determine the microbial stability of food, as well as the rate of some chemical reactions/changes in food such as lipid oxidation, browning, and vitamin loss. Most spoilage bacteria do not grow below a_w of 0.91 and most do not persist below a_w of 0.85. Common spoilage bacteria encountered are *Pseudomonas* spp., *Lactobacillus* spp., and *Flavobacterium* spp. Halophilic bacteria, such as some *Vibrio* spp. and *Micrococcus* spp., may grow and spoil food products with high salt contents (e.g., salted fish) at a_w levels as low as 0.75. Whereas yeasts and molds can grow at a_w as low as 0.61 (xerophilic molds and osmophilic yeasts), and are the primary spoilage microorganisms at a_w levels <0.85 (Beuchat 1983). Yeasts such as *Zygosaccharomyces* spp. and molds such as *Aspergillus* spp. are frequently associated with food spoilage. The mold-free shelf-life (MFSL) of foods (in days) at 21°C can be estimated using the Eq. 1.4 (Cauvain and Seiler 1992):

$$MFSL = 10^{7.91-8.1(a_w)} \tag{1.4}$$

For example, the estimated MFSL at 21 °C of foods with water activities of 0.75, 0.85, and 0.90 will be 68, 10.6, and 4.2 days, respectively.

For pathogenic bacteria, *Staphylococcus aureus* can grow at a_w as low as 0.86. *C. botulinum* cannot grow below an a_w of 0.94, with type E seeming to be less resistant to low water activity compared to that of types A and B (Beuchat 1981; Jay et al. 2005). Although an a_w level may be lower than the requirement for pathogenic bacterial growth, the low water activity may send the bacteria into a survival mode and remain in a dormant state until they are able to resuscitate. Some microorganisms, including foodborne pathogens may survive in very low a_w environments, depending on their osmotic and/or dry stress resistance. For example, *Cronobacter sakazakii* can survive in powdered infant formula with an a_w of about 0.2 (Breeuwer et al. 2003). In a recent study, Gill et al. (2020) have shown that Shiga toxin-producing *Escherichia coli* (STEC) can survive in wheat flour (a_w below 0.5) for up to 2 years of storage. Bacteria can protect themselves against increasing osmolarity and survive in low a_w environments by using different mechanisms, such as a rapid intracellular accumulation of ions, mainly K^+, followed by an accumulation of compatible solutes such as proline, glycine betaine, and trehalose (Kempf and Bremer 1998; Burgess et al. 2016). In addition, the a_w can be influenced by varying pH levels and temperatures. Microorganisms can grow and survive in a wide range of water activity, requiring careful evaluation of the water activity within the food matrix to minimize the risks.

In many food products, especially those that are ready-to-eat (RTE), the low a_w contributes to the control of food stability and preventing microbial growth. Bacteria, yeasts, and molds have varying limiting levels of a_w below which they do not grow or produce toxins (Fig. 1.2). Different ranges of water activity are observed for minimum growth levels of common microorganisms of concern in food products (Table 1.2). In a laboratory setting, minimum a_w levels are frequently analyzed using prepared media with the addition of salts and/or sugars. Although water activity

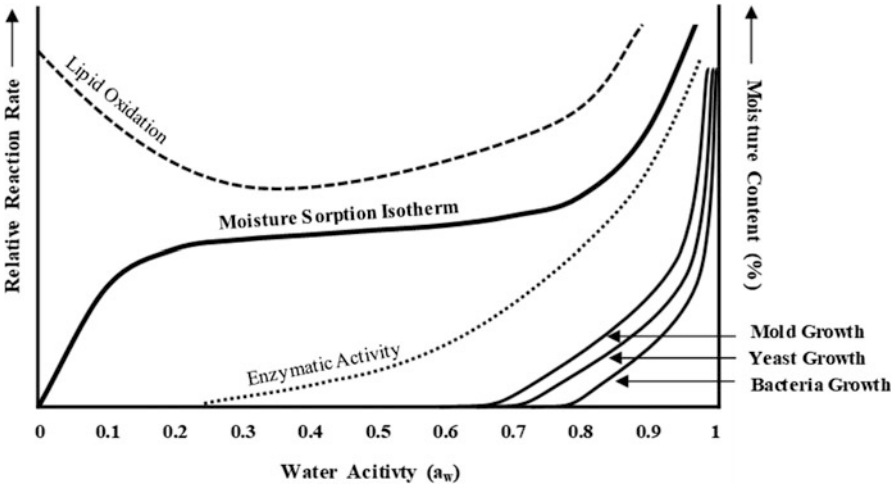

Fig. 1.2 Rates of degradative reactions and stability of microbial growth in correlation with water activity. (Adapted from Labuza and Altunakar 2007)

Table 1.2 Minimum water activity (a_w) values for the growth of selected microorganisms and examples of associated food products

Microorganisms	Minimum a_w	Associated food products
Clostridium botulinum Group II (nonproteolytic: type E and some of types B and F)	0.97	Minimally heated, chilled foods, vacuum-packaged smoked fish
Pseudomonas spp.	0.97	Fresh vegetables, refrigerated meat and poultry products, fish, eggs, milk and dairy products
Acinetobacter spp.	0.96	Fresh meat and poultry, fish, shellfish, eggs, milk, vegetables, soft drinks
Escherichia coli O157:H7	0.96	Raw or undercooked ground beef, unpasteurized milk and beverages
Bacillus subtilis	0.95	Bakery products, synthetic fruit drinks, mayonnaise, meat, seafood with rice
Candida spp.	0.90	Dairy products, salad dressings, meat products, seafood, fruit products, including fruit juices
Clostridium botulinum Group I (proteolytic: type A and some of types B and F)	0.94	Home-preserved foods (e.g., canned vegetables and cured meats)
Salmonella spp.	0.94	Raw meat and poultry, eggs, unpasteurized milk
Vibrio parahaemolyticus	0.94	Raw or undercooked seafood, including fish and shellfish
Mucor spp.	0.90	Fruits and vegetables, yogurt, cheese, cereals
Rhizopus spp.	0.93	Fruits and vegetables
Listeria monocytogenes	0.92	Unpasteurized milk, soft cheeses made from unpasteurized milk, raw meat and poultry, deli meats, pâté, fermented sausages, raw fruits and vegetables, seafood
Staphylococcus aureus	0.86	Raw meat and poultry, ham, seafood, unpasteurized milk and dairy products, egg products, salads, cakes, pastries
Alternaria spp.	0.84	Cereals, fruits and vegetables
Penicillium griseofulvum (formerly P. patulum or P. urticae)	0.81	Cereals, nuts, meat and meat products
Eurotium repens	0.72	Dried foods, high-sugar products (e.g., confectionery, dried fruits, and jams), cheese, meat products
Aspergillus spp.	0.70	Cereals, dried foods, fruits and vegetables
Zygosaccharomyces rouxii	0.62	Confectionery products, jams, jellies, fruit concentrates, syrups, honey, oriental fermented products (e.g., soy sauce), dried fruits
Xeromyces bisporus	0.61	Dried fruits, confectionery products

Adapted from Jay et al. (2005), Wareing et al. (2010b, c, d)

levels can be controlled to a defined limit, the use of prepared media does not provide an exact representation of a realistic food system. This can have implications as the food matrix itself and other conditions play important roles for the behavior of water activity and microorganisms. Other factors such as pH, temperature conditions/fluctuations, oxygen concentration, and nutrient availability can influence microbial growth (Tapia et al. 2007).

The relationships of temperature-resistant microorganisms and water activity levels have been studied. Cells may become heat tolerant when slightly dehydrated and exposed to high temperatures compared to those in higher a_w levels (Goepfert et al. 1970; Villa-Rojas et al. 2013; Finn et al. 2013; Smith and Marks 2015). The proteins and other cellular components become more resistant to heat injury at lower a_w levels, especially in spore-forming organisms. Heat resistance of spores has shown to increase as the a_w decreases. Cells with very low moisture (but not completely desiccated) exhibit greatest resistance. An a_w range of 0.2–0.4 was shown to provide the maximum thermal stability in most of the microorganisms (Murrell and Scott 1966).

Products may have a low enough water activity level to inhibit the growth of microorganisms. However, bacterial cells may still be viable and reproduce when products are rehydrated, potentially allowing these microorganisms to revert back to their normal state once returning to optimal conditions. This response can be attributed to stress adaptations occurring from the exposure to a mild form of the same or different stressors through cross-tolerance (Gupte et al. 2003). Dried products are frequently exposed to multiple stress conditions within the production process, being of particular interest to low-moisture, shelf-stable products. The duration of dehydration process has also shown to influence the survival of bacterial cells. An increase in the rate of dehydration has shown to provide greater lethal effects on *Salmonella*, attributed to the *de novo* synthesis of proteins necessary for survival in harsh conditions (Gruzdev et al. 2012).

The moisture sorption isotherm (MSI) of a food is acquired from the equilibrium moisture contents determined at multiple a_w levels while maintaining a constant temperature. This can be viewed within a graph as moisture content of a food compared to water activity (p/p_o) at a constant temperature (Reid and Fennema 2007). The beneficial information gained from establishing a MSI include: details on controlling concentration and dehydration processes, formulating food mixtures to prevent moisture transfer, determine the moisture content to hinder the growth of microorganisms, define moisture barrier properties required from packaging materials, and to predict chemical/physical stability with changing moisture content (Reid and Fennema 2007). Each product has its own unique moisture sorption isotherm, with the shape determined by the different interactions between water and solid components at various moisture contents. The low moisture segment of a MSI is most applicable, as this region typically shows the critical a_w value related to a phase transition. Resorption and desorption isotherms can be created through addition of water to dried samples or dehydration of a sample, respectively (Fig. 1.3). Isotherms generally follow a sigmoidal shape; however, certain foods with large amounts of

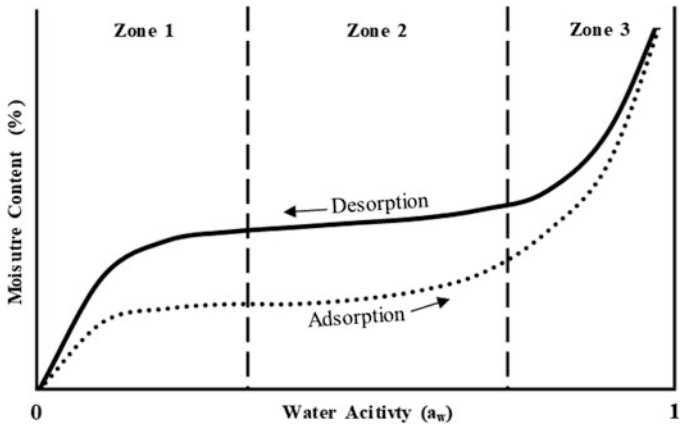

Fig. 1.3 Moisture sorption isotherm showing resorption (adsorption) and desorption with hysteresis. (Adapted from Berk 2013)

sugar and other small soluble molecules may take on J-shaped curve (Barbosa-Cánovas and Juliano 2007).

The shape and positions of the isotherms are dependent on sample composition, sample structure, pretreatments of the sample, temperature, and treatment method. MSIs are often divided into zones: Zone 1 contains the lowest water concentrations, Zone 2 is at intermediate water concentrations, and Zone 3 is at high water concentrations (Fig. 1.3). Water in Zone 1 is strongly bound and the least mobile, acting as part of the solid and very difficult to remove. Zone 2 contains loosely bound water, interacting through hydrogen bonding with adjacent water molecules and solutes. The boundary between Zones 1 and 2 is referred to as the Brunauer-Emmett-Teller (BET) monolayer and represents the monolayer moisture value. At this value, all available polar sites of the dry matter are permeated with water in a single monolayer. If water is continuingly added, full hydration of macromolecules will be reached and progression into bulk-phase water occurs. In Zone 3, water is easily removed with decreased viscosity and increased molecular mobility. Water in Zone 3 is available as a solvent and supports microbial growth (Reid and Fennema 2007).

Though both resorption and desorption isotherms are used, they are not necessarily overlaid onto one another for the same food product. This gap between resorption and desorption is referred to as a "hysteresis". At a given water activity, the water content within a sample will be greater during desorption than during the resorption process. Inversely, at a given moisture content, the water activity during desorption can be notably lower than that of resorption (Sperber 1983). The extent of the hysteresis, shape of the curves, and beginning/end of the hysteresis loop is dependent on factors involving the natural state of the food, physical changes caused from water removal or addition, fluctuation in temperature, and the rate/degree of water removal during desorption (Reid and Fennema 2007). At higher temperatures, hysteresis is less noticeable and more pronounced as temperatures decrease.

1.2.4 Oxidation-Reduction (Redox) Potential (Eh)

The oxidation-reduction potential (redox or Eh) of a substrate measures the ability of the substrate to gain or lose electrons. When a compound loses an electron, this is described as the substrate being oxidized. In contrast, when a compound gains an electron, this is described as the substrate being reduced. A substance that donates the electron is called the reducing agent. A substance that takes up the electron is called the oxidizing agent. The transfer of electrons creates a potential difference between the compounds, measured in electrical units of millivolts (mV). Redox is expressed as +mV when in the oxidizing range and expressed as –mV when in the reducing range. Oxidation may also be caused by the addition of oxygen, but the presence of oxygen is not required for redox reactions (Ray and Bhunia 2008).

Within a food system, the Eh is influenced by the chemical composition, processing treatments, and storage conditions. Specifically, the natural Eh of the food system, resistance of the system to a change in the Eh (*poising capacity*), oxygen tension in the atmosphere, and contact of the atmosphere with the food product (Jay et al. 2005). Fresh foods from plant and animal origin are in a reduced state. This is due to the reducing substances such as ascorbic acid, reducing sugars, and –SH group of proteins. Processing using thermal treatments can increase or decrease reducing compounds and change the Eh. Chopping, grinding, and mincing of products will increase the Eh. Foods stored with air can have a higher Eh (+mV) than those that are vacuum-packaged or in modified gases. Oxygen can exist in its gaseous state or dissolved form within a food system (Ray and Bhunia 2008; Kornacki 2010).

1.2.5 Antimicrobial Components

Certain food products have a natural defense against foodborne microorganisms that cause spoilage and disease. These defense strategies are commonly observed in plant species which contain natural antimicrobial substances. Examples include essential oils which are found in cloves, garlic, cinnamon, mustard, sage, and thyme (Wareing et al. 2010a). Siroli et al. (2018) studied the response mechanisms of *E. coli* K12 towards sublethal applications of thyme essential oil, carvacrol, 2-(E)-hexanal, and citral. Results demonstrated disruptions in fatty acid biosynthesis and membrane composition, along with mechanisms involved with energy metabolism and oxidative stress protection.

Natural antimicrobials are also found in milk and egg whites. Cow's milk contains lactoferrin and conglutinin proteins, along with the lactoperoxidase enzyme system, all of which demonstrate antimicrobial activity (Clare et al. 2003; Jay et al. 2005). A study conducted by Baron et al. (1997) investigated the growth of *S. enteritidis* within egg whites and the inhibitory mechanisms. Results suggested that egg white proteins, in specific, ovotransferrin (conalbumin), play a major role in preventing bacterial growth. Ovotransferrin is suspected of causing nutrient

deficiencies in bacterial cells through sequestering the iron necessary for proliferation. In addition, milk and egg whites also contain an enzyme called lysozyme which is able to lyse the carbohydrate chains in the peptidoglycan layer of Gram-positive bacteria and destroy the bacterial cell wall.

1.2.6 Biological Structures

One of the more obvious defenses against the microorganisms is the natural covering which provides protection from damage and reduces the risk of spoilage. Structures such as the outer coverings on fruits, the shells of nuts, and shells of eggs prevent the entry of foodborne pathogens and spoilage microorganisms (Jay et al. 2005).

In addition, individual confined microenvironments within a food product can result in different microbial survival and persistence. Li et al. (2014) demonstrated the effects of the immediate microenvironment in relation to *Salmonella* inactivation in a multi-ingredient food matrix. Introduction of the pathogen to different ingredients provided dissimilar levels of inactivation, suggesting certain ingredients allow for more favorable conditions for *Salmonella* survival. This could be caused by differing nutrient availability for microorganisms in the local microenvironments or alternative water activities present in the ingredients in contrast to the finished product. Additionally, ingredients with higher lipid compositions may have a protective effect on *Salmonella* compared to those with lower fat content.

1.3 Extrinsic Factors

Factors which are controlled through external conditions are described as *extrinsic factors*. These are related to the food processing and storage parameters. Examples include temperature, relative humidity, gaseous environments, the presence of other microorganisms, and processing operations. Typically, a combination of intrinsic and extrinsic factors are used to maintain food quality and safety.

1.3.1 Temperature

Temperature can influence enzymatic reactions and microbial growth in food systems. Foodborne pathogens and spoilage microorganisms are able to grow in a range of temperatures. The following categories of microorganisms have been established based on their growth temperature ranges: psychrophiles (subzero – 20 °C), psychrotrophs (0–20 °C), mesophiles (20–45 °C), and thermophiles (55–65 °C) (Jay et al. 2005). Psychrotrophic microorganisms are considered cold-tolerant and ubiquitous in natural food environments, whereas psychrophilic microorganisms are

cold-loving and permanently restricted to extreme cold environments (Gounot 1986). Growth temperatures for yeasts and molds demonstrates a broad range of 10–35 °C, with some species growing at temperatures below and above these thresholds (Tournas et al. 2001).

Psychrotrophs are most commonly observed within the *Pseudomonas* and *Enterococcus* genera, growing well at refrigerated temperatures and causing spoilage of refrigerated products such as meats, poultry and vegetables (Jay et al. 2005). With the refrigeration of food products, a reduction in the growth and types of microorganisms occurs once temperatures begin to decrease. For instance, at approximately 12 °C, growth of strict anaerobes is stalled along with *C. perfringens* and proteolytic strains of *C. botulinum* (type A and some of types B and F). At approximately 3 °C, nonproteolytic strains of *C. botulinum* (type E and some of types B and F) are unable to grow. Although 3 °C was once thought to prevent the growth of all pathogens, *Listeria monocytogenes* and *Yersinia enterocolitica* have shown capable of growth at temperatures below 1 °C (Gould 2000). Storage and preservation of foods through freezing at a temperature of approximately −18 °C prevents microbial growth but cannot be considered a kill step alone and commonly used in combination with other inactivation treatments (Gould 2000; Montville et al. 2012).

Thermophiles are frequently associated with canning/retort products, belonging to the genera such as *Bacillus*, *Clostridium,* and *Geobacillus* (Jay et al. 2005). Heat pasteurization techniques are commonly used in industrial processing, targeting the inactivation of spoilage microorganisms and vegetative, non-spore-forming pathogens. Commercial sterilization (canning/retort) is used to provide a lethal treatment for all microorganisms, however, *C. botulinum* spores may still be present though unable to germinate in the low pH or low a_w environment of the product (Montville et al. 2012).

The above classification is applicable to foodborne microorganisms. There are other microorganisms (bacterial and archaeal species) which can grow optimally at temperatures between 80 and 110 °C, called hyperthemophiles. They have been isolated from all types of hot terrestrial and marine environments, including natural and man-made environments. Their enzymes have unique structural and functional properties which provide high thermostability and optimal activity at temperatures above 70 °C (Vieille and Zeikus 2001).

1.3.2 Relative Humidity

As mentioned in the water activity Sect. 1.2.3, relative humidity can influence the water activity (a_w) level especially on the food surface and therefore can influence the growth of microorganisms. Packaging solutions are frequently used to prevent the exchange/migration of moisture from the environment into the product as it can increase the a_w of the product. Additionally, control of relative humidity is commonly used in industrial sanitation techniques through air and surface dehumidification systems following disinfection procedures (Esbelin et al. 2018).

1.3.3 Gaseous Environment

Similar to microbial behavior towards pH and water activity, microorganisms demonstrate a range of sensitivity related to the gaseous environment they are in contact with. Aerobic microorganisms (requiring oxygen for growth) necessitate a positive redox potential (oxidized) for growth. During aerobic respiration, oxygen is used as the final electron acceptor. Anaerobic microorganisms (not requiring oxygen for growth) require a negative redox potential value (reduced). During anaerobic respiration, a molecule other than oxygen is used as the final electron acceptor. For example, certain microorganisms use nitrate or sulfate ions for anaerobic respiration. These ions have smaller reduction potentials than oxygen and therefore releases less energy when oxidized. Certain microorganisms are obligate aerobes (*Pseudomonas* spp., *Bacillus* spp., *Flavobacterium* spp., and most yeasts and molds, etc.) requiring oxygen and high redox potential from the food environment in which they are present (Ray and Bhunia 2008). On the other hand, strict anaerobes such as *Clostridium* spp. cannot tolerate the presence of oxygen and require low redox potential (Kornacki 2010).

Anaerobic microorganisms can be facultative anaerobes and aerotolerant anaerobes. Facultative anaerobes include microorganisms that have greater growth in the presence of oxygen but are able to grow under anaerobic conditions. Examples of facultative anaerobes are *Lactobacillus* spp., *Pediococcus* spp., and enteric pathogens of *Salmonella* spp., *E. coli*, and *Listeria* spp. Aerotolerant anaerobes include those that tolerate oxygen in their environment but do not utilize it, such as *Enterococcus* spp. An additional category includes microaerophiles, which are those that require less oxygen than that of atmospheric conditions. *Campylobacter* spp. is a microaerophile (Jay et al. 2005). Figure 1.4 demonstrates the typical growth pattern of different categories of microorganisms based on the presence of oxygen.

Obligate aerobes grow at the Eh (redox potential) range of +500 to +300 mV, facultative anaerobes at the Eh range of +300 to +100 mV, and obligate anaerobes at the Eh range of +100 to −250 mV or lower (Ray and Bhunia 2008). The growth of microorganisms within a food package can be controlled by altering the package atmosphere. Vacuum packaging (i.e. removing available air within a package) is a common method for preventing the growth of aerobic microorganisms; however, aerobic microorganisms such as *C. botulinum* may still grow in vacuum-packaged foods. Foods of plant origin typically have an Eh value of between +300 and +400 mV favoring the growth of aerobic bacteria and molds, whereas solid meat has an Eh value of −200 mV favoring the growth of anaerobic microorganisms (Wareing et al. 2010a). Depending on the product and target microorganism of concern, packaging environments can be adjusted to include specific levels of oxygen (O_2), carbon dioxide (CO_2), and nitrogen (N_2). Referred to as modified atmosphere packaging (MAP), this technology allows for the control and prevention of the growth of spoilage and disease-causing microorganisms, while allowing fermenting bacteria to continue to grow (Gould 2000). MAP is a standard method for extending the shelf-life and preserving the quality attributes of a variety of

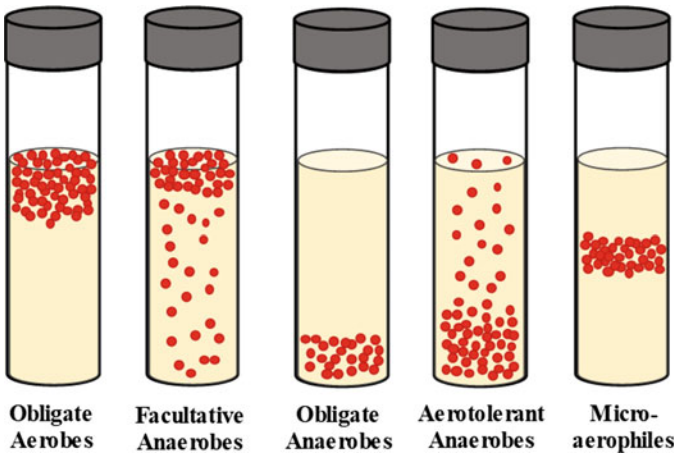

Fig. 1.4 Oxygen growth requirements. (Adapted from 2006 Pearson Education, Inc., publishing as Benjamin Cummings)

packaged food products without the need for using chemical preservatives or stabilizers. It is commonly used for raw meat and seafood, minimally processed fruits and vegetables, pasta, prepared foods, cheese, baked goods, cured meats, and dried foods (Modified Atmosphere Packaging 2012). The O_2 level in the package can affect bacterial growth, oxidation processes, and the color of fresh products such as raw meat. The CO_2 gas is known to have some bacteriostatic properties. The bacteriostatic effect of CO_2 within MAP is primarily influenced by CO_2 absorption into the food (Devlieghere et al. 2001; Meredith et al. 2014). When carbon dioxide is included within packaging, it is partially dissolved in the water-phase and the fat-phase of the product (Devlieghere et al. 1998). With the use of carbon dioxide as a preservative, an ideal concentration is sought to inhibit the growth of spoilage bacteria without providing an environment for anaerobic pathogens to propagate (Daniels et al. 1985). More detailed information on modified atmospheric packaging (MAP) can be found in the chapter entitled "modified atmospheric packaging".

1.3.4 Presence of Other Microorganisms

Bacteria can co-exist, dominate, or synergize with other species occupying the same environment. Cooperation in inter-species relationships has been observed. Through quorum sensing, groups of bacteria are able to regulate gene expression in response to extracellular signals (Hense et al. 2007; Hibbing et al. 2010). Multiple microorganisms within a food system can result in the competition for the same nutrients as well as production of toxic byproducts which can hinder the growth of the contending species. Byproducts such as bacteriocins, organic acids, harmful metabolites, and antibiotics can be produced by a single type of bacteria causing limiting

effects on others present. "Scramble competition" and "contest competition" are two common competitive relationships between microorganisms. Scramble competition pertains to rapid uptake of a limiting resource without direct interaction between competitors. Contest competition occurs when there is a direct, antagonistic interaction between microorganisms vying for a mutual resource (Hibbing et al. 2010; Nicholson 1954). In bacterial predation, predator microorganisms actively track down prey microorganisms to destroy and consume their macromolecules as an energy source (Perez et al. 2016).

The chemical characteristics of the food system may also be altered, resulting in inhibition of one or more competing types of microorganisms (Kornacki 2010). Conversely, a reduced natural background population in a product following processing may provide less stringent conditions for other microorganisms to proliferate (Genigeorgis 1981).

1.4 Other Factors

1.4.1 Stress Adaptation

Microbial cells are exposed to multiple types of intrinsic and extrinsic factors, which can alter their physical and chemical environments. This leads to varying forms of stress applied towards the cells during production, processing, preservation, storage, transportation, and consumption. During these treatments, if a stress is applied within a suboptimal range (Fig. 1.5), this may enable cells to develop mechanisms which allow them to resist subsequent exposures of the same or harsher treatment.

The mechanisms involved with stress adaptation have been observed in various foodborne pathogens and spoilage microorganisms during numerous processing and storage conditions (cold/warm water, low a_w, high pressure processing, bacteriocins, antibiotics, etc.). Environments at suboptimal pH levels have been frequently studied and these studies observed acid resistance, acid tolerance response (ATR), and acid shock response (ASR). Acid resistance (or acid adaptation) refers to cells being subjected to a mild acidic environment (pH 5.0–5.8) for an extended length of time which enables them to develop resistance to subsequent exposures of acidic conditions (pH $\leq$ 2.5). Acid tolerance response refers to a brief exposure of cells to mild acidic conditions, enabling them to survive subsequent exposure to pH 2.4–4.0. Acid

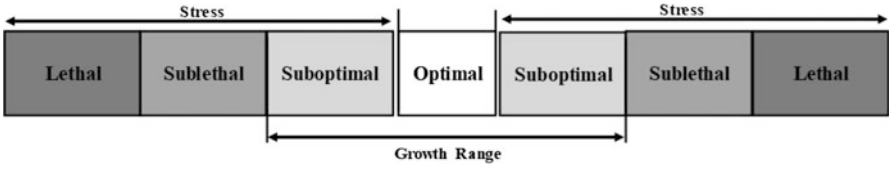

Fig. 1.5 Range of environment stresses on bacterial cells. (Adapted from Ray and Bhunia 2008)

shock response describes the response of cells to a low pH environment without previously adapting the cells to a mild pH (Ray and Bhunia 2008).

Cheville et al. (1996) studied the relation of *rpoS* genes and survival of *E. coli* O157:H7 during acid, heat, and salt treatments using a *rpoS* mutant. The *rpoS* mutant had diminished capabilities to survive within the various matrices. Spore-forming microorganisms have also shown to have ATR mechanisms. A study was conducted with *Bacillus cereus* which was either acid-adapted or pre-exposed to sublethal levels of heat, ethanol, salt, and hydrogen peroxide followed by an acid treatment at pH 4.6. Acid-adapted bacterial cells were able to endure a pH of 4.6 and lethal levels of heat and ethanol. In addition, pretreatments with sublethal heat, ethanol, salt, and hydrogen peroxide were able to protect cells during the acid treatment at a pH of 4.6 (Browne and Dowds 2002). Cross-protection over multiple stresses is observed when sublethal levels of one stress causes protection against a lethal level of another stress (Ray and Bhunia 2008).

1.4.2 Sublethal Injury

Similar to stress adaptation, sublethal injury can result in the survival of foodborne pathogens and spoilage microorganisms. Rather than withstanding a treatment within the suboptimal range, the microbial cells are exposed to a stress in a sublethal range (Fig. 1.5). The cells are not able to grow during a sublethal treatment but may be able to survive and resuscitate from reversible alterations (Wu 2008). Evidence of sublethal injury has been observed in many foodborne pathogens, such as *L. monocytogenes*, *Salmonella* spp. *E. coli* O157:H7, and *C. botulinum*. Cellular effects of sublethal injury include the down regulation of gene products responsible for cell division, increased sensitivity to compounds, loss of cellular materials, and lengthened lag phase for repair. Three microbial subpopulations are generated from a sublethally injured population: uninjured (normal) cells, reversibly injured (injured) cells, and irreversibly injured (dead) cells. In addition, the cytoplasmic membrane within cells may be damaged and lead to decreased permeability barrier functions and leakage of cellular material. This loss in permeability may allow potentially toxic chemicals to enter the cell and result in the loss of divalent cations. Similarly, lipopolysaccharide (LPS) layer within the outer membrane of Gram-negative bacteria can be sublethally damaged. Sublethal damage to lytic enzymes, loss of permeability, and damage to DNA in bacterial spores can result in delayed or lack of germination (Ray and Bhunia 2008).

A study done by Foster and Hall (1990) demonstrated the acid tolerance response in *Salmonella* Typhimurium cells during in vitro experiments using minimal glucose medium. The use of intermediate pH levels (pH 7.6 and pH 5.8) allowed the survival of *S.* Typhimurium once reaching a medium at pH 3.3. However, cross protection was not observed for other stressors such as oxidative, DNA damage, or heat shock (Foster and Hall 1990).

1.5 Conclusions and Future Trends

The intrinsic and extrinsic factors within the physical, chemical, and biological environments of food products influence the growth and resistance of microorganisms. By impacting the metabolic requirements and energy sources, control of potentially harmful and/or spoilage microorganisms can be achieved. The intrinsic and extrinsic parameters within a food system can be modified through either natural or external processes to provide a microbiologically safe product and maintain food safety and quality during storage.

Further studies should be conducted using physiological, molecular, and genetic approaches to improve the understanding of the responses of microorganisms to different intrinsic and extrinsic factors in foods. Advancing the knowledge in this area will assist with better prediction of microbial stability and safety of new products and effective use of a combination of hurdles to prevent the growth of microorganisms in various food systems.

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Chapter 2
Foodborne Pathogens

Hassan Gourama

2.1 Introduction

In every society, ensuring that the food supply is safe is a critical and fundamental right. Despite many significant advances in food science and food safety, food-borne illnesses continue to be a major public health issue around the world. Throughout history, humans have used microorganisms and their by-products to produce and preserve foods using food fermentations, while at the same time some of these microbes are known to cause significant food spoilage and foodborne illnesses. In the United States, the Centers for Disease Control (CDC) estimates that every year, 48 million individuals become sick by consuming contaminated foods. This leads to 128,000 hospitalizations and 3000 deaths (Scallan et al. 2011). Although, there are foodborne illnesses that are not reported or documented. However, with the introduction of food safety surveillance system, more of these un-detected cases are being examined. A conservative financial cost estimate of foodborne illness in the United States was reported to be around $55.5 billion (Scharff 2015). This financial cost is due to hospitalizations, loss of productivity, economic losses, and various other causes. Generally, these official estimates do not take into consideration other burdens such as life-long health consequences caused by foodborne pathogens. Certain segments of the population are more susceptible to food-borne illnesses, including the elderly, immuno-compromised individuals, pregnant women, infants, homeless people, and others of low socioeconomic status. Sources of these bacterial pathogens are numerous, including plant and animal food products, soil, water, air, processing equipment, and humans. These microorganisms contaminate numerous food products including meat, dairy, fruits, vegetables, seafood, grains, and water.

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Changes in the food system within the last 60–70 years have contributed to the rise of foodborne illnesses. In the past, foods were harvested, processed and distributed locally. However, the current food system is global, where foods are grown in one state or country, processed in another and distributed to many national and international markets for consumption. In addition, the increase in the number of mega food processing plants, has also contributed to the rise of foodborne illnesses, where contamination of a food product during processing can affect large groups of consumers. Moreover, improvement in recent years in food safety surveillance systems has resulted in the reduction of un-reported cases.

Many of these foodborne pathogens have also developed certain degrees of resistance to traditional food processing technologies such as thermal-processing, acidification, antimicrobials, and refrigeration. An increase in the demand by the consumers for more fresh fruits and vegetables has also contributed to the rise of foodborne illnesses. The foodborne pathogens responsible for most outbreaks include Norovirus, *Campylobacter* spp., *Salmonella* spp., *Listeria monocytogenes*, and Shiga toxin-producing *Escherichia coli*. Foodborne illness outbreaks occur around the world, see selected examples of these outbreaks in Table 2.1. In the United States, between 2009–2015, Norovirus caused the highest number of outbreaks followed by *Salmonella*. However, *L. monocytogenes* caused the highest number of deaths (Dewey-Mattia et al. 2018). Based on the mechanism by which these foodborne pathogens cause disease, foodborne illnesses are categorized into three types: foodborne infections, foodborne toxico-infections, and foodborne intoxications.

Symptoms of foodborne infections are caused by the pathogens themselves when they are ingested. Examples of these organisms include *Salmonella* spp., *Campylobacter jejuni*, *Shigella* spp., and *L. monocytogenes*. Symptoms of toxico-infections are caused by toxins produced inside the host by ingested pathogens. Examples of these organisms are *Clostridium perfringens*, Enterotoxigenic *E. coli* and *Vibrio cholerae*. In the case of intoxications, the symptoms are due to the ingestion of the microbial toxins that were produced in foods. Examples of these toxins are enterotoxins produced by *Staphylococcus aureus*, botulinum toxins produced by *Clostridium botulinum* and mycotoxins produced by some mold species (Jay et al. 2005).

2.2 *Staphylococcus aureus*

Staphylococcus aureus is a significant bacterial pathogen that can cause numerous diseases including skin infections, infection of wounds, toxic shock syndrome, and food poisoning. The Centers for Disease Control and Prevention (CDC) estimates that a third of the U.S. population carries *S. aureus* on their skin and in noses. Globally, Staphylococcal foodborne poisoning is one of the most common foodborne illnesses. Staphylococcal food poisoning results from the consumption of foods containing toxins produced by *S. aureus*. The genus *Staphylococcus* contains about 40 different species. Staphylococci are spherical Gram-positive

Table 2.1 Selected Foodborne Illness Outbreaks

Year	Location	Foodborne illness	Food involved	Number of cases	Number of deaths	References
1975	Airline Flight	Staph. food poisoning	Ham	197	0	Eisenberg et al. (1975)
1989	England	Botulism	Hazelnut yogurt	27	1	O'Mahony et al. (1990)
1996	Scotland	*E. coli* O157:H7	Meat pie	496	21	Pawsey (2002)
1998	Brazil	Staph. food poisoning	Chicken, roasted beef, rice, beans	4000	–	Do Carmo et al. (2004)
2010	Texas	Listeriosis	Diced celery	10	5	Gaul et al. (2013)
2011	Germany	*E. coli* O104:H4	Sprout	3816	54	Buchholz et al. (2011)
2011	Multistate (USA)	Listeriosis	Cantaloupe	147	33	CDC (2011)
2014	USA	Listeriosis	Mung bean sprouts	5	2	CDC (2015a)
2014	Utah	Campylobacteriosis	Raw milk	99	0	Davis et al. (2016)
2015	Multistate (USA)	Salmonellosis	Bean sprout	115	0	CDC (2015b)
2019	Multistate (USA)	Salmonellosis	Pre-cut melons	137	0	CDC (2019)

bacteria that are non-motile, non-endospore formers, and are known to grow in media containing up to 15% salt (NaCl) concentrations. *S. aureus* is known to grow over wide range of temperatures (7–49 °C) with an optimal temperature of 30–37 °C and pH range of 4–9 with an optimum pH of around 7 (Jay et al. 2005).

Among the group of Staphylococci, *S. aureus* is considered the most virulent. The other Staphylococci species such as *S. epidermidis*, *S. saprophyticus*, and *S. haemolyticus* are not considered serious pathogens but have been associated with human diseases. However, as far as food microbiology is concerned, *S. aureus* is the most significant pathogen. The virulence of *S. aureus* is characterized by the production of coagulase that causes the blood serum to form a clot. Coagulase is probably forming a blood clot around *Staphylococcus* cells to protect them from the host's immune defenses.

S. aureus is also known to develop resistance to antibiotics such as methicillin. Currently MRSA (Methicillin Resistant *S. aureus*) is a major health issue in the Unites States and around the world. CDC estimates that 5% of patient population in the U.S. hospitals carry MRSA in their noses and skin. Staphylococcal food poisoning is characterized by a rapid onset of symptoms within 30 minutes to 8 hours after consumption of the contaminated foods. The most common symptoms of

staphylococcal food poisoning are nausea, vomiting, stomach cramps, and diarrhea. Other possible symptoms include chills, sweating, weak pulse, and subnormal body temperature. In most patients, recovery is typically achieved within 1 to 3 days. Although a severe illness is rare, in some patients such as infants and immunocompromised individuals, the disease can have more severe consequences.

Since *S. aureus* is commonly found on human skin and hair, foods that require handling can easily become contaminated if proper food handling practices are not followed. The growth of *S. aureus* and production of Staphylococcal toxins (Enterotoxins) are typically found in foods rich in proteins such as meat, meat products, milk, dairy products, poultry products, eggs, clam chowder, salads, and cream filled baked goods (Jay et al. 2005; Gourama et al. 1991).

Staphylococcal food poisoning is caused by the consumption of enterotoxins produced by *S. aureus*. These Staphylococcal enterotoxins (SEs) are a family of 13 structurally related toxins (SEA, SEB, SEC_1, SEC_2, SED, SEE, SEG, SEH, SEI, SEJ, SEK, SEL) (Jay et al. 2005). SEA is the most common toxin implicated in Staphylococcal poisoning outbreaks. These enterotoxins are proteinic in nature with aspartic, glutamic, lysine, and tyrosine as the dominant amino acids. SEs are relatively resistant to heat, freezing, and drying conditions. SEs are also resistant to proteolytic enzymes such as pepsin or trypsin and low pH, which allows them to be active in the gastrointestinal tract. The heat resistance of these enterotoxins presents a significant challenge to the food industry and food service establishments. Thus, the focus should be on the prevention of contamination and growth of *S. aureus* in foods. The production of these enterotoxins by *S. aureus* has been shown to occur during all phases of bacterial growth. Although, many studies have shown that production of enterotoxins starts when the population of *S. aureus* reaches 10^6 to 10^7 cfu/g or ml.

Globally, numerous Staphylococcal food poisoning outbreaks have occurred during the last few decades. One well known outbreak occurred in 1975, when 197 people aboard a Japan Airlines (Boeing 747) traveling from Anchorage, Alaska to Copenhagen, Denmark fell ill after consuming ham omelets for breakfast (Eisenberg et al. 1975). One hour after eating breakfast people experienced typical symptoms of Staphylococcal poisoning including nausea, vomiting, diarrhea, and abdominal cramps. The investigation has revealed that one of the cooks who prepared the meal had lesions on his hand that were infected with *Staphylococcus aureus*. Prior to serving, the breakfast food was stored at temperatures within the temperature danger zone for more than 24 hours.

Prevention of Staphylococcal food poisoning requires adhering to proper food handling practices. This includes preventing food handlers with infected sores from handling foods. Foods should not be held at the temperature danger zone (5–57 °C or 41–135 °F) for more than 2 hours. In addition, proper food preparation, proper food cooking, proper food storage, and proper hand washing should also be followed.

2.3 *Clostridium botulinum*

Clostridium botulinum is an anaerobic spore forming Gram-positive bacteria. *C. botulinum* is naturally found in soil, aquatic sediments, and dust. Endospores formed by *C. botulinum* are heat resistant. While, *C. botulinum* is one of the most common producers of these toxins, other *Clostridium* species such as *C. butyricum*, *C. baratii* and *C. argentinense* (Sobel 2005) have been associated with the production of botulinum toxins (Sobel 2005). There are seven botulinum neurotoxins that are produced by *Clostridium species*: A, B, C, D, E, F, and G. These neurotoxins are immunologically distinct and have different physiological characteristics (Jay et al. 2005). Ingestion or exposure to these botulinum toxins results in botulism. Typical symptoms of botulism include double vision, vomiting, constipation, difficulty in swallowing, and difficulty in speaking. In severe cases, death can result from heart and respiratory failures. Botulinum toxins are neurotoxins which affect the nerves that control some of the key body functions such as dilation of blood vessels, breathing, and activity of the heart.

There are six types of botulism: foodborne botulism, wound botulism, infant botulism, intestinal botulism, inhalational botulism, and iatrogenic botulism. Foodborne botulism results from the ingestion of foods contaminated with botulinum toxins. Wound botulism results when wounds or cuts get contaminated with *C. botulinum* spores. The anaerobic conditions of the wound trigger germination of *Clostridium* spores and production of the toxin. Infant botulism occurs when spores of *C. botulinum* colonizes the intestines of infants aged 1 year and younger and produces the toxin. In rare cases, *C. botulinum* can colonize the intestines of adults, produce toxins, and causes intestinal botulism. In the past, aerosolization of botulism toxins in a laboratory setting has caused inhalational botulism. While iatrogenic botulism is caused by the injection of higher doses of botulism toxins in the case of cosmetic or therapeutic treatments (Sobel 2005).

However, as far as food safety is concerned, the major focus is foodborne botulism where most cases of botulism are related to canned foods especially home canned foods. If home canned foods are not processed properly, they provide a suitable environment for the germination of *C. botulinum* spores and subsequent production of toxins in foods. Outbreaks of botulism caused by commercially canned foods are extremely rare. *C. botulinum* does not grow at acidic pH (< 4.6), consequently acid foods (pH < 4.6) such as fruits are not suitable for the growth of *C. botulinum* and production of botulinum toxins. While the spores of *C. botulinum* are heat resistant, botulinum toxins are easily destroyed by heat. Bringing the temperature of the food to 176 °F (80 °C) for 10 minutes will destroy botulinum toxins (Jay et al. 2005).

Humans are more susceptible to botulinum toxin types A, B, and E (Gupta et al. 2005). In the United States, toxin type A is mainly prevalent in the western states, while outbreaks caused by type B are reported more often in the eastern states. Outbreaks caused by type E are commonly reported from Alaska and the Great Lakes area. Foods that have been involved in botulism outbreaks in the past include

baked potatoes, potato salads, potato soup, chopped garlic stored in oil, home-canned vegetables, and sautéed onions (Jay et al. 2005).

2.4 *Clostridium perfringens*

Clostridium perfringens causes a foodborne illness or perfringens poisoning. *C. perfringens* is an anaerobic Gram-positive bacterium that is widely distributed in soil, water, foods, dust and the intestinal tract of animals. As in the case of *C. botulinum*, *C. perfringens* is an endospore former. However, endospores produced by *C. perfringens* are not as heat resistant as *C. botulinum* spores. *C. perfringens* grows over a wide range of temperatures from 20–50 °C with an optimal range of 37–45 °C. An optimal pH range for the growth of *C. perfringens* is 5.5–8.0. A minimum water activity (a_w) of 0.95 was reported to be needed for the growth of *C. perfringens*. Heat resistance of endospores produced by *C. perfringens* varies depending on the strains. A D-value at 100 °C varies from 0.7 to 38.4 minutes depending on the strain and growth medium. Vegetative cells of *C. perfringens* do not survive well at freezing conditions while dried spores have a better survival rate.

Based on the production of enterotoxins, *C. perfringens* is divided into five types: A, B, C, D, and E. The majority of reported foodborne illnesses caused by *C. perfringens* are due to type A. The production of enterotoxins by *C. perfringens* is associated with the sporulation process, where conditions that favor sporulation also favor enterotoxin production. Enterotoxin type A is heat sensitive and is resistant to proteases such as trypsin, chymotrypsin and papain.

The symptoms associated with perfringens poisoning include diarrhea and abdominal pain, while fever and vomiting are rare. Typically, the foods involved in perfringens poisoning are meat dishes. Meat destined for human consumption often gets contaminated with *C. perfringens* during the animal slaughter process. The main reason why these foodborne illnesses occur is that *C. perfringens* endospores survive the cooking process, germinate and grow when these meat dishes are held at improper holding temperatures. To prevent perfringens poisoning, meat dishes or gravies should be cooked to the minimum required cooking temperature. If the food is not immediately consumed it should be refrigerated ($\leq$41 °F) shortly after cooking. If the food is going to be held at warm temperatures for later service, it should be held at a temperature of 135 °F or higher (Jay et al. 2005) (Garcia et al. 2019).

2.5 *Bacillus cereus*

Bacillus cereus is a Gram-positive, spore-forming, and rod-shaped bacteria. It is readily found in different environments including soil, plants, dust and water. *B. cereus* is an aerobe that can grow in a temperature range of 8–55 °C with an

optimum range of 28–35 °C. Minimum a_w for growth is approximately 0.95. *B. cereus* grows over a pH range of 5–9. The spores formed by *B. cereus* are also heat resistant.

B. cereus causes two types of foodborne illnesses: one is diarrheal and another is emetic that leads to nausea or vomiting. The diarrheal illness often involves meats, vegetables, fish, mashed potatoes, soups, puddings, and milk. While the emetic illness is often associated with rice and rice products. Emetic illness has also been reported to be associated with other products such as spaghetti, mashed potatoes, and vegetable sprouts.

Diarrheal disease is relatively mild by comparison with the emetic illness and has an incubation period of 8–16 hours. Symptoms consist of abdominal pain and watery diarrhea and last for 12–24 hours. The diarrheal illness is classified as a toxico-infection where *B. cereus* cells produce enterotoxins while they are in the small intestine. Diarrhea occurs when they reach a level of 10^5–10^8 of viable cells or spores. Three types of enterotoxins have been identified: hemolysin BL, nonhemolytic enterotoxin, and cytotoxin K.

The incubation period for the emetic illness is 30 minutes to 6 hours with symptoms (vomiting and nausea) that are more severe than the diarrheal illness. A level of 2×10^9 cells/g is necessary to produce the illness, which is much higher than the level needed for diarrheal disease. The toxin responsible for the emetic illness has been characterized as a water-insoluble peptide called cereulide. The toxin production was found to be independent of the sporulation process. Symptoms for both illnesses usually clear after 24 hours, however more severe complications can result in the case of immune-compromised individuals and intravenous drug users (Lindback and Granum 2019; Jay et al. 2005).

2.6 *Listeria monocytogenes*

Foodborne infection caused by *L. monocytogenes* (Listoriosis) is still one of the most significant foodborne illnesses in the U.S. and globally. *L. monocytogenes* is a Gram-positive, facultative anaerobe bacterium, that can grow from 0 to 45 °C with an optimum range of 30-35 °C. *L. monocytogenes* is also known to be able to grow at refrigeration temperatures, which is of concern to food safety. The pH range for the growth of *L. monocytogenes* is 4.1 to 9.6. The minimum growth pH is a function of other factors such as temperature, nutritional composition of food or substrate, water activity, and the presence of solutes such as salt. The minimum water activity for the growth of *L. monocytogenes* varies between 0.90 and 0.93 depending the humectant used in the study.

L. monocytogenes has been isolated from many environments and foods, including decayed vegetation, soils, sewage, silage, and water. Regarding human foods, *L. monocytogenes* has been isolated from many fresh animal or plant food products. It has been found in fruits, vegetables, meat, poultry, seafood, raw milk, and dairy products. *L. monocytogenes* has been reported to be relatively resistant to heating

and drying by comparison with other foodborne pathogens. Foodborne outbreaks due to *L. monocytogenes* have been reported during the last four decades involving soft cheese, pre-cut celery, cantaloupe, ice cream, bean sprouts, shellfish, and apples (Buchanan et al. 2017). In 1985, consumption of Mexican-style cheese contaminated with *L. monocytogenes* caused 142 cases of listeriosis with 34% fatality rate. Between 1983 and 1987, 31 individuals died of listeriosis by consuming contaminated swiss cheese. The time between the consumption of foods contaminated with *L. monocytogenes* and the onset of symptoms is relatively long and can take up to 3 months. The most common symptoms related to listeriosis include miscarriage for pregnant women and sepsis, pneumonia, and meningitis in newborns. The most significant virulence factor responsible for listeriosis associated with *L. monocytogenes* is listeriolysin O (LLO). There are thirteen *L. monocytogenes* serotypes, however most human cases are caused by three serotypes 1/2a, 1/2b, and 4b. (Buchanan et al. 2017; Jay et al. 2005; Radoshevich and Cossart 2018).

2.7 *Salmonella* spp.

Consumption of foods contaminated with *Salmonella* spp. leads to foodborne infection Salmonellosis. *Salmonella* bacteria are Gram-negative, non-spore forming rods that are facultative anaerobes. *Salmonella* is part of the *Enterobacteriaceae* family that also includes *E. coli*, *Yersinia* and other Gram-negative bacteria. Salmonellosis is one of the leading causes of foodborne illnesses in United States and around the world. Most *Salmonella* serotypes can grow at temperatures of 5–45 °C with an optimum temperature of around 35–37 °C and at pH that varies from 4.0 to 9.5 with an optimum pH of 6.5–70. The minimum water activity for the growth of *Salmonella* was reported to be 0.94. *Salmonella* is typically heat sensitive and, destroyed at 60 °C or higher. Thus, any food contaminations with *Salmonella* are due to post process contamination or an undercooking process. Typical food sources for *Salmonella* include undercooked poultry, eggs and meat products, raw milk, dairy products made from raw milk, and vegetable salads. Direct contact with infected animals and their environments can also lead to Salmonellosis (Jay et al. 2005).

Based on serotyping there are more than 2500 serotypes of *Salmonella*. These serotypes are characterized based on the lipopolysaccharide on their cell wall (O antigen) and flagellar antigen (H antigen). The *Salmonella* serovars are divided into six groups: A, B, C_1, C_2, D, and E_1 (Jay et al. 2005). *Salmonella* bacteria are causing more than 90 million diarrheal diseases world-wide, and most of these illnesses are related to contaminated foods. In the United States, the number of cases related to *Salmonella* infection is estimated to be around one million cases per year, resulting in around 20,000 hospitalizations and 400 deaths (Chlebicz and Slizewka 2018).

The pathogenicity of *Salmonella* has been shown to be related to many factors including the serotype, the state of the host immune system, adherence to host cells, invasion and replication inside the host, and toxin production. The pathogenicity of

Salmonella is controlled by chromosomal and plasmid genes. Salmonellosis is caused by the ingestion of *Salmonella* cells that reach small intestine via stomach. From the small intestine, the cells reach the lymph nodes, blood, and other organs such as kidneys, spleen, and gall bladder (Chlebicz and Slizewka 2018). If patients are not treated, the infection can be fatal. The incubation period of Salmonellosis can vary from 6 to 48 hours. The types of Salmonellosis in humans include typhoid fever and nontyphoid fever either invasive or noninvasive. Typhoid fever is caused by *S. typhi*, that is transmitted between individuals due to the lack of personal hygiene. Typical symptoms of typhoid fever are fever, diarrhea, headache, and loss of appetite. Other possible symptoms can occur including respiratory complications, injury of the intestine, and neurological changes (Fabrega and Villa 2013). The nontyphoid serotypes responsible for most of the foodborne illnesses are *S.* Typhimurium, *S.* Enteritidis, *S.* Newport, and *S.* Heidelberg. These serotypes enter the host when they consume contaminated foods or water. The infective dose necessary to cause the symptoms was reported to vary from 10^6 to 10^8 *Salmonella* cells. Although, in some cases, ten *Salmonella* cells were enough to cause the illness. The symptoms of nontyphoid Salmonellosis are abdominal pain, diarrhea, fever, chills, cramps, and loss of appetite. Adult patients can shed Salmonella in feces for up to 4 weeks even when symptoms cease to appear. This can last for up to 7 weeks in the case of children. In the case of severe diarrhea, hospitalization with fluid therapy may be required. The use of antibiotics should be allowed only in the case of severe complications (Antunes et al. 2016).

2.8 *Shigella*

The illness, shigellosis, often called bacillary dysentery is caused by the genus *Shigella*. *Shigella* is a member of the family *Enterobacteriaceae* like *Salmonella* and *E. coli*. Although genetically *Shigella* is closer to *Escherichia* than *Salmonella*. Based on serological characteristics *Shigella* genus contains four distinct species: *S. dysenteriae*, *S. flexneri*, *S. sonnei*, and *S. boydii*. *S. dysenteriae* causes most of the bacillary dysentery cases. *Shigella* species are Gram-negative, non-motile rods that are oxidase negative. *Shigella* grows over a wide range of temperatures from 10 to 48 °C, and at pH of 6–8.

Shigella and enterotoxigenic *E. coli* are the major causes of the diarrheal disease around the world, especially in sub-Saharan Africa and South East Asia. It is estimated that *Shigella* infection results in 125 million diarrheal cases per year, causing around 160,000 deaths. Most of these cases involve young children. In the United States, there are around 500,000 cases of shigellosis annually. Shigellosis is typically caused by ingestion of a low infectious dose of 10^1 to 10^4 *Shigella* cells. The symptoms of shigellosis are characterized by fever, cramps, chills, and watery diarrhea that is often bloody. The symptoms last from 1to7 days, or longer in severe cases. Death can easily be avoided by replacing fluids and electrolytes in patients. Humans are the primary carriers of *Shigella*. Virulence factors associated with the

pathogenicity of *Shigella* involve the invasion of epithelial cells of the intestine, intracellular multiplication, and spreading. Some *Shigella* species produce a proteinic toxin that is released in the gut. Transmission happens from one person to another via fecal-oral route or via contaminated water. Flies are also known to transmit *Shigella* to foods. Foodborne illnesses caused by *Shigella* are always due to contaminated water or handling of foods by an infected person. The foods implicated in shigellosis are typically moist foods handled by bare hands such as salads, raw produce, fruits, shellfish, and water. (Jay et al. 2005; Faherty and Lampel 2019).

2.9 *Escherichia coli*

Escherichia coli is a Gram-negative, facultative anaerobic rod that belongs to the *Enterobacteriaceae* family. *E. coli* is a commensal flora of the intestinal tract of humans and warm-blooded animals. *E. coli* can also survive outside the intestinal tract as in soil for a long period. Many of these *E. coli* strains are not pathogenic, others can cause a variety of diseases in humans and animals (Gonzales-Escalona et al. 2019). Pathogenic *E. coli* strains are known as one of the major causes of blood infections, urinary tract infections (UTIs), intestinal gastroenteritis, infections in pregnant women, and meningitis in newborns (Villa et al. 2016). The serological classification of *E. coli* strains is based on the somatic antigen (O), the flagellar antigen (H), and the capsule antigen (K). Currently there are more than 700 serotypes of *E. coli*. *E. coli* are also classified based on their virulence factors such as adhesins, toxins, invasins, iron-acquisition systems, antiphagocytis surface structures, and polysaccharide coats. Since *E. coli* is a part of the normal flora of the intestinal tract of humans and animals, it has been exposed to antibiotics over long period of time, which lead to the development of resistance to various antibiotics including multi-drug resistance. *E. coli* has also the ability to form biofilms in natural environments and processing surfaces (Vila et al. 2016).

Based on the mechanism by which gastrointestinal pathogenic *E. coli* cause illnesses, they are divided into five categories: Enteroinvasive *E. coli* (EIEC), Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E coli* (EPEC), Enteroaggregative *E. coli* (EAEC) and Shiga toxin-producing *E. coli* (STEC).

2.9.1 *Enteroinvasive* E. coli *(EIEC)*

The illness caused by EIEC is like the one caused by *Shigella* spp. EIEC strains are not known to produce enterotoxins, but due to their outer membrane proteins they access and multiply in the epithelial cells in the colon and spread to other cells. The symptoms are like shigellosis involving mild to severe dysentery. Older individuals and young children are more susceptible to EIEC. The infective dose of EIEC is estimated to be around 10^9 cells, with an incubation period that can vary from 2 to

48 hours. The transmission route of EIEC includes the consumption of contaminated foods and person-to-person transmission (Gonzales-Escalona et al. 2019; Kaper et al. 2004).

2.9.2 *Enteropathogenic* E. coli *(EPEC)*

EPEC strains cause watery diarrhea, vomiting and mild fever. EPEC strains are not known to produce enterotoxins. The mechanism of pathogenicity of EPEC involves colonization of the intestinal membranes causing attaching-and-effacing lesions in epithelial cells which leads to the effacement of the intestinal microvilli. The infective dose is reported to be 10^8–10^9 cells. EPEC is known to cause traveler's diarrhea in many parts of the world such as Latin America and North Africa (Gonzales-Escalona et al. 2019).

2.9.3 *Enterotoxigenic* E. coli *(ETEC)*

ETEC is one of the leading causes of infantile diarrhea in many underdeveloped and developing countries due to the lack of sanitary facilities and hygienic practices. ETEC is also one of the main causes of traveler's diarrhea. Typical symptoms include sudden and explosive non-bloody diarrhea, with possible vomiting and abdominal cramps. It has been reported that an infective dose of 10^8–10^{10} cells is needed to cause these symptoms. The mechanism of pathogenicity of ETEC involves colonization of small intestine and production of enterotoxins that are proteinic in nature, and that are heat-labile (LT) or heat-stable (ST) toxins. The LT toxin is a protein that is like the cholera toxin (CT). It is inactivated in 30 minutes at 60 °C, while ST toxin can survive at 100 °C for 15 minutes (Jay et al. 2005; Gonzales-Escalona et al. 2019).

2.9.4 *Enteroaggregative* E. coli *(EAEC)*

This is a group of *E. coli* that are related to EPEC; however, their adherence pattern is different from the other pathogenic *E. coli* strains. EAEC strains form a pattern that looks like stacked bricks when they attach on epithelial cells. EAEC strains have been found to produce hemolysin and a heat-stable toxin, however their virulence role is still unclear. EAEC is not the cause of traveler's diarrhea, but it has been reported to be the cause of persistent diarrhea, especially in children that endured for over 2 weeks. In 2011, a sprout associated outbreak of EAEC in Germany resulted in the infection of 4000 people with 23% of hemolytic-uremic syndrome (HUS) cases and 54 deaths (Gonzales-Escalona et al. 2019; Frank et al. 2011).

2.9.5 *Shiga Toxin-Producing* E. coli *(STEC)*

STEC are a group of pathogenic *E. coli* which used to be known as Enterohaemorrhagic *E. coli* (EHEC). One of the first well documented outbreaks related to STEC occurred in 1982, when *E. coli* O157:H7 caused two outbreaks of hemorrhagic colitis. Other serogroups belonging to this group are O26, O111, O45, O145, O113, O121, and O157: NM. *E. coli* O157:H7 is the one that caused most of the STEC associated outbreaks. STEC produces toxins known as verotoxins or Stxs. Stxs resemble toxins produced by *Shigella dysenteriae*. STEC also have pathogenic properties like EPEC, by producing attaching and effacing lesions. The gastrointestinal disease caused by STEC is characterized by intense abdominal pain and watery bloody diarrhea. In some cases, involving children, Hemolytic Uremic Syndrome (HUS) can occur, causing acute failure of kidneys, reduction in blood platelet and hemolytic anemia. This can lead to seizures, coma, and death. The infectious dose of STEC was reported to be very low, as low as 10 cells in some cases (Gonzales-Escalona et al. 2019).

Investigations have shown that *E. coli* O157:H7 can survive acidic conditions by comparison with other foodborne bacterial pathogens using known resistance mechanisms such as the decarboxylase/antiporter-dependent systems (Foster 2004). The minimum pH required for the growth of *E. coli* O157:H7 was reported to be 4.0 to 4.5. However, growth of *E. coli* O157:H7 is dependent on the interaction of pH with other factors such as type of food, media, and incubation temperature. Studies on the effect of heat on *E. coli* O157:H7 have shown that it has similar heat sensitivities as other foodborne pathogens ($D_{60°C} = 45$ seconds). *E. coli* O157:H7 did not survive well in manure under fluctuating temperatures (Semenov et al. 2007). Outbreaks related to *E. coli* O157:H7 have initially been linked to the consumption of contaminated undercooked ground beef and unpasteurized milk. However, now it is known that many other types of foods have been linked to infections with *E. coli* O157:H7, including salad vegetables (lettuce, sprouts, and spinach) and contaminated drinking water (Gonzales-Escalona et al. 2019). Contacts with animals and animal feces have also been reported to cause *E. coli* O157:H7 outbreaks (Licence et al. 2001). *E. coli* O157:H7 can also be transmitted from one person to another. Shedding of the organism by HUS patients can last from 3 weeks to 3 months.

2.10 *Campylobacter*

In many parts of the world, *Campylobacter* is responsible for most of the foodborne illnesses. The number of cases caused by *Campylobacter* is 3–4 times higher than the ones caused by *Salmonella* or pathogenic *E. coli* (Facciola et al. 2017). *Campylobacter* genus is a member of the *Campylobacteriaceae* family in addition to other genera, *Arcobacter* and *Helicobacter* (Fitzgerald and Nachamkin 2011). There are 29 species within the genus *Campylobacter* (Habib et al. 2019). Among these

species, *C. jejuni* and *C. coli* are the ones responsible for human gastroenteritis. The genus *Campylobacter* is a curved rod, Gram-negative, non-spore former that is highly motile. One of the key metabolic characteristics of *Campylobacter* is that it is microaerophilic, it grows best in an environment with low oxygen level (5%), 10% carbon dioxide and 85% nitrogen (Garenaux et al. 2008). *Campylobacter* grows at an optimal pH range of 6.5 to 7.5. *Campylobacter* species are considered thermotolerants, they grow between 37 and 42 °C but not at or above 55 °C or below 30 °C. The optimal water activity for the growth of *Campylobacter* was reported to be 0.997 (Silva et al. 2011).

Pathogenesis of *Campylobacter* involves adherence and colonization of the intestinal epithelium. This colonization is facilitated by the motility characteristics of *Campylobacter*. Symptoms of Campylobacteriosis include bloody stools, fever and abdominal pain. Infections by *Campylobacter* spp. has been shown to trigger other complications including the Guillain-Barre Syndrome, arthritis, and irritable bowel syndrome (IBS) (Facciola et al. 2017). The incubation period for Campylobacteriosis can vary from 2 to 7 days and last for up to 10 days. The infectious dose was reported to be around 500 *Campylobacter* cells. Natural reservoirs of *Campylobacter* include cattle, pigs, birds, and pets such as dogs and cats. Poultry birds are typically colonized with a high number of *Campylobacter* species after hatching. This colonization remains high until the time of slaughter (El-Shibiny et al. 2005). Typical transmission of *Campylobacter* involves fecal-oral route by the consumption of contaminated foods. Other foods such as raw milk, fruits, and vegetables have been implicated in *Campylobacteriosis* outbreaks. Many recent studies have reported that *C. jejuni* is becoming resistant to many antibiotics including erythromycin, tetracycline, and ciprofloxacin (Facciola et al. 2017). Investigators have proposed ways to prevent *Campylobacter* infections including surveillance of Campylobacteriosis within human populations, potential use of vaccines against *Campylobacter* in humans and animals, prevention and control of *Campylobacter* in poultry farms, insect and rodent control, use of hygienic and sanitary practices by workers in poultry farms and processing plants, in addition to the chlorination of water (Newell and Fernley 2003).

2.11 *Yersinia enterocolitica*

Yersinia genus is a member of the *Enterobacteriaceae family*. There are three species of *Yersinia* that are pathogenic to humans, *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica*. *Y. pestis* is the agent that causes the plague (Black death), while *Y. pseudotuberculosis* is an intestinal pathogen that targets rodents and occasionally humans. However, the one *Yersinia* species that is of interest in foods is *Y. enterocolitica*, which is a Gram-negative, short rod, facultative anaerobe that can grow over temperatures of – 1 to 40 °C with optimum growth around 29 °C, and over pH range of 4 to 8 with an optimum pH of 7.0. One unique characteristic of *Y. enterocolitica* is that it is motile at 30 °C or below but not at 37 °C (Jay et al. 2005).

Y. enterocolitica is ubiquitous in many environments including water streams, lakes, wells, soil, and intestinal tracts of animals. It has been found in many animals such as swine, cattle, chickens, horses, birds, deer, oysters, and many others. *Y. enterocolitica* has been isolated from vacuum-packaged meats, milk, fruit, and vegetables. However, most of these isolates have been found to be non-pathogenic, except for the isolates that were isolated from pigs (McNally et al. 2004).

Symptoms associated with Yersiniosis include gastroenteritis, pseudo-appendicitis, arthritis, and abscesses in colon and neck. The gastroenteritis syndrome cases occur more in the fall than the other seasons. The illness is more likely to affect young children and elderly. Young children are more susceptible than adults. The incubation period of yersiniosis can take from 1 to 11 days and last for up to 14 days. Symptoms include abdominal pain, diarrhea, and sometimes mild fever. Infection with *Y. enterocolitica* can also result in bacteremia in immunocompromised patients and cause many immunological complications.

The virulence characteristics associated with *Y. enterocolitica* pathogenicity have been found to be due to the production of heat-stable enterotoxins (ST). ST has been found to withstand 100 °C for 20 minutes. However, other investigators reported that the production of enterotoxins is not the only virulence factor in some *Y. enterocolitica* serovars. (Okuku and Bari 2019).

2.12 *Vibrio*

The genus *Vibrio* is ubiquitous in fresh water and marine environments. *Vibrio* species are Gram-negative bacteria, with rod or curved shapes (pleomorphic), are facultative anaerobes, and can grow over temperatures of – 5 °C to up to 45 °C with an optimum of 37 °C. The known pathogenic *Vibrio* spp. are *Vibrio cholerae*, *V. parahaemolyticus*, *V. vulnificus,* and *V. alginolyticus*. *V. parahaemolyticus* is the most significant species, when it comes to foodborne illnesses. It causes severe gastroenteritis due to the consumption of contaminated raw or undercooked seafood, especially along the coastal waters. *V. parahaemolyticus* is not known to spread from one individual to another or through fecal-oral route (Baker-Austin et al. 2010). Initially infections caused by *V. parahaemolyticus* have been limited to Japan, but during the last 40 years this infection has been encountered all the over the world. In the United States, the number of infections is estimated to be around 30,000 cases per year (Scallan et al. 2011). Incubation time of infections caused by *V. parahaemolyticus* can vary from 2 to 4 days and last up to 8 days. Symptoms include watery diarrhea, abdominal pain, vomiting, fever, nausea, and chills. The most common seafood vehicles of this infection include oysters, crabs, lobsters, shrimps, and shellfish. The gastroenteritis caused by *V. parahaemolyticus* is believed to be caused by the production of hemolysins that functions as cytotoxin and an enterotoxin (Ceccarelli et al. 2019). *V. parahaemolyticus* is tolerant to salt concentration of 0.5–8%, with best growth at 3% salt. The minimum water activity is between 0.94 and 0.99. *V. parahaemolyticus* grows well over a pH range of 7.5–8.5, and it can tolerate up to a pH of 11.0 and as low as a pH of 4.5. *V. parahaemolyticus*

typically grows well in coastal marine waters, while the other *Vibrio* species such as *V. cholerae* is common in fresh waters.

V. cholera is known to be the agent that causes cholera, through the drinking of polluted waters. Globally, it is estimated that every year 3–5 million people are diagnosed with cholera, causing around 100,0000 deaths (Zuckerman et al. 2017). Most of these deaths affect children under 5 years of age. The incubation period of cholera is 1–3 days. *V. cholera* cells produce an enterotoxin in the intestine, which in turn causes an extensive secretion of ions such as Na^+, Cl^- and K^+. This leads to the production of profuse watery diarrhea and vomiting without fever. Typical treatment of cholera involves the replacement of fluid and electrolytes. Without the treatment many complications can occur including renal failure, loss of blood, and death. *V. vulnificus* is typically isolated from seawater and seafoods especially in warm summers. Infection by *V. vulnificus* causes septicemia and cirrhosis in immunocompromised patients. *V. vulnificus* is very invasive that produces cytotoxin and hemolysin.

2.13 Viruses

Viruses are acellular infectious agents that need to invade and multiply inside a living host's cells, thus they are obligate intracellular parasites. Viruses are very small agents so they cannot be observed using a light microscope. Viruses consist of nucleic acid (RNA or DNA) surrounded by a proteinic capsid. In addition to the capsid, some viruses have a lipid bilayer membrane called an envelope. Enteric viruses are a major cause of foodborne illnesses worldwide. The two most important foodborne viruses are hepatitis A virus and Norovirus. These viruses are typically dispersed in feces and vomit and are transmitted via fecal-oral routes. These viruses do not grow in foods or water, but they can be transmitted to people who consume contaminated foods. Globally, Noroviruses are the leading cause of all foodborne illnesses (Gibson et al. 2019). Many of the foodborne illnesses in the United States are due to the ingestion of Noroviruses, causing around five million cases that lead to 150 deaths annually (Gibson et al. 2019). Enteric viruses are known to be stable in the gastrointestinal environments and various processing conditions. The infectious dose of enteric viruses is low. Foodborne illnesses caused by these enteric viruses are the result of consuming contaminated shellfish, fresh produce, and ready-to-eat foods. The sources of the viruses in these foods are contaminated waters or infected food handlers (Jay et al. 2005).

2.13.1 Norovirus

Norovirus is a member of the *Calciviridae* family and consists of 40 genotypes and 7 genogroups (Vinje 2015). Norovirus is easily transmitted from one infected person to another, which leads to the contamination of water and foods such as leafy

vegetables such as lettuce, fruits, and shellfish. Most Norovirus outbreaks occur in food service establishments, where infected food service workers transmit the virus to ready-to-eat foods or foods that do not receive a kill step (cooking) before consumption. Infected food handlers without symptoms can also transmit the virus. Norovirus outbreaks are common occurrences in cruise ships. The enclosed living space of cruise ships, the presence of large number of passengers and the sharing of dining facilities make it difficult to control Norovirus on cruise ships. The incubation period of Norovirus infection can vary from 15 to 50 hours before the start of symptoms. Typical symptoms include nausea, vomiting, non-bloody diarrhea, and abdominal cramps. This can last for 1–4 days. Norovirus outbreaks are very costly. In the United States it is estimated that the cost reaches $2–4 billion annually (Gibson et al. 2019).

2.13.2 Hepatitis A Virus

Hepatitis A virus is a member of *Picoranviridae* family. It is an RNA enveloped virus. The incubation period for Hepatitis A virus varies from 14 to 50 days, producing symptoms of nausea, vomiting, fatigue, and jaundice. At the global level, it is estimated that 1.5 million cases of Hepatitis A virus occur every year. These cases are more concentrated in underdeveloped countries where the sanitary infrastructure is not well developed (Gibson et al. 2019). The most effective measure to prevent and control these enteric viruses, is to reinforce strict personal hygiene by all food handlers including proper hand washing. Food handlers should also stop working when they start experiencing gastrointestinal symptoms.

2.14 Toxigenic Fungi

Filamentous fungi or molds are ubiquitous in many products and environments including foods, agricultural commodities, processing environments, animal feed (Gourama et al. 2015), and even in space (De Middeleer et al. 2019). Fungi have the capacity to grow or survive on a wide variety of products and conditions such as high acidity, dry conditions, and low temperatures. The growth of fungi in foods and crops can have both beneficial and harmful effects depending on the type of the fungal species, growth substrate and environmental conditions (Gourama et al. 2015). Historically, fungi are used to produce many food products including ripened cheeses, soy sauce, tempeh, and other products. On the other hand, filamentous fungi cause the spoilage of many food products and crops throughout the world. It is well accepted that this spoilage leads to high economic loss. In addition, to the spoilage issue, some mold species present health hazards to humans and animals by causing infections or producing toxic substances called mycotoxins. Mycotoxins are considered secondary fungal metabolites that are naturally produced by some mold

species. The term mycotoxin is derived from the Greek word "mykes," which means fungus and the Latin word "toxicum," which means toxin, or poison. The main mold genera that produce mycotoxins are *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria*. The major mycotoxins that are of significant concern to human and animal health are aflatoxins, ochratoxins, fumonisins, deoxynivalenol, T-2 toxin, and zearalenone. Other mycotoxins that are becoming of concern are sterigmatocystin, patulin, cyclopiazonic acid, and moniliformin.

These toxins, even at very small levels, can cause many chronic and acute toxic effects on humans and animals that range from gastrointestinal complications, nephropathies, immunotoxicity, mutagenicity, and carcinogenicity. Toxigenic molds have caused outbreaks throughout history, although the first mycotoxin that was chemically identified was aflatoxin in 1960, the cause of the "Turkey X Disease" (Bennet and Klich 2003). In 2004, an outbreak of aflatoxicosis occurred in Kenya due to the consumption of contaminated corn. Of the 317 people who became ill, 125 died. It has been estimated that 0.5–2 mg of aflatoxin/kg can cause death. (Lewis et al. 2005).

The production of mycotoxins by these mold genera depends on many factors such as moisture, temperature, relative humidity, type and state of substrate, pH, and competition from background flora. Crops can be contaminated with mycotoxins before harvest, after harvest, during drying, and during storage. Commodities that are frequently contaminated with mycotoxins are corn, wheat, barley, rice, oats, peanuts, cottonseed, cassava, raisins, cocoa, milk, cheese, and other products. Mycotoxins are heat resistant and can survive many post-harvest processes that inactivate molds. For more information on mycotoxins, see Chap. 3.

2.15 Parasites

Throughout history, animal parasites have caused enteric illnesses in humans and animals. Unlike foodborne bacteria, animal parasites do not grow in foods and in laboratory culture media. Therefore, they are detected based on screening for specific antibodies, special staining methods or by checking the animal hosts. Currently the number of illnesses caused by these parasites is below the number of illnesses caused by pathogenic bacteria and viruses. However, due to increased global food trade, international travel, increase in the number of immunocompromised individuals, and consumption of more fresh fruits and vegetables, the number of parasitic illnesses is expected to raise (Dixon et al. 2011). The incubation period of illnesses caused by these parasites can take 1–2 weeks. Foods involved in outbreaks are usually determined using epidemiological data, because in many cases foods involved in these parasitic outbreaks are consumed or already disposed of before the investigation is accomplished. There are three groups of animal parasites that can cause illnesses in humans: protozoa, flatworms, and roundworms (Jay et al. 2005).

2.15.1 Protozoa

Protozoa are considered the smallest and most primitive of the animal parasites. The protozoa parasites of concern to food and water are *Cryptosporodium* spp., *Giardia* spp., *Taxoplasma* spp., and *Cyclospora* spp.

2.15.1.1 *Cryptosporodium*

The most prevalent *Cryptosporodium* species is *C. parvum*, which is an obligate intracellular parasite. *C. parvum* cells (oocysts) are oval to spherical in shape. Major sources of *C. parvum* are cattle, sheep, goats, and deer. Other products such as fruits and vegetables get contaminated during irrigation or post-harvest wash. After ingestion of the oocysts, diarrhea occurs after an incubation time of 1–2 weeks and can last for up to 3 weeks. On occasions, abdominal pain, vomiting, and low-grade fever may occur. Symptoms can be more severe in immunocompromised patients. Transmission to other hosts can occur via many routes; fecal-oral route, zoonotic, or by ingestion of foods or drinking water contaminated with these cells. It was reported that milk pasteurization (HTST) destroys *C. parvum* cells. Oocysts of *C. parvum* have been found to be resistant to disinfectants such as chlorine (Jay et al. 2005; Dixon et al. 2011; Ortega 2019).

2.15.1.2 Giardia

Giardia intestinalis (also known as *G. duodenalis* or *G. lamblia*) is one of the most prevalent causes of parasitic diseases worldwide. Each year, there are approximately 2.5 million giardiasis cases in the United States (Dixon et al. 2011). After ingestion, the parasite multiply in the upper intestinal tract causing symptoms of diarrhea, cramps, fever, vomiting, and weight loss. The incubation period of Giardiasis takes 1–2 weeks and can persist for up to 1 year. Giardiasis is very contagious. Cysts of *G. intestinalis* are also resistant to disinfectants like chlorine, but they are easily inactivated by heat during cooking. Transmission occurs primarily by contaminated water and food or by coming in contact with feces containing the parasite (Dixon et al. 2011; Ortega 2019).

2.15.1.3 *Toxoplasma gondii*

Toxoplasma gondii is commonly found in livestock, birds, cats, and humans. The oocysts are typically formed in the intestine and shed in feces. After ingestion, the oocysts release eight sporozoites, which pass from the intestines to the circulatory system of the host. These sporozoites become embedded in the host tissue as bradyzoites. Symptoms of toxoplasmosis include skin rash, headaches, pain in the

joints, and swelling of the lymph nodes. Although, in many cases these symptoms are very mild, in immunocompromised patients the infection can cause serious complications. Outbreaks related to *T. gondii* have been associated with the consumption of undercooked pork and venison, raw goat milk, raw ground beef, raw oysters, raw clams, and contaminated drinking water. The inactivation of *T. gondii* in meats can be achieved by cooking, freezing, irradiation, and high-pressure processing (Dixon et al. 2011; Ortega 2019).

2.15.1.4 *Cyclospora cayetanensis*

Humans appear to be the main host for this parasite. When shed in feces, oocysts undergo sporulation in the environment. After ingestion, the parasite goes through many transformations and penetrate the intestinal wall. Typical symptoms include watery diarrhea, weight loss, dehydration, and abdominal pain. In the United States, around 15,000 cases of cyclosporiasis occur every year, mainly due to the consumption of contaminated fruits and vegetables. Control measures of cyclosporiasis should include using good personal hygienic practices by workers on farms and by consumers. Oocysts of *C. cayetanensis* have been found to be resistant to chemical disinfectants and freezing (Dixon et al. 2011).

2.15.2 Flatworms

Flatworms include two specific parasites, *Taenia saginata* and *Taenia slium*. These parasites are typically associated with beef and pork as intermediate hosts and humans as the final host. Infection of humans by these parasites causes irritation of the gut producing symptoms of anemia, nausea, and abdominal pain. In extreme cases the nervous system can be infected which can result in death (Dorny et al. 2009).

2.15.3 Roundworms

The most significant roundworm parasite for humans is *Trichinilla spiralis*, which is responsible for trichinosis. Intermediate and definitive hosts for *T. spiralis* are humans and pigs. Eggs produced by females of *T. spiralis* are found in soil or feces. Once they are consumed, they develop into larvae in the intestinal tract of the host. After burrowing through the intestinal wall, they settle in different muscles including tongue, diaphragm and biceps. The larvae develop later into cysts in the muscles. Symptoms of trichinosis include muscle pain, and swelling, with occasional diarrhea, vomiting and fever. Trichinosis can be prevented by cooking meat (pork, bear, etc.) to a minimum internal temperature of 145 °F for 4 minutes. Freezing is also known to inactivate *T. spiralis* larvae (Dorny et al. 2009; Gamble et al. 2000)

2.16 Other Foodborne Pathogens

Other less common and potentially emerging foodborne pathogens are *Aeromonas* spp., *Plesiomonas shigelloides*, *Brucella* spp., *Mycobacterium* spp., and *Cronobacter* spp. A brief description about each of the pathogens are given below.

2.16.1 Aeromonas *Species*

Aeromonas species, mainly *A. hydrophila*, *A. caviae* and *A. sobria* are considered foodborne pathogens that have begun to attract the attention of food and clinical microbiologists. They are motile Gram-negative rods that produce catalase and oxidase. *Aeromonas* is known to be non-tolerant to salt and acidic conditions. Its optimal temperature is around 28 °C. However, one of the main characteristics of *A. hydrophyla* is its ability to grow in cold temperatures. Aquatic sources such as lakes, streams, and wastewater centers are known reservoirs of *Aeromonas* species. Because of their psychrophilic properties, *Aeromonas* have been found to spoil chilled products such as meat, fish, poultry, and raw milk. Watery diarrhea and occasional vomiting are some of the common symptoms associated with *Aeromonas* gastroenteritis. Young children and immunocompromised individuals are more susceptible to this foodborne pathogen. Virulence factors associated with *Aeromonas* pathogenicity are cytotoxic enterotoxins such as aerolysin and Beta-hemolytic enterotoxin (Skwor and Kralova 2019).

2.16.2 Plesiomonas shigelloides

Many of the characteristics of *P. shigelloides* are like *Aeromonas* spp. *P. shigelloides* is a Gram-negative motile rod, that is catalase and oxidase positive. It grows over a temperature range of 8–45 °C, with an optimal temperature of 37 °C. Minimum pH for the growth of *P. shigelloides* is 4.5. It has been isolated from water and soil and from various animals such as frogs, snakes, fish, cattle, pigs, poultry, and dogs. The foods that are commonly associated with *P. shigelloides* are fish and shellfish (crab, shrimp, cuttle fish, and oysters). The usual symptom related to an infection with *P. shigelloides* is mild watery diarrhea. This infection is common in countries with warmer climate (Janda et al. 2016).

2.16.3 Brucella *Species*

Brucella species are Gram-negative, short oval non-motile rods, that produce catalase and oxidase enzymes. They grow optimally at 37 °C and are not tolerant to high

temperatures (>60 °C). The animal hosts associated with *Brucella* species are cattle (*B. abortus*), sheep and goats (*B. melitensis*), pigs (*B. suis*), and dogs (*B. canis*). Thus, people who are in contact with these hosts such as farmers are at a much higher risk to contract brucellosis. Brucellosis can also be contracted by consuming contaminated raw milk, cheese made from unpasteurized milk, and undercooked meat. Symptoms of brucellosis include fever, sweat, headaches, constipation, and weight loss (Dadar et al. 2019).

2.16.4 Mycobacterium *Species*

Mycobacterium species are Gram-positive, pleomorphic, non-spore forming aerobic bacteria. By contrast with most of the Gram-positive bacteria, their cell wall is made of a thick lipid layer. They are resistant to lysozyme, phagocytes, and drying conditions. The two most significant *Mycobacterium* species are *M. tuberculosis* and *M. bovis*. *M. tuberculosis* is the primary cause of pulmonary tuberculosis disease. The transmission of tuberculosis is an aerial one that occurs from an individual to others through coughing and sneezing. *M. bovis* causes tuberculosis in animals, especially cattle. Humans can get *M. bovis* causing what is called foodborne tuberculosis through the consumption of contaminated raw milk. *M. bovis* accesses the human body through the intestinal tract. Introduction of pasteurization has significantly reduced the incidence of foodborne tuberculosis. However, the recent emergence of drug-resistant strains of *Mycobacterium* and the increase in the consumption of raw milk in many parts of the world may lead to more cases of this disease. The incubation period of tuberculosis can take months or even years. Some of the key symptoms of tuberculosis are fever, chills, and weight loss, in addition to other complications (Forbes et al. 2018).

2.16.5 Cronobacter *Species*

Cronobacter genus, formerly known as *Enterobacter sakazaki*, is a group of 11 species that are Gram-negative, non-spore formers, facultative anaerobes that are oxidase negative, catalase positive and motile. C*ronobacter* is considered an opportunistic pathogen that has historically been implicated in newborn and infant infections causing three known diseases: Necrotizing Enterocolitis (NEC), Septicemia, and Meningitis. C*ronobacter* has also been linked to infections in adults. C*ronobater* survives well in dry conditions and in low water activity foods. It has been detected in dried milk powder, legumes, dried meats, cheese powder, dried flours, spices, and others. However, only powdered infant formula has been epidemiologically linked to C*ronobacter* foodborne outbreaks. C*ronobacter* has also been found to be heat and acid tolerant (Jaradat et al. 2014).

2.17 Conclusion and Future Trends

The food system is now globalized, and consumer demographics and behavior are changing. A significant portion of the population is becoming older or immunocompromised.Consumers are demanding more fresh fruits and vegetables and minimally processed foods. With these changes, we should expect that the challenges caused by foodborne pathogens are going to continue. The only way to meet these challenges, is for stakeholders throughout the food system to be ready to apply new and innovative approaches. Food safety professionals should "expect the unexpected" (Swerdlow and Altekruse 1998). The ability of pathogens to adapt to new environments and new niches, and the emergence of new pathogens should never be overlooked. Scientists, food producers, food industry, and health professionals can meet these challenges by applying new and innovative measures in every part of the food system, from farm to table. New preventive approaches and technologies need to be developed to help with food safety at the production level. Examples of these approaches include proper composting of manure to inactivate pathogens and controlling pathogens in farm animals by using vaccinations and competitive exclusion. At the processing and distribution levels, pathogens can be controlled by expanding the use of already proven or developing technologies such as ionizing radiation, high-pressure processing, ultrasound, smart food packaging, and others. Microbiologists and health professionals should continue to use innovative detection methods such as genetic methods and effective surveillance strategies. Education of consumers and food preparers on proper and safe food handling practices should also be an integral part of any food safety program.

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Chapter 3
Microbial Toxins

Yuksel Cetin

3.1 Introduction

Bacteria produce two kinds of toxins, endotoxins: cell-associated lipopolysaccharide (LPS) toxins released after disruption of the cell; exotoxins: toxic proteins synthesized inside the cells and then released to the target cells. In general, endotoxins are part of the outer membrane or the cell wall of Gram-negative bacteria, whereas, exotoxins are soluble proteins excreted by both Gram-positive and Gram-negative bacteria, fungi, algae, and protozoa. Enterotoxins are a type of exotoxin released in the intestine, which alters the permeability of the epithelial cells of the intestinal wall. *Escherichia coli*, *Clostridium perfringens*, *Vibrio cholerae*, and *Staphylococcus aureus* often produce pore-forming toxins that induce pores in cell membranes leading to cell death (Martin 2012). Bacterial toxins cause toxic damage in a specific organ of the host by targeting inhibition of protein synthesis, destruction of cell membranes, activation of secondary messenger, activation of immune system, septic shock, or acting as an enzyme (Table 3.1) (Kumar et al. 2019). *Clostridium* spp. and *Bacillus* spp. are spore-forming, remarkably adaptable to different environment, found throughout nature, particularly in soil, water, and gastrointestinal tracts of various mammals, insects, and various other hosts. Their toxins and spores are commonly involved in food poisoning, antibiotic-associated diarrhea, gas gangrene, and enterotoxemia (Table 3.1) (Barth et al. 2004; Borriello and Carman 1983; Lindback and Granum 2015). A synergistic binary mechanism of several proteins including *C. botulinum* C2 toxin, *C. difficile* toxin (CDT), *C. perfringens* iota toxin, *C. spiroforme* toxin (CST), *B. anthracis* edema, and lethal toxins are involved in intoxication of eukaryotic cells. The protein components of these toxins are

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Table 3.1 Bacterial toxins, their associated sources, and toxicity (Barth et al. 2004; Stiles et al. 2014; Hernández-Cortez et al. 2017; Miller and Wiedmann 2016)

Organisms	Toxins	Sources	Toxicity
C. perfringens	Alpha, Beta, Epsilon, & Iota *C. perfringens* enterotoxin, CPE	Soil, water, mammalian intestines	Gas gangrene, food poisoning, enterocolitis, antibiotic-associated diarrhea, puerperal septicemia
C. spiroforme	*C. spiroforme* toxin, CST	Mammalian intestines	Enterocolitis, antibiotic-associated diarrhea
C. difficile	C. difficile toxin, CDT	Mammalian intestines, meat products, pets, water, healthcare facilities	Enterocolitis, antibiotic-associated diarrhea
C. botulinum	C2 toxin	Soil, water, meat products, honey, mammalian intestines	Food poisoning, infant & adult botulism
C. botulinum	Botulinum neurotoxins (BoNTs)	Cultivated and forest soils; bottom sediments of streams, lakes, & coastal waters; in the intestinal tracts of fish and mammals	Botulism, muscle weakness, paralysis of the arms, legs
B. anthracis	Protective antigen, Edema toxin, Lethal toxin	Soil, contaminated animal products, such as bones or hides	Edema & skin necrosis, shock, severe respiratory dysfunction, and hypotension, cardiac failure
B. cereus	Hemolysin (Hbl), Nonhemolytic enterotoxin (Nhe), cytotoxin (CytK)	Soil, water, insect and mammalian intestines, meat & grain products	Food poisoning, diarrheal and vomiting type of illness
H. pylori	Cytotoxin-associated gene A (cagA)	Contaminated drinking water, brewers, raw chickens & tuna, raw milk	Chronic inflammatory diseases, severe gastric diseases, including ulcers, gastric cancer
E. coli	Shiga toxin	Contaminated food products, animals, water, person and environment	Mild non-bloody diarrhoea to bloody diarrhoea, haemolytic uraemic syndrome, kidney failure
S. aureus	Hemolysin-α (Hla/α-toxin), Hemolysin-β (Sphingomyelinase C), Leukotoxins	Meat and meat products cooked at high temperatures, poultry, salad with mayonnaise	Immunosuppression, toxic shock syndrome, high fever, hypotension, erythematous rash

(continued)

Table 3.1 (continued)

Organisms	Toxins	Sources	Toxicity
L. monocytogenes	Cholesterol-dependent cytolysin listeriolysin O (LLO), phosphatidylinositol-specific phospholipase C (PlcA), broad-range phospholipase C (PlcB)	Dairy products, soft cheeses, cheeses made with unpasteurized milk, celery, cabbage, ice cream, hot dogs, and processed meats	Gastroenteritis, meningitis, meningoencephalitis in immunocompromised individuals, abortions in pregnant women
Salmonella spp. *S. enterica* *S. Typhimurium* *S. Typhi*	Typhoid toxin/*Salmonella* cytolethal distending toxin (CDT) Typhoid toxin/A_2B_5 toxin Toxic Shock Syndrome Toxin (TSST-1)	Poultry meat products, and eggs, undercooked meat or ground beef, and dairy products	Nausea, vomiting, diarrhea, septicemia or bacteremia, and reactive arthritis
V. cholera, O1/O139	Cholera toxin (Ctx)	Contaminated water and raw seafood	Cholera
Campylobacter spp. *C. jejuni, C. coli*	Cytolethal distending toxin (CDT)	Poultry products, un pasteurized milk, and water	Campylobacteriosis, arthritis, meningitis, Guillain-Barré syndrome, acute diarrhea, abdominal pain, fever, intestinal bloody diarrhea

produced as separate A and B molecules which are preformed as A-B complex in solution and do not bind cells. Intoxication of these toxins initialy involves receptor binding of B components that form heptamer-receptor complex on the cell surface which acts as a docking platform for translocation of A component into the cytol where a binary form of components occured and can inhibit cell functions (Barth et al. 2004). However, other multiple-chain toxins composed of proteins produced by *B. cereus* and *S. aureus* do not associated in solution and pore-forming cytolysins remain on the cell surface and are attributed to their enzymatic activity (Barth et al. 2004). The microbial toxins from bacteria and fungi, and their characteristics, habitats and food sources, and general disease symptoms and complications, and outbreaks have been summarized in this chapter.

Mycotoxins are a group of secondary metabolites produced by filamentous fungi which have been implicated as causative agents of adverse health effects in humans and animals including livestock as a result of consuming fungus-infected agricultural products (Chandra et al. 2008). The Food and Agriculture Organization (FAO) estimates that one quarter of the world's crop are affected by mycotoxins each year. These mycotoxins commonly produced by *Aspergillus, Fusarium,* and *Penicillium* genera are diverse in their structure and biological activity, and can be produced on cereals and grains during their growing stage or harvesting and post-harvesting stages (Martinović et al. 2016).

3.2 Clostridium Toxins

Clostridium species are found throughout the nature and have developed unique mechanisms for survival within and outside of numerous hosts resulting in illneesses. *Clostridium* is an anaerobic bacterial genus and widely spread in the environment: soil, dust and water, comprising of more than 120 described species (Baldassi 2005). *Clostridia* produce more protein toxins than any other bacteria, and involved in severe diseases in humans and animals. The clostridial toxins cause neurotropic disorders (primarily affected nervous system), enterotoxemias (affecting intestinal tract and parenchymatous organs), and gas gangrene (myonecrosis with toxemia) (Baldassi 2005). *C. perfringens* ε-toxin and *C. septicum* α-toxin are the prototypes of clostridial toxins that form small pores. The cell intoxication mechanisms of *Clostridium* and *Bacillus* binary toxins involve activation of cell binding "B" precursors by proteolytic cleavage on the cell surface or solution. The activated B components interact with the specific cell surface receptors and form heptamers. B heptamer-receptor complex acts as a docking platform that translocates enzymatic A components into the cytosol and can inhibit normal cell functions by (i) mono-ADP-ribosylation of G-actin, which induces cytoskeletal disarray and cell death; (ii) proteolysis of mitogen-activated protein kinase kinases (MAPKK), which inhibits cell signaling; or (iii) increasing intracellular levels of cyclic AMP (cAMP) (Fig. 3.1) (Barth et al. 2004). Other toxins are involved in the degradation of specific cell membrane or extracellular matrix components due to their enzymatic activity (e.g. phospholipase C and collagenase). Clostridial neurotoxins inhibit neurotransmission at neuromuscular junctions, which have an essential cellular function (Popoff and Bouvet 2009). Due to this specification, clostridial neurotoxins have been used as powerfull pharmacological and biological tools for treatment of several diseases.

3.2.1 Clostridium perfringens *Enterotoxins, CPE, Alpha, Beta, Epsilon, and Iota*

Clostridium perfringens is a Gram-positive, rod-shaped, anaerobic, sporulating, and heat-resistant enterotoxin producing bacterium, which is relatively cold tolerant. It has been widely distributed throughout the environment and found in the intestine of healthy humans and animals (Petit et al. 1999). *C. perfringens* has been associated with various systemic and enteric diseases in humans and animals including food poisoning, enteritis, necrotic enteritis, enterotoxaemia, gangrene, and puerperal septicemia (Heida et al. 2016). *C. perfringens* is divided into five toxinotypes (A, B, C, D and E) depending upon their ability to produce main lethal toxins (alpha, beta, epsilon, and iota) (Petit et al. 1999; McClane et al. 2006; Popoff and Bouvet 2009). *C. perfringens* enterotoxin (CPE) is responsible for causing the gastrointestinal symptoms (Kokai-Kun et al. 1994). Most CPE-positive strains are classified as

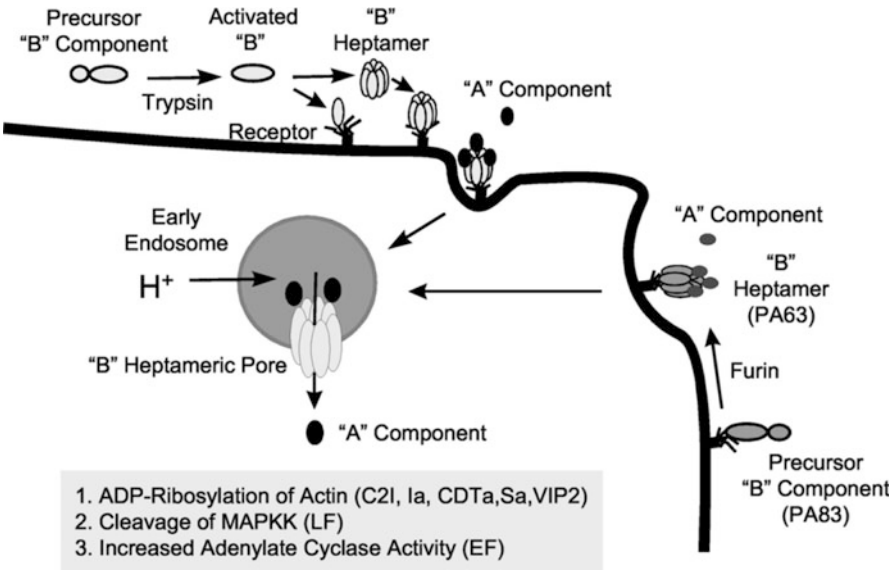

Fig. 3.1 The cell intoxication mechanisms of *Clostridium* and *Bacillus* binary toxins. B precursor components of binary toxins are activated by proteolytic cleavage on the cell surface and then activated B components interact with the specific cell surface receptors and subsequently form heptamers. B heptamer-receptor complex acts as a docking platform that translocates an enzymatic A components into the cytosol and can inhibit normal cell functions (Barth et al. 2004)

type A, although types C and D strains producing this enterotoxin are also fairly common. *C. perfringens* produces several virulence factors in the lumen of the gastrointestinal tract which increase mucosal colonization and cause cell alterations. Consequently, enterotoxins are absorbed into systemic circulation, and acts on distant organs such as kidney, lung, and brain. *C. perfringens* Alpha Toxin (CPA) is produced by all toxinotypes especially, A type. *C. Perfringens* is usually isolated from the intestine of apparently healthy humans and animals (McClane et al. 2006).

Clostridium perfingens Epsilon Toxin (ETX): It is synthesized by toxinotypes B and D. It is the causative virulence factor of all symptoms due to toxinotype B and lesions due to toxinotype D (Petit et al. 1999). ETX is a heptameric β-pore-forming toxin like *Aeromonas* aerolysin and *Clostridium septicum* alpha toxin (Knapp et al. 2010) but it is a much more potent toxin, which is responsible for enterotoxemia in animals, mainly in sheep (Finnie 2004). ETX induces perivascular edema in various tissues and accumulates particularly in the kidneys and in the brain, where it causes edema and necrotic lesions (Soler-Jover et al. 2007). ETX is able to pass through the blood-brain barrier (BBB) and to stimulate the release of glutamate, which accounts for the nervous excitation symptoms observed in animal enterotoxemia (Wioland et al. 2015). Recently, ETX-secreting *C. perfringens* type B strain has been isolated from a patient suffering from multiple sclerosis. ETX could specifically target the degeneration of central nervous system and lead to the cell death therefore, it could be involved in multiple sclerosis (Rumah et al. 2013). ETX is one of the most potent

toxins considered a potential biological weapon classified as a category B agent, after the botulinum neurotoxins. Vaccines based on chemically detoxified toxins using formalin against enterotoxemia due to *C. perfringens* ETX are extensively used in veterinary medicine.

Clostridium perfringens Iota Toxin: The type E strains of *C. perfringens* produces iota toxin including two separate proteins (iota A or Ia and iota B or Ib) and when proteins are combined, a potent cytotoxin is formed (Sakurai and Kobayashi 1995). These binary protein enterotoxins produced by some *Clostridium* and *Bacillus* species form an AB complex on a cell's surface that consists of ADP-ribosyl transferase (A) and cell-binding (B) components, initially released as separate proteins from the bacterium (Fig. 3.1). Following receptor-mediated endocytosis and endosomal trafficking, the A component mono-ADP-ribosylate globular actin in turn destroys the cytoskeleton and causes cell death (Barth et al. 2004, 2015). The iota toxin is associated with some diarrheic outbreaks among calves and lambs (Redondo et al. 2015). The other spore-forming bacilli use a similar binary mechanism for intoxicating the intestines of insects, animals, and humans that include: *C. perfringens* (iota toxin and binary enterotoxin), *C. spiroforme* (*C. spiroforme* toxin, CST), *C. difficile* (*C. difficile* toxin, CDT), *C. botulinum* (C2 toxin), as well as *Bacillus cereus* (vegetative insecticidal protein, VIP) (Stiles et al. 2014).

3.2.2 Clostridium spiroforme *Toxin (CST)*

C. spiroforme causes severe diarrhea, enterocolitis, and eventually death of host especially in rabbits (Carman and Borriello 1982). In 1970s a strong correlation was established between colony outbreaks in rabbits and *C. spiroforme*. The virulence of *C. spiroforme* strongly correlates with the production of an enterotoxin, CST including Sa and Sb components which are analogous to Ia and Ib of the iota toxin (Borriello and Carman 1983). CST's enzyme component (Sa) harbors mono (ADP-ribosyl) transferase activity and its separate binding component (Sb) forms heptamers wich is responsible for the cell entry and binds to target cell by forming pores in endosomal membranes. After that, the translocation of the enzyme component into the cytosol of target cells occurred (Barth et al. 2004). A component of CST inhibit the functions of the cell by mono-ADP-ribosylation of G-actin, thereby leading to cytoskeletal disarray and death (Stiles et al. 2014). Furthermore, the components of CST and iota toxin are interchangeable and form biologically active chimeras as analogous structures.

3.2.3 Clostridium difficile *Toxin (CDT)*

Over the past 15 years, hypervirulent strains of *C. difficile* have emerged causing increased morbidity and mortality particularly in health-care settings (Gerding et al. 2014). These hypervirulent strains possesing CDT are more antibiotic resistant, more

readily sporulate and increase production of large molecular weight toxins A and B. *C. difficile* is the cause of antibiotics-associated diarrhea and pseudomembranous colitis, which are quite problematic and life-threatening for some unfortunate patients. *C. difficile* can also colonize in many animals used for food production (cattle, chickens, pigs, rabbits, and sheep), wildlife (elephants), and even pets (cats and dogs) besides of humans (Keessen et al. 2011). In addition, *C. difficile* and its spores were isolated from commercially available meats and vegetables, which were foodborne sources for human colonization (Metcalf et al. 2010; Harvey et al. 2014). *C. difficile* toxins A (TcdA) and B (TcdB), two large secreted proteins that contain four structurally homologous domains, and *C. difficile* transferase toxin have much structural homology with iota toxin and CST (Pruitt et al. 2010). TcdA and TcdB containing glucosyl transferase domains at the amino terminus mediate toxicity by glycosylating. Therefore, inactivation of host GTPases in the cytosol of targeted cells disrupts the cytoskeleton and leads to the disassociation of tight junctions between colonic epithelial cells and the loss of epithelial integrity (Hunt and Ballard 2013; Abt et al. 2016).

3.2.4 Clostridium botulinum *C2 and BoNTs Toxin*

C. botulinum is a Gram-positive, obligate anaerobic, rod-shaped, spore-forming, heat-resistant bacrerium and commonly found in soils and marine sediments throughout the world (Rossetto et al. 2014). *C. botulinum* was first described in a food poisoning due to consumption of contaminated sausage (Devriese 1999). The neurotoxin types (A–G) of *C. botulinum*, Botulinum Toxins (BoNTs) are produced during vegetative growth and classically are determined by mouse lethal assays with toxin-specific antisera (Poulain et al. 2015). The non-neurotoxic C2 toxin of *C. botulinum* produced by types C and D during sporulation induces adverse effects in animals such as vascular permeability, necrotic-hemorrhagic lesions, as well as a lethal fluid accumulation in lungs and intestinal tracts (Stiles et al. 2014). C2 toxin is composed of two separate proteins, which are the cell binding and translocation component C2II and enzyme component C2I (Nagahama et al. 2009). The B domain protein C2II binds target cells and translocates the A domain C2I. The A domain is an ADP-ribosyltransferase that causes cell rounding and apoptosis initiated by ADP-ribosylation of cytoplasmic actin C2II monomers are proteolytically processed to remove a 20 kDa segment from the N-terminus (Fig. 3.1) (Ohishi and Tsuyama 1986; Aktories et al. 1986). These proteins act in binary combinations to produce toxic, cytotoxic, and lethal effects and they influence vascular permeability (Pavlik et al. 2016).

The toxins are produced during vegetative growth and classically are determined by mouse lethal assays with toxin-specific antisera (Poulain et al. 2015). There are seven distinct forms of BoNTs, types A–G. Four of these (types A, B, E and rarely F) cause human botulism and types C, D and E cause illness in other mammals, birds and fish. The spores are heat-resistant and can survive in foods when they are improperly processed and inadequately cooked home-preserved foods. BoNTs are

produced by different *C. botulinum* strains, which belong to four phylogenetically distinct groups, and by *C. butyricum* and *C. barati*. BoNTs are produced together with nontoxic accessory proteins (NAPs) to form progenitor toxin complexes (PTCs) of various sizes. NAPs including a nontoxic non-hemagglutinin component form with the neurotoxins a hand-in-hand-shaped heterodimer, and several hemagglutinin components (HAs) (Popoff and Marvaud 1999). Nontoxic non-hemagglutinin component is a protective role of the neurotoxin primarily from the many proteases and protein-modifying agents (Benefield et al. 2013; Lee et al. 2013). Therefore, BoNT can be produced secondarily in the harsh environment of the gastrointestinal tract (Rossetto et al. 2014). Conversely, HA proteins of PTCs present multiple carbohydrate-binding sites which are likely to act as binding sites on the intestinal mucus layer and the polarized intestinal epithelial cells of the intestinal wall through which BoNTs enter into the lymphatic circulation and then in the blood circulation (Simpson 2013; Fujinaga et al. 2013). In the PTC, the proteins are not covalently linked, but their association occurs in the bacterial cultures and in naturally contaminated food. The complex is stable at acidic pH, but dissociates at pH 7 (Eisele et al. 2011). The median lethal dose (LD_{50}) of BoNTs for susceptible mammals, including humans, range from 0.1 to 1.0 ng/kg of body weight. *Botulinum* neurotoxins are included as Category A bioweapon select agents by the US Centers for Disease Control and Prevention (CDC). On the other hand, the BoNTs have been developed as therapeutics for the treatment of many human disorders characterized by hyperexcitability of peripheral nerve terminals and hypersecretory syndromes (Hallett et al. 2013).

3.3 Bacillus Toxins

The *Bacillus* species and related genera are the causative agents of foodborne diseases due to their heat resistant endospores (Tewari and Abdullah 2015). The most important pathogenic species consist of *B. cereus*, *B. mycoides*, *B. thuringiensis*, *B. anthracis, B. weihenstephanensis* (Lechner et al. 1998), *B. pseudomycoides,* and *B. cytotoxicus* (Nakamura 1998; Stiles et al. 2014). The most species are found in the natural habitat in soil therefore, direct contamination of agricultural products from soil increases the risks of food-borne infection or intoxication and food spoilage (Tewari and Abdullah 2015). *Bacillus thuringiensis* (Bt) is a Gram-positive, spore-forming bacterium that synthesizes parasporal crystalline inclusions containing insecticidal Cry- and Cyt-proteins, produced during sporulation which are toxic against a wide range of insects and nematodes (Palma et al. 2014; Lindback and Granum, 2015). *B. weihenstephanensis* is a psychrotrophic strain, and thus can grow at refrigerated temperatures. Foods such as beef, turkey, rice, beans, and vegetables were associated with outbreaks. *B. cereus* was recorded to be the third most common cause of the food-poisoning outbreaks in Hungary (117 outbreaks) between 1960 and 1968, followed by Finland (50 outbreaks), Netherlands (11 outbreaks), and Canada (9 outbreaks) (Tewari and Abdullah 2015).

3.3.1 Bacillus anthracis *Toxins*

Bacillus anthracis, an agent of anthrax, is Gram-positive, spore-forming bacterium that causes an acute and commonly lethal infection. It significantly affects grazing livestock, wild ungulates, and other herbivorous mammals, but also causes a serious threat to human health (Hugh-Jones and Blackburn 2009). The main virulence factors of *B. anthracis* are edema and lethal toxins formed by three components which are protective antigen (PA, 83 kDa, the binding component), edema factor (EF, 89 kDa, calmodulin-dependent adenylate cyclase), and lethal factor (LF, 90 kDa, zinc-dependant metalloprotease). The pathogenicity of *B. anthracis* is linked to plasmid encoded poly γ-D-glutamic acid, and the two toxins, edema toxin (ET) and lethal toxin (LT) (Fig. 3.1) (Moayeri et al. 2009). ET disrupts endothelial homeostasis and induces a strong disruption of the actin cytoskeleton (Trescos et al. 2015). ET and LT set up a complex immune evasion strategy by targeting key role in the immune defenses of the host by acting on neutrophils, macrophages, monocytes, dendritic cells (DCs), and T and B cells in the early stage of anthrax (Tournier et al. 2009). LT primarily targets cardiomyocytes and vascular smooth cells, while hepatocytes are the major targets of ET (Liu et al. 2013). In the late stage of anthrax, toxic shock-like symptoms, severe respiratory dysfunction, and hypotension are observed and followed by cardiac failure. *B. anthracis* spores are highly resistant to weather extremes and can remain viable in soil and contaminated animal products, such as bones or hides, for many years. Humans can become infected from exposure to infected animals or contaminated animal products (including meat, hides, and hair) (Shadomy and Smith 2008). Inhalation of spore-contaminated soil has been suggested as a possible source of infection for bison in anthrax outbreaks in Canada (Dragon et al. 1999). Outbreaks and epidemics in human upon exposure to anthrax via inhalation, dermal, and ingestion are highest in Africa, the Middle East, and central and southern Asia (WHO 1998; 2008a). *B. anthracis* is a veterinary disease affecting livestock as well as a major agent of biological warfare and is thus of interest for biodefense (Mock and Fouet 2001). In 2001, its potential use as a weapon of bioterrorism in the United States was occurred as the attacks related to letters contaminated with anthrax spores (WHO 2008a). The anthrax vaccines for animals utilize the toxigenic, non-capsulating *B. anthracis* strain derived from a virulent bovine isolate in the 1930s. In the United States vaccine for human use, now commonly referred to AVA (anthrax vaccine adsorbed), a study of mill workers when the vaccine was first being introduced recorded a protection rate of 93% (Brachman et al. 1962).

3.3.2 Bacillus cereus *Enterotoxins*

Bacillus cereus is a Gram-positive, facultatively anaerobic, heat resistant endosporeforming, large rod bacterium. The ability to form heat resistant endospores which can survive pasteurization and cooking processes and produce toxins in a

wide variety of foods significantly increased the importance of *B. cereus* with respect to food safety (Lindback and Granum 2006). *B. cereus* is one of the most ubiquitous bacteria in the natural environment such as decaying organic material, fresh water, soil, marine water, vegetables, and the intestinal tract of invertebrates (Tewari and Abdullah 2015). *B. cereus* produces an emetic toxin (ETE) and three different enterotoxins responsible for the diarrhoeal type of food poisoning (Hemolysin BL (Hbl), Non-haemolytic enterotoxin (Nhe), and Cytotoxin K (CytK)). Hbl is composed of L2, L1 (lytic proteins) and B proteins (hemolysin), whereas Nhe is composed of NheA, NheB, and NheC protein components. The emetic syndrome, due to ETE, is an intoxication caused by a single toxin highly resistant to heat, proteolysis, acid, and alkali (Shinagawa et al. 1995). Cereulide is a thermostable small ring-formed dodecadepsipeptide that is produced during the stationary phase and causes emesis. Cereulide also forms ion channels and holes in membranes. The heat-labile diarrhoeagenic enterotoxin Nhe and/or hemolytic enterotoxin Hbl mediated foodborne poisoning cause intestinal fluid secretion probably due to pore formation and activation of adenylatecyclase enzymes (Lindback and Granum 2006). Hbl is a proteinaceous toxin that also has dermonecrotic and vascular permeability activities and causes fluid accumulation in animal studies. Nhe is another three-component proteinaceous, pore-forming toxin. CytK is a single-component, b-barrel pore forming toxin that is dermonecrotic, cytotoxic, and haemolytic, and nearly 90% of *B. cereus* strains may carry the gene for it (Ngamwongsatit et al. 2008). The cases with both diarrhoeal and emetic symptoms caused by strains to produce toxin(s) influence the infective or intoxicating dose in either types of illness. Food-borne outbreaks of *B. cereus* from a large variety of foods in many countries including the USA, UK, European countries, Scandinavia, Japan, and China are characterized by generally mild and self-limiting, more severe and fatal forms of diseases (Lindback and Granum 2006). The ingestion of *B. cereus* contaminated food is the primary mode of transmission. The diarrheal type is transmitted mostly by milk products, vegetables, and meat while emetic type of food poisoning has been largely associated with the consumption of rice and pasta (Murray et al. 2007). The major factors that contribute to food poisoning by *B. cereus* and its toxins are commonly inadequate cooking temperatures, contaminated equipment, and poor hygiene conditions during food processing.

3.4 Other Bacterial Toxins

Food poisoning or foodborne disease is one of the main problems in public health worldwide. According to the WHO, each year 600 million people around the world become ill after consuming contaminated food. Among all these people, 420,000 die, including 125,000 children under 5 years of age (WHO 2015). A total of 66% of foodborne diseases is caused by illnesses such as botulism caused by *C. botulinum*, gastroenteritis caused by *E. coli* strains, *Salmonellosis* and *Staphylococcal* poisoning. Moreover, *B. cereus* and *Vibrio colera* are bacteria frequently reported as

causative agents of toxic infection by food (Hernández-Cortez et al. 2017). Cholera toxin (Ctx) (*Vibrio cholerae*), thermolabile toxin (LT), thermostable toxin (ST) (Enterotoxigenic *E. coli*), Shiga Toxin (*Shigella dysenteriae* and *E. coli* O157:H7) have also been involved in foodborne diseases (Lindback and Granum 2006; Hernández-Cortez et al. 2017). In addition, *Campylobacter* and *Salmonella* are pathogenic and their reservoirs are livestock and domestic animal. Contamination with *Campylobacter* and *Salmonella* is produced due to bad practices in the food production chain and by cross-contaminations (Lamas et al. 2018: Lai et al. 2016).

3.4.1 Helicobacter Toxins

Helicobacter pylori is a Gram-negative, spiral-shaped, flagellated, and microaerophilic bacterium that have colonized in the stomach, intestine, and liver of mammals, birds, and reptiles. Some strains are more virulent than others and are more often associated with chronic inflammatory response, severe gastric diseases, including ulcers and cancer. Its prevalence in developing countries is 70–90% whereas it is about 25–50% in more developed countries (IARC 2011). The transmission of *H. pylori* has been reported as fecal–oral or oral–oral routes with water and food as possible vehicles of infection (Tanih et al. 2010). The presence of *H. pylori* genes in the oral cavity has been associated with their presence in the stomach and symptomatic gastric disease (Liu et al. 2009; Silva et al. 2010). Epidemiological studies have revealed positive associations between untreated or fecally contaminated drinking water and incidence of *H. pylori* infection (Bellack et al. 2006). Foodborne transmission of *H. pylori* was reported to be present within cells of brewers' and bakers' yeast (Siavoshi et al. 2011) and in drinking water (Goh et al. 2011), raw chickens, tuna (Meng et al. 2008), raw bovine milk (Fujimura et al. 2002), raw sheep, goat, and cow milk (Quaglia et al. 2008). There is an increasing evidence from epidemiological studies of the association of *H. pylori* infection and specific virulence factors with gastric cancer (Park et al. 2018). The virulence factor of *H. pylori*, the cag pathogenicity island (cag PAI) in the *H. pylori* genome has been identified (Cover 2016). It consists of Cag family proteins, which constitute a type IV secretion system (T4SS) which is responsible for injecting bacterial factors, cytotoxin-associated gene A (cagA), into the host cell. They can be phosphorylated by the host enzymes and initiates a series of events, which may dramatically interfere with cell morphology, motility, polarity, proliferation, and differentiation, leading to invasive phenotypes of host cells (Backert et al. 2015). It contributes to the activation of proinflammatory signaling cascade through interaction with the cytosolic pathogen recognition which initiates host defense against cag PAI-positive *H. pylori* strains (Viala et al. 2004; Kwok et al. 2007). In epidemiological studies, a clear link between CagA and the development of precancerous lesions and eventually gastric cancer was found in humans, as well as studies in animals infected with CagA-positive *H. pylori* strains, or in transgenic mice expressing CagA (Liu et al. 2009). The discovery of *H. pylori* and of its link to peptic ulcer led to 2005 Nobel

Prize in Medicine to Barry Marshall and Robin Warren (Ahmed 2005). The development of malignancy is also linked to host factors such as proinflammatory genetic background. The International Agency for Research on Cancer (IARC) has classified *H. pylori* as a group 1 carcinogen (IARC 1994). The risk of malignant outcome of the *H. pylori* infection would be decreased by vaccines targeting CagA (Park et al. 2018).

3.4.2 Escherichia coli *Shiga Toxin*

The enterohemorrhagic *Escherichia coli* (EHEC), a subset group of Shiga toxin-producing *E. coli* (STEC), are pathogenic strains, with *E. coli* O157:H7. *E. coli* is a Gram-negative, facultative anaerobe, non-sporulating rod within the family Enterobacteriaceae (Feng 2013). The type three secretion systems (T3SS) and Shiga toxin (Stx) are two main virulence factors of EHEC, T3SS encoded by the locus of enterocyte effacement is involved in the formation of attaching and effacing (A/E) lesions on the colonic epithelium through the injection of specific effectors into epithelial cells. Interactions of bacterial effectors with eukaryotic signal transduction pathways lead to host cytoskeleton reorganization that is characterized by two key markers of A/E lesions: an effacement of microvilli and formation of pedestals beneath adherent EHEC (Schmidt 2010). Shiga toxins (stx 1 or stx 2) encoded by genes located on lysogenic lambdoid phages is an A1:B5 toxin that binds specifically to a receptor at the surface of intestinal and glomerular endothelial cells. Stx blocks translation in intoxicated cells resulting in cell death by apoptosis and renal dysfunction (Griffin et al. 1988; Karmali et al. 1983; Croxen et al. 2013). STEC are an important cause of foodborne disease and infections have been associated with a wide range of human clinical illnesses ranging from mild non-bloody diarrhoea to bloody diarrhoea (BD) and haemolytic uraemic syndrome (HUS) which often includes kidney failure (Tarr et al. 2005). The modes of STEC infection is transmitted to human from contaminated foods, animals, water, environment, and through person-to-person contact (DuPont 2007). Shiga toxin-producing *E. coli* including O157 and many non-O157 serogroups, are important causes of foodborne diseases. Although many outbreaks throughout the world has been attributed to O157:H7, approximately 400 STEC serotypes are considered to be implicated in the disease (Karmali et al. 2010). STEC causes 2,801,000 acute illnesses annually worldwide and leads to 3890 cases of HUS, 270 cases of end-stage renal disease, and 230 deaths in the USA, costing more than $1 billion each year in direct and indirect costs (Majowicz et al. 2014). The most frequently attributed sources of STEC cases globally are beef (11%,) and dairy products (7%) (FAO and WHO 2018). There were a total of 919 STEC outbreaks based on surveillance received from 27 countries between 1998 and 2016. Outbreakes were caused by a single food category (328 outbreaks, 36%), a complex food (79 outbreaks, 9%) and were not attributed to a source (512 outbreaks, 56%) (FAO and WHO 2018). It is not easy to eliminate enteropathogens. However, it is possible to

reduce the risk of contamination by reduction of the pathogens in farms, application of sublethal multiple hurdles in the food processing and preservation, proper cooking of the food products, and avoiding the consumption of raw/uncooked ani-mal products.

3.4.3 Staphylococcus aureus *Enterotoxins*

Staphylococcal species are Gram-positive, facultative anaerobe, catalase-positive, spherical and small bacteria (cocci), which are commonly found in foods due to environmental, human, and animal contamination. *S. aureus* is one of the most resistant non-sporeforming human pathogens and can survive for extended periods in a dry state. *S. aureus* is an opportunistic human pathogen known to colonize the respiratory tract of approximately 30% of the USA population (Gorwitz et al. 2008). *S. aureus* is a versatile human pathogen capable of causing staphylococcal food poisoning, toxic shock syndrome, pneumonia, postoperative wound infection, and nosocomial bacteremia. The *S. aureus* toxins can be divided into three major groups; the pore-forming toxins (PFTs), exfoliative toxins (ETs), and superantigens (SAgs). Pore-forming toxins can be further divided into four types which are Hemolysin-α (Hla or α-toxin), Hemolysin-β, leukotoxins, and phenol-soluble modulins (PSMs) (Oliveira et al. 2018). *S. aureus* toxins are associated with some diseases such as toxic shock syndrome (TSS), staphylococcal scalded skin syndrome (SSSS), necrotizing pneumonia, or deep-seated skin infections (Dinges et al. 2000). The toxins are capable of damaging the cell membranes of the host, either by degrading intercellular connections or by modulating immune responses (Grumann et al. 2014). Staphylococcal enterotoxins are stable in the gastrointestinal tract and indirectly stimulate the emetic response. When they are ingested, the enterotoxin may rapidly produce symptoms, which commonly include nausea, abdominal cramping, vomiting, and diarrhea. The commonly implicated food poisoning associated with staphylococcal enterotoxins includes meat and meat products, poultry, bakery products, and salads with mayonnaise. *S. aureus* is the cause of sporadic food poisoning episodes around the world. CDC estimates that, in the United States, staphylococcal food poisoning causes approximately 241,188 illnesses, 1064 hospitalizations, and 6 deaths each year. For instance, multiple methicillin-resistant *Staphylococcus aureus* infections were reported in correctional facilities in Georgia, California, and Texas between 2001–2003 (CDC 2003). Multiple staphylococcal foodborne diseases in 1989 were associated with canned mushrooms (CDC 1989).

3.4.4 Listeria monocytogenes *Exotoxins*

Listeria monocytogenes is a Gram-positive, rod-shaped, facultatively anaerobic, flagellated, ubiquitous, and intracellular pathogen that is among the leading causes

of death from foodborne illness. *L. monocytogenes* can be found in moist environments, soil, and decaying vegetation and can adopt a planktonic life or form biofilms (Cossart 2011). This bacterium can grow at 4 °C, at extreme pH, and in high salt concentration, ready-to-eat food products being usually involved in listeriosis outbreaks reported worldwide (Cossart 2011). *L. monocytogenes* is mainly present in dairy products, soft cheeses, cheeses made with unpasteurized milk, celery, cabbage, ice cream, hot dogs, and processed meats (CDC 2016). It is able to cross the intestinal barrier, the blood–brain barrier, and the fetoplacental barrier and infects animals and humans (Cossart 2011). *L. monocytogenes* secrates four exotoxins: the cholesterol-dependent cytolysin listeriolysin O (LLO), a phosphatidylinositol-specific phospholipase C (PlcA), a broad-range phospholipase C (PlcB), and a hemolysin listeriolysin S (LLS). The main pathogenicity islands of *L. monocytogenes* strains are LIPI-1, LIPI-3, and LIPI-4. LLO is a pore-forming hemolytic toxin responsible for bacterial phagosome escape into the cytosol of an infected host cell and spreading infection (Seveau 2014). LIPI-1 is orchestrating regulatory factors which have roles in actin polymerization and metalloprotease associated activation of pathogenicity (Cossart 2011). LIPI-3 contains listeriolysin S (LLS), a posttranslational modified peptide that exhibits properties of both hemolytic–cytotoxic factors and bacteriocins (Cotter et al. 2008; Quereda et al. 2016). LIPI-4 encodes for a cellobiose PTS system necessary for central nervous system infection (Maury et al. 2016). *L. monocytogenes* is able to cross the intestine invading phagocytic and non-phagocytic cells and then pass from primarily infected cells to neighboring cells. It can be disseminating within tissues without being exposed to antimicrobial molecules and phagocytes of the immune system in the extracellular environment (Stavru et al. 2011). To successfully colonize the intestine, *L. monocytogenes* has developed an elaborated exotoxins therefore, it can invade eukaryotic cells, escape from the internalization vacuole, resist autophagic killing, and multiply and spread from cell to cell (Cossart 2011; Stavru et al. 2011). *L. monocytogenes* is causative agent of self-limiting gastroenteritis in healthy individuals as well as meningitis and meningoencephalitis in immunocompromised individuals, and abortions in pregnant women. Although epidemics can occur with a high mortality rate (20–30%) and neurological sequelae in at-risk populations, its cases are sporadic (Cossart 2011). An outbreak of listeriosis from cantaloupes in Colorado, USA, in 2011 resulted in infection of 147 people and 33 deaths (CDC 2016). In the USA, CDC estimates that 1600 illnesses and 260 deaths caused by listeriosis annually (CDC 2016) and also in the EU, a total of 2161 cases of listeriosis were recorded (EFSA 2010a). *L. monocytogenes* infection cases can be treated with antibiotics such as ampicillin, penicillin, and gentamicin suggested for impaired immunity, including neonates, and in cases of meningitis and endocarditis.

3.4.5 Salmonella *spp. Enterotoxin*

Salmonella is a Gram-negative, rod-shaped, motile, non-sporeforming, bacterium in the family *Enterobacteriaceae*. *Salmonella* is widely dispersed in nature and can

colonize the intestinal tracts of vertebrates, including livestock, wildlife, domestic pets, and humans (Ricke et al. 2013). It may contaminate meat, farm-irrigation water, soil, insects, factory equipment, hands, kitchen surfaces, and utensils. *Salmonella* can cause two types of illnesses, depending on the serotype: nontyphoidal salmonellosis and typhoid fever. Nontyphoidal salmonellosis is caused by serotypes other than *S.* Typhi and *S.* Paratyphi A. The subspecies *enterica, Salmonella* Typhimurium and *Salmonella* Enteritidis, is responsible for more than 99% of human salmonellosis (Lamas et al. 2018). The symptoms of salmonellosis include nausea, vomiting, abdominal cramps, diarrhea, fever, and headache which are generally self-limiting among healthy people with intact immune systems. *S. enterica* subsp. *enterica* serovar *Javiana* produces typhoid toxin or *Salmonella* cytolethal distending toxin (CDT) which plays an important role in the DNA damage and systemic host colonization (Miller and Wiedmann 2016). The genotoxin CDT is one of the major virulence factors of *S. Typhi*, which causes typhoid fever in humans. However, CDC is not commonly found in major nontyphoidal salmonellosis causing serotypes. *S.* Enteritidis, *S.* Typhimurium, *S.* Newport, *and S.* Heidelberg are among the top serotypes causing nontyphoidal salmonellosis (Miller and Wiedmann 2016). Nontyphoidal *Salmonella* infection or salmonellosis is a major public health concern throughout the world and approximately 93.8 million cases of gastroenteritis are globally estimated to occur each year. A majority of salmonellosis are foodborne resulting in an estimated at 80.3 million cases per year (Majowicz et al. 2010). Typhoid fever is caused by the typhoid toxin produced by serotypes *S.* Typhi and *S.* Paratyphi A, both of which are found only in humans. The toxin can intoxicate the infected cell or be released into the extracellular environment and intoxicate noninfected, bystander cells (Song et al. 2013). *Salmonella* Typhi causes life-threatening typhoid fever in humans and it has a small locus encoding typhoid toxin, an A2B5 toxin with several distinct characteristics that contribute to *S.* Typhi's pathogenicity (Chong et al. 2017). The symptoms of typhoid fever are abdominal pains and diarrhea or constipation; headache; achiness; loss of appetite. Septicemia, with colonization of other tissues and organs, may lead to endocarditis (Chong et al. 2017). CDC estimates that *Salmonella* causes about 1.35 million illnesses, 26,500 hospitalizations, and 420 deaths in the USA every year. Food is the source for about one million of these illnesses. This highlights the significant public health importance of *Salmonella* infections and the urgency of control, particularly for invasive infections in low- and middle-income settings where most of the mortality occurs (Majowicz et al. 2010).

3.4.6 Vibrio Cholera *Enterotoxin*

Vibrio cholera is a Gram-negative, facultative anaerobic, non-spore forming curve-shaped bacterium naturally occurring in salty water, but survives and exists in aquatic environments ranging from freshwater to open ocean (Maheshwari et al. 2011; Hernández-Cortez et al. 2017). It is a causative agent for gastroenteritis and septicemic infections among people with predisposing conditions including chronic

liver disease (cirrhosis, hepatitis, liver transplantation, and cancer of the liver), elevated serum iron levels (hemachromatosis), compromised immune system, and other chronic illnesses (Hernandez-Cortez et al., 2017). *V. cholerae* O1 and O139, causative serogroups of Cholera and CTX toxin (Cholera toxin) is the main virulence factor. CTX toxin is responsible for the harmful effects of cholera infection. Each of the subunits of A-B subunit group of toxins has a specific function. The B subunit binds the holotoxin to the eukaryotic cell receptor. The effect is dependent on a specific receptor and monosialosyl ganglioside present on the surface intestinal mucosal cells (Guidolin and Manning 1987). A subunit possesses a specific enzymatic function for adenylate cyclase activation in small intestinal epithelial cells. It causes profuse diarrhea resulting in dehydration electrolyte imbalance, and death (Hernandez-Cortez et al., 2017). Cholera has been categorized as one of the emerging and re-emerging infections in developing countries (Satcher 1995) and is classified as a Category B bioterrorism agent by CDC (WHO 2008b). The most substantial burden due to foodborne cholera occurs in African and Asian Regions (WHO 2015). Approximately 1.3 billion people are at risk for cholera in endemic countries. It is estimated that 2.86 million cholera cases occur annually in endemic countries resulting in an estimated 95,000 deaths among these cases (Ali et al. 2015). The fatality rate is about 5%, generally among people with the predisposing conditions. CDC estimates that these Vibrio species cause 17,564 cases of foodborne illness from annually in the USA.

3.4.7 Campliobacter *spp. Cytolethal Distending Toxin (CDT)*

Campylobacter spp. is a non-sporeforming, curved Gram-negative rod, microaerophilic bacterium. It is one of the most common causative agents of foodborne diarrheal disease in humans worldwide (Silva et al. 2011). According to European Food Safety Authrotiy (EFSA), 20–30% of human cases of campylobacteriosis may occur due to handling, preparation, and consumption of broiler meat while 50–80% may be attributed to the chicken reservoir (EFSA 2010b). Campylobacteriosis is characterized by acute onset of diarrhea, abdominal pain, and fever, and it is usually self-limiting (WHO 2012). However, a range of other serious conditions within the gastrointestinal tract have been reported, including intestinal bloody diarrhea, esophageal diseases, periodontitis, functional gastrointestinal disorders, celiac disease, cholecystitis, and colon cancer. *C. jejuni* infection in humans usually occurs upon consumption of contaminated poultry products (Corry and Atabay 2001). *C. jejuni*-associated enterocolitisis is typically associated with a local acute inflammatory response that causes intestinal tissue damage (Black et al. 1988). The important virulence factor of *C. jejuni* is cytolethal distending toxin (CDT), a genotoxin which is composed of three subunits: CdtA, CdtB, and CdtC. CDT cell elongation, cell distention, irreversible cell cyle arrest, and consequently leads to cell death (Yamasaki et al. 2006). CdtA and CdtC serve as carriers for delivering the active subunit, CdtB, into host cells. CdtB is subsequently internalized

while CdtA and CdtC remain associated with the membrane receptor (Lai et al. 2016). Current experimental and epidemiological studies support critical roles of CDT in *C. jejuni*-induced pathogenesis, including cell adhesion, invasion, and inflammation. Bacteria that cause persistent infections associated with chronic inflammatory responses may possess a high risk of promoting carcinogenesis (Lai et al. 2016). It is estimated that *Campylobacter* causes more than two million illnesses (1% of the population), 13,000 hospitalizations, and 100 deaths each year in the USA (Anonymous 2007).

3.5 Mycotoxins

Mycotoxins are a group of secondary metabolites produced by filamentous fungi, which has been implicated as causative agents of adverse health effects in humans and animals including livestock as a result of consuming fungus-infected agricultural products (Chandra et al. 2008; Alshannaq and Yu 2017). The Food and Agriculture Organization (FAO) estimates that one quarter of the world's crop are affected by mycotoxins each year. These mycotoxins commonly produced by *Aspergillus, Fusarium*, and *Penicillium* genera are diverse in their structure and biological activity, and can be produced on cereals and grains during their growing, harvesting, and post harvesting stages. Ingestion of mycotoxin-contaminated grains can cause severe health effects in humans and animals including livestock (Martinović et al. 2016). The most common mycotoxin-contaminants are aflatoxins, fumonisins, ochratoxins, and trichothecenes such as deoxynivalenol and zearalenones (Fig. 3.2). Eventhough good agricultural, storage, and processing practices are implemented, they are not easily eliminated during food processing due to their heat stability, physical, and chemical properties (Cetin and Bullerman 2006).

The ingestion of mycotoxin-contaminated grains can cause severe health effects in humans and animals including livestock. For instance, consumption of Brazilian peanuts contaminated with aflatoxin caused the outbreak of Turkey-X disease and led to death of 100,000 poults in England in 1960 (Bullerman 1979). Among the mycotoxin-producing fungal species, *Fusarium* species are one of the most considerable ones due to their incidence, diverse toxigenic nature, and its health effects ranging from acute skin lesions to cancers incidence. Fungi grow on a variety of different crops and foodstuffs including cereals, nuts, spices, dried fruits, apples, and coffee beans, often under warm and humid conditions and can pose adverse health effects on human and animals range from acute poisoning to long-term effects such as immune deficiency and cancer (Chandra et al. 2013; Ramana et al. 2013, 2014; Priyanka et al. 2014). There is also a public health concern over the potential ingestion of animal-derived food products, such as meat, milk, or eggs, containing residues or metabolites of mycotoxins (Alshannaq and Yu 2017). Food-borne fungi are capable of producing hundreds of secondary metabolites but only a relative few are regulated, considering their adverse effects on human and animal health (JECFA 2017). Aflatoxins, fumonisins, tricothecenes (particularly deoxynivalenol),

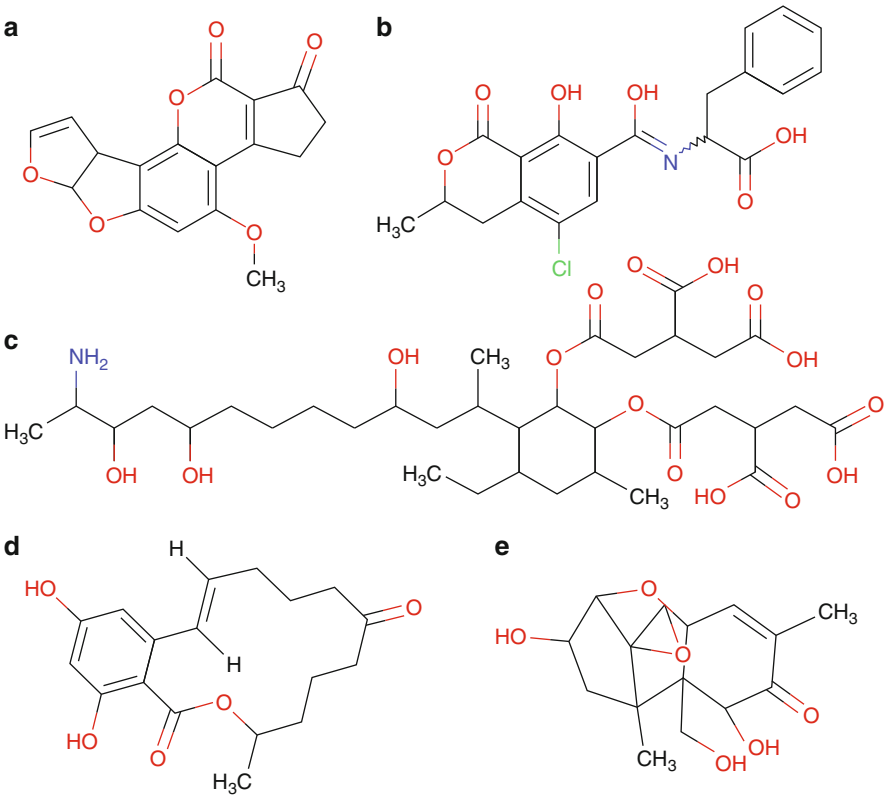

Fig. 3.2 Chemical structures of most prevalent mycotoxins. (**a**) Aflatoxin B1, (**b**) Ochratoxin A, (**c**) Fumonisin B1, (**d**) Zearalenone, (**e**) Deoxynivalenol. (The Metabolomics Inovation Centre, http://www.t3db.ca)

ochratoxins, and zearalenone are widely regulated mycotoxins whereas other mycotoxins including ergot alkaloids, patulin and T-2 and HT-2 toxins are not widely regulated (Table 3.2) (Van Egmond et al. 2007). Although many methods have been validated and used for the analysis of mycotoxins in food and feed, there are still major challenges and drawbacks to these analytical methods due to difficulties in detecting low-level of mycotoxin contamination in complex food matrices, the great diversity of mycotoxin chemical structures, and the co-occurrence of mycotoxins (Alshannaq and Yu 2017). Mycotoxin exposures not only affect human and animal health in the developed countries of Western Europe, Canada, and the United States, but also cause increased costs of foods, increased cost of health expenses, and accumulating effects on environment. Because of the harmful effects of mycotoxins, the prevention of fungal growth, food decontamination, and detoxification strategies are very crucial for health and economy (Cetin and Bullerman 2005a, b, 2006).

Table 3.2 Major mycotoxins in food and feed (Alshannaq and Yu 2017)

Mycotoxins	Fungal Species	Food Commodity	Toxicity	Limits (μg/kg)
Aflatoxins B1, B2, G1, G2 M1, M2	*A. flavus, A. parasiticus*	Maize, wheat, rice, peanut, sorghum, pistachio, almond, ground nuts, figs, cottonseed, spices	Carcinogenic, teratogenic, hepatotoxic, mutagenic, immunosuppressive	20 for total 0.5 for milk
Ochratoxin A	*A. ochraceus, P. verrucosum*	Cereals, dried vine fruit, wine, grapes, coffee, cocoa, cheese	Nephrotoxic, hepatotoxic	Not set
Patulin	*P. expansum*	Apples, apple juice, & concentrate	Ulceration, hemorrhage	50
Fumonisins B1, B2, B3	*F. verticillioides, F. proliferatum*	Maize, maize, products, sorghum, asparagus	Equine leukoencephalomalacia, porcine pulmonary edema carcinogenic	2000–4000
Zearalenone	*F. graminearum, F. culmorum*	Cereals, maize, wheat, barley	Reproductive toxicity	Not set
Deoxynivalenol	*F. graminearum, F. culmorum*	Cereals, cereal products	Neurotoxic, immunosuppresive	1000

3.5.1 Aflatoxins

Aflatoxins are produced primarily by the common fungus *Aspergillus flavus* and the closely related species *Aspergillus parasiticus*. There are two broad categories of aflatoxins according to their structures. Aflatoxins B1, 2 (AFB1, AFB2) and aflatoxins M1, 2 (AFM1, AFM2) in milk and milk products are within the difurocoumarocyclopentenone series whereas Aflatoxins G1, 2 (AFG1, AFG2) are of the difurocoumarolactone series (Fig. 3.2a). Aflatoxins have been detected in a variety of other foods and feeds including corn (maize), peanuts, cottonseed, almonds, figs, milk, milk products (non-fat dry milk, cheese, and yogurt), eggs, and meat products (Dennings 1987). AFB1 is a potent liver carcinogen in humans and is acutely toxic at high levels of exposure (JECFA 2017; Wild et al. 2015). Mammals that consume dietary aflatoxin AFB1 convert it to AFM1, which is excreted in animal and human milk. AFM1 is as cytotoxic as AFB1 (Zhang et al. 2015) but has less than 10% of AFB1 carcinogenicity and mutagenicity (JECFA 2001). The reactive metabolite, AFB1 epoxide is thought to be responsible for both the acute and chronic toxicity of AFB (Hsieh and Wong 1982). It is highly electrophilic and reacts with the DNA guanine moiety to form covalent bonds at the N-7

guanine residue leading to depurination and carcinogenesis (Smela et al. 2001). Aflatoxins have been classified as group I carcinogens by International Agency for Research on Cancer (IARC) (IARC 2002). Aflatoxins can be detected using thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC-MS), or liquid chromatography–electrospray ionization tandem-mass spectrometry (LC-ESI-MS-MS) (Pohland and Trucksess 2001; Gilbert 1993) and enzyme-linked immunoassay (ELISA) test kits. The limits for human consumption is allowed to contain 20 ppb of AF. Milk has a lower limit of 0.5 ppb AF. High levels of AF (up to 300 ppb) are allowed in feed for cattle, hogs, and poultry (Table 3.2) (Pitt and Miller 2017).

3.5.2 Ochratoxins

Ocratoxin A (OTA) was initially isolated from *Aspergillus ochraceous* and later it was found in other *Aspergillus* and *Penicillium* species such as *P. verucosum* associated with Balkan endemic nephropathy (Pohland 1993). In general, *A. ochraceus* can produce OTA in hot-tropical regions whereas *P. verrucosum* more likely grow and produce OTA under cool-temperate conditions (Scudamore 2005). OTA is a pentaketide-derived dihydroisocoumarin moiety and B, and C analogues are the alkyl esters of ochratoxins (Fig. 3.2b). QTA has been found as a contaminant in a wide variety of foods such as corn, wheat, barley, flour, coffee, rice, oats, rye, beans, peas, and mixed feeds, and also found in wine, grape juice, and dried vine fruits. OTA can also be found in animal-derived products, such as meat and milk, and in human milk (Stoev 2013). OTA is very stable in acidic conditions and also can survive under high thermal processing; thus, it can be found in processed food products, beer, and roasted coffee (Alshannaq and Yu 2017). OTA was easily absorbed through the gastrointestinal tract and bind to plasma protein with affinity based on animal studies. It can also accumulate in the organs due to its lipophilic nature therefore, it was found in decreasing order of concentrations in kidney, liver, fat, and muscle tissues (Fung and Clark 2004). The toxicity of OTA involves inhibition of protein synthesis as well as DNA and RNA synthesis (Creppy et al. 1984). OTA also disrupts hepatic microsomal calcium homeostasis by impairing the endoplasmic reticulum membrane via lipid peroxidation (Omar and Rahimtula 1991). It has been caused immunotoxicity, genotoxicity, neurotoxicity, teratogenicity, and embryotoxicity in both human and animals. OTA is classified by IARC in Group 2B (possible human carcinogen). FDA has not set any limits for OTA in foods and feeds (Table 3.2) (IARC 2012). On the other hand, EU has established limits of OTA in the ranges of 5–50 parts per billion (ppb) in several foodstuffs (Regulations (EC) No. 1881/2006).

3.5.3 *Patulin*

Patulin is produced by several species of *Aspergillus*, *Byssochlamys,* and *Penicillium* and commonly found as a contaminant in fruit juices, particularly apple, pears, and grapes juice (Puel et al. 2010). Toxicity of patulin includes congestion and edema of pulmonary, hepatic, and intestinal blood vessels and tissues (JECFA 1995). The injection of high doses of patulin into the animals resulted in sarcomas. There have been concerns over the possibility of carcinogenicity to children and adults who drink large amounts of fruit juice, especially apple juice, for many years (JECFA 1995). The recent JECFA evaluation confirmed patulin's association with gastrointestinal problems. These problems are likely attributable to the toxin's ability to bind sulfhydryl groups, thereby inhibiting the activity of many enzymes. The FDA limits patulin to 50 ppb in food for human consumption (Table 3.2) whereas EU committee has set a maximum level of 50 ppb for fruit juices and concentrated fruit juices, 25 ppb for solid apple products, and 10 ppb for juices and foods consumed by babies and infants. WHO Codex Alimentarius Commission recommends a limit of 50 μg/kg of patulin given in Table 3.2.

3.5.4 Fusarium *Toxins*

Fusarium is a large genus of filamentous fungi which are capable of producing a wide range of secondary metabolites, mycotoxins including trichothecenes (T-2 toxin, deoxynivalenol (DON/vomitoxin), nivalenol (NIV), zearalenone (ZEN), fumonisin (B1, B2, B3, B4), diacetoxyscirpenol (DAS), moniliformin, enniatin, fusaric acid, fusarin C, fusaproliferin, aurofusarin, fuscofusarin, and their respectable derivatives (Chandra et al. 2010; Ramana et al. 2011, 2012, 2014; Desjardins 2003). The most important mycotoxins, in terms of natural occurrence and toxicity, have been grouped into toxins derived from *F. sporotrichioides* (T-2 toxin and related trichothecenes); toxins derived from *F. graminearum*, *F. culmorum*, and *F. crookwellense* (Deoxynivalenol, Nivalenol, Fusarenone X, and Zearalenone); and toxins derived from *F. moniliforme* (fumonisins and fusarin C). Their infection of important crops such as wheat, barley, oats, rice, and maize pose a serious problem as infection leads to yield loss through lowered growth rate, reduction of grain size, and weakening of the straw (foot rot) (Ramana et al. 2014; Samson et al. 2000). *Fusarium* spp. are also a causative agent of storage rot of sugar beet, potatoes, and apples (Goswami and Kistler 2004). Further investigations on mechanism of patogenicity, prevention, detoxification processes of *Fusarium* toxins are critical (Cetin and Bullerman 2005b).

3.5.4.1 Fumonisins

Fumonisins are a group of mycotoxins mainly produced by species within the *Gibberella fujikuroi* complex of species, *F. verticillioides, F. proliferatum, F. subglutinans, and F. nygamai*. Fumonisins commonly contaminates maize kernels and can also affect sorghum, wheat, barley, soybean, asparagus spears, figs, black tea, and medicinal plants (Yazar and Omurtag 2008). The toxicological significance and accurance of Fumonisins B1 (FB1) (Fig. 3.2c) and B2 (FB2) are very high while the others (B3, B4, A1, and A2) occur in very low concentrations (Ramana et al. 2012, 2014). A single outbreak of acute food-borne disease in India possibly caused by FB1 has been reported (Bhat et al. 1997). FB1 induces hepatocellular carcinoma, cholangiofibrosis, and cholangiocarcinoma in rats and it has been shown to be a strong tumor promoter, but only a weak initiator. Fumonisins exert low levels of acute toxicity and exposure via ingestion can cause equine leukoencephalomalacia and porcine pulmonary edema (Marasas et al. 1988; Voss et al. 2007). Fumonisins are found to be responsible for human esophageal cancer observed in South Africa, China, and Northeast Italy (Peraica et al. 1999). The chemical structure of FB1 is very similar to the primary component of sphingolipids such as lipid sphinganine and sphingosine. FB1 competitively inhibits sphinganine and sphingosine, which are key enzymes in de novo ceramide synthase in the sphingolipid biosynthetic pathway (Fig. 3.2c). This inhibition causes growth retardation and developmental abnormalities to the embryos of hamsters, rats, mice, and chickens (Lumsangkul et al. 2019). FB1 has also been associated with neural tube defects in experimental animals and may therefore be involved in cases of spina bifida in humans (Hendricks 1999). The toxin has been categorized as a 2B carcinogen (possibly carcinogenic to humans) status by the IARC (IARC 1993).

3.5.4.2 Zearalenone

Zearalenone (ZEN) is produced mainly by *F. graminearum* and related species, principally in wheat, maize, sorghum, barley, rye grown in cooler and moist regions worldwide and also compounded feeds. Zearalenone is a nonsteroidal, oestrogenic mycotoxin that induces hyperoestrogenic responses in mammals and can result in severe reproductive and infertility problems, when they are fed to domestic animals in sufficient amounts (Zinedine et al. 2007; Tessari et al. 2006). The chemical structure of ZEN (Fig. 3.2d) is similar to the naturally-occurring estrogens. Therefore, ZEN induces estrogenic effects in humans and animals (Bennett and Klich 2003). High concentrations of zearalenone have been associated with infertility and development of atypical secondary sexual characteristics in heifers (Choi et al. 2012). Zearalenone has also been shown to be immunotoxic, mutagenic, hemotoxic, and hepatotoxic, but the mechanisms of toxicity are not fully understood (Zinedine et al. 2007). Zearalenone and its analogues are capable of inhibiting mitogen-stimulated lymphocyte proliferation and can induce thymic atrophy and macrophage

activation (Choi et al. 2012; Tessari et al. 2006). The biotransformation of ZEN in animals involves the formation of two metabolites α-zearalenole and β-zearanol, which are subsequently conjugated to glucuronic acid. In addition, Venkataramana et al. (2014) also reported the neurotoxic potential of zearalenone in in vitro cell line models. ZEN is classified as a Group 3 carcinogen by IARC. However, the European committee has regulated the maximum levels of ZEN ranging between 20–100 ppb in various food commodities [(EC) No. 1126/2007]. There are no advisory levels of ZEN set by FDA in USA (Table 3.2).

3.5.4.3 Trichothecenes

Trichothecenes are sesquiterpenoid mycotoxins which share the 12, 13-epoxytrichothecene skeleton as the common structural feature. The presence or absence of an 8-keto moiety leads to differentiation of group B and group A trichothecenes, respectively. Various trichothecene compounds such as type B-trichothecenes deoxynivalenol (DON) and nivalenol (NIV), type-A-trichothecenes T-2 toxin, HT-2 toxin, neosolaniol (NEOS), and diacetoxyscirpenol (DAS) produced by *Fusarium* spp. are the most common and/or toxic compounds isolated from natural sources.

T-2 toxin causes immunosuppressive effects especially in livestock (Li et al. 2011; Sokolovic et al. 2008). Clinical signs of T-2 toxicosis in pigs include emesis, posterior paresis, lethargy, and frequent defecation (Meissonnier et al. 2008). T-2 toxin at natural levels of contamination in the diet causes reduced feed intake and animal performance (Pandey et al. 2006). It produces diarrhea, emesis, and feed refusal when ingested at high concentrations in the diet. In poultry, T-2 toxicosis causes oral lesions, reduced feed consumption, and growth rate in young animals, and reduced egg production in laying hens (Pandey et al. 2006; Sklan et al. 2003). At dietary concentrations above 2 mg/kg, the toxins have an impact on poultry production. T-2 toxicosis in ruminants results in a wide range of responses, such as feed refusal, leucopenia, depression, diarrhea, coagulopathy, enteritis, and posterior ataxia (Pandey et al. 2006; Sklan et al. 2003). Exposure to low concentrations of T-2 toxin in the diet frequently reduce humoral immunity of pigs, poultry, and ruminants (Meissonnier et al. 2008; Pandey et al. 2006).

Deoxynivalenol (DON, vomitoxin) (Fig. 3.2e) mainly produced by *Fusarium graminearum* and *Fusarium culmorumis* is one of the most acutely toxic among trichothecenes. DON has a high incidence rate in cereals, including maize, wheat, barley, and oats (Ramana et al. 2011, 2014). At low dietary concentrations, it induces food consumption and weight gain, while higher doses induce feed refusal, diarrhea, and vomiting in animals (Bonnet et al. 2012; Sobrova et al. 2010). Deoxynivalenol alters brain neurochemicals and the serotonergic system which appears to play a role in mediation of the feeding behavior and emetic response (Bonnet et al. 2012). Deoxynivalenol can be immunosuppressive inhibiting protein synthesis or immunostimulatory interfering normal regulatory mechanisms, depending upon the dose and the duration of exposure (Pestka 2010; Solcan et al. 2012). There

have been developing alternative methods to analyze deleterious effects on immune system for hazard assessment (Roggen et al. 2008). Although, the various detoxification methods for deoxynivalenol containing cereals have been investigated, they had limited success due to its heat stability (Cetin and Bullerman 2006). The FAO/WHO Joint Expert Committee recommends a provisional maximum tolerable daily intake of 1 mg/kg body weight of DON and 60 mg/kg of body weight of T-2 toxin (Table 3.2).

3.6 Concluding Remarks and Future Trends

Food poisoning results from the consumption of contaminated food or water containing various bacteria, viruses, or toxins of biochemical or chemical nature. *Salmonella, Campylobacter,* and Enterohaemorrhagic *Escherichia coli* are among the most common foodborne pathogens that affect millions of people annually sometimes with severe and fatal outcomes. *Listeria* infection leads to unplanned abortions in pregnant women or death of newborn babies. Although disease occurrence is relatively low, Listeria's severe and sometimes fatal health consequences, particularly among infants, children and the elderly. *Vibrio cholerae* infects people through contaminated water or food. Symptoms include abdominal pain, vomiting, and profuse watery diarrhoea, which may lead to severe dehydration and possibly death. Rice, vegetables, millet gruel, and various types of seafood have been implicated in cholera outbreaks. (WHO 2015).

Bacterial pathogens, viruses, phycotoxins, mycotoxins, and other microbiological issues adversely affect human and animal health and safety, food and feed quality, food industry economics, and international trade. A multidisciplinary research approach involving microbiology, epidemiology, molecular biology, genetics, toxicology, and other related disciplines needs to be implemented to reduce, avoid, and prevent comtamination and exposures to microbial toxins. In general, identification of microorganisms and toxins, elucidating their genetics and regulation of toxin biosynthesis pathways, mechanisms of action, methods of detection and quantification of toxins, investigation of disease outbreaks, monitoring and surveillance, and mitigation strategies are all essential (Voss et al. 2018, Scallan et al. 2011).

Pathogenic bacteria have developed sophisticated virulence factors, which allow them to invade, replicate, and colonize within an immune competent host in the process of evolution for survival. The specificity and uniqueness of bacterial pathogenesis such as morphological adaptations (e.g., elongation; formation of spore, biofilm, or filament), growth characteristics, motility, toxicity, ability to replicate, and ability to avoid degradation in eukaryotic cells suggests the existence of evolutionary learning processes for survival (Kumar et al. 2019). The existence of diversity in bacterial toxins poses a major challenge in defining the infection of host cells by the toxins. Other challenges include specific recognition of host cells, their toxin receptors, and determination of the mechanism of action (Kumar et al. 2019). Understanding the evolutionary lineage of bacterial toxins allows us to rethink the

current paradigm of evolutionary traits and provides a better understanding of predator-prey relationship, host pathogenesis, mimicry, transfer of molecular properties, and the fundamental biochemical understanding (Kumar et al. 2019). Bacterial toxins, highly potent molecules, are capable of performing some of the most remarkable tasks, such as formation of amazing nanomachines, specific targeting, learning and utilizing cellular processes, or modifications of cellular components, better understanding of biochemical processes, or designing future medical treatments (Kumar et al. 2019).

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Chapter 4
Conventional and Novel Rapid Methods for Detection and Enumeration of Microorganisms

Purnendu C. Vasavada, Alvin Lee, and Roy Betts

4.1 Introduction

Microbiological analysis of foods is an important and essential part of food safety and quality management. Routine food microbiology analysis involves detection and enumeration of microorganisms present in food and food plant environment. Although not routine, identification and characterization of microorganisms related to food spoilage, food preservation, food fermentation, food safety, and foodborne pathogens isolated from ingredients and raw materials, food products and processing environments are becoming increasingly important, especially in the context of food safety assurance and regulatory compliance. Conventional methods for the detection, enumeration, isolation, identification, and characterization of pathogenic microorganisms in foods mostly rely on metabolism, growth, and colony forming ability of microorganisms. These, so-called culture-based methods are based on general principles of enrichment and isolation of microorganisms, and are useful in the detection of a very low initial level of microorganisms occurring in foods. Also, they are useful for the recovery of injured or stressed and viable but non culturable (VBNC) microorganisms. Conventional methods are usually simple, inexpensive but they can be slow, tedious, material- and labor intensive, and can require specialized skills. Also, they are retrospective and may lack sensitivity and specificity. However, the need for timely, specific, and sensitive methods for

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detection, enumeration, isolation, and monitoring the numbers, kinds of microorganisms and metabolites related to food spoilage, food preservation, and food safety, has emerged in recent years. Hence, a variety of rapid and automated methods have been developed for microbiological analysis of food. Advances in the areas of immunology, molecular biology, and instrumentation have resulted in the advancement and availability of diagnostic technologies that can be exploited for rapid detection, enumeration, and characterization of foodborne microorganisms. The developments in these rapid methods and automation in microbiology have been reviewed and discussed in detail in a large number of books, book chapters, and refereed publications (Fung 2002; Vasavada 2001; Vasavada 1993a; Foong-Cunningham et al. 2006; Vasavada et al. 1993; Dwivedi and Jaykus 2011; Dwivedi et al. 2015; Harbottle 2018; Eden 2014a; Sperber et al. 2015; Holbrook 2000; Brehm-Stecher and Tortorello 2015; Tortorello 2014; Petran et al. 2015; Wehr and Frank 2004). Many technologies provide similar levels of sensitivity and specificity to culture-based methods, but with faster sample turnaround times and a substantial reduction in manual labor and are increasingly used in meeting the food quality and safety requirements in the industry today. Some of these methods are discussed below.

4.1.1 Conventional Methods

4.1.1.1 Detection and Characterization

Food and food plant environmental samples generally contain a mixed culture containing microorganisms of different kinds. Detection of microorganism by conventional culture methods involves isolation of the microorganism(s) as different species of micoorganisms may be present. Several simple, inexpensive, and well-established conventional cultural methods are available for isolation, detection, and enumeration of microorganisms in foods. These methods rely on the growth of microorganisms in one or more growth media and can be applied to both quantitative and qualitative analysis. In a qualitative method, a small amount of food or environmental sample is directly streaked onto agar plates to obtain separated colonies for isolation of target microorganisms (Holbrook 2000; Petran et al. 2015; Wehr and Frank 2004). Detection of microorganisms may also be done by microscopic observation of a stained smear preparation. Direct detection of microorganisms using a light microscope is very difficult due to their small size and almost transparent appearance in the suspended medium unless they are stained with certain dyes that aid their detection by providing color contrast. Some staining techniques, such as the Gram and Ziehl-Neelsen stains also have diagnostic value because of their differential staining properties for specific bacteria. Specialized staining technique such as spore staining, flagella staining, and negative staining also help observation of cellular structures such as spores, flagella, and capsules and help direct detection of microorganisms. Also, specialized techniques such as hanging drop technique

may be used for microscopic observation of motility of living bacteria (Brehm-Stecher and Tortorello 2015; Tortorello 2014).

4.1.1.2 Enumeration

Quantitative analysis of food sample involves enumeration of microorganisms present in a sample using agar plate count procedures to estimate the number of viable microorganisms present in the sample using either the spread plate or pour plate method. The pour plate method (Holbrook 2000; Petran et al. 2015; Wehr and Frank 2004; The FDA's Bacteriological Analytical Manual On line (BAM) 2018) is designed to enumerate aerobic and facultative anaerobic bacteria in food that are capable of growth under the conditions employed (growth medium, time, and temperature of incubation). The spread plate method (Petran et al. 2015) involves spreading a small aliquots or dilutions of the sample (0.1–0.2 ml) uniformly on the surface of solid agar in a Petri dish using a sterile bent-glass rod or "a hockey stick". In order to improve detection and precision or where low counts are expected, larger sample aliquot, up to 0.3 ml can be surface plated or larger Petri dishes may be used to spread up to 1.0 ml of food aliquot or dilution.

The spiral plate method (Spiral Systems Instruments, Inc., Bethesda, MD) is another method used for enumerating microorganisms in which a known volume of sample is dispensed onto a rotating agar plate in an Archimedes spiral. The volume of sample decreases as the spiral moves out toward the edge of the plate, thus "diluting" the sample. A modified counting grid, which relates the area of the plate to sample volume, is used to count the colonies in an appropriate area of the plate.

The agar plate count is the simplest and most commonly used conventional method for enumeration of microorganisms in food (Holbrook 2000; Petran et al. 2015). Also, minor variations in procedures can alter the results obtained with the colony count and other enumeration methods. Thus, sterility of media, materials, and equipment, aseptic techniques, and competency of the technicians are very important (Petran et al. 2015; Wehr and Frank 2004; The FDA's Bacteriological Analytical Manual On line (BAM) 2018). However, it should be noted that there is no single method and universal set of conditions that will allow enumeration of all bacteria that may be found in food.

In addition to the agar plate count methods, enumeration of the number of viable microorganisms present in the samples may be estimated using, the most probable number (MPN) procedures (Brehm-Stecher and Tortorello 2015; Tortorello 2014; Betts et al. 1989), and the membrane filtration plate count method (Eden 2014b). Rapid, quantitative analysis for enumeration of microorganisms in food, ingredients, water, and food plant environment can also be done using microscope (Brehm-Stecher and Tortorello 2015; Tortorello 2014; Betts et al. 1989), especially when applying preparative steps, such as concentration. Counting chambers, dried films on microscope slides, and membrane filters are some of the accessories

needed to quantify microbial populations by microscopy. However, the sensitivity or limit of detection often cannot match that of agar plate counts (Brehm-Stecher and Tortorello 2015).

4.1.1.3 Classification, Identification, and Characterization

The microbiological analysis of food typically involves qualitative assessment as to presence/absence of bacterial contamination and quantitative assessment of contamination or load i.e. enumeration of microorganisms in terms of total number of microorganisms present per unit (g, ml, cm^2) of food or food contact surface. Routine microbiological analysis does not typically involve identification, characterization, and classification of isolates, which may be necessary for foodborne pathogens, food safety recalls, troubleshooting of cause of spoilage, etc. However, it is important to know which microorganisms may be associated with a particular food or food ingredient in its natural state and which of the microorganisms present are not normal for that particular food or food ingredient. Also, species identification and strain typing is often necessary for pathogenic bacteria. Conventional methods for the detection, identification, and characterization of pathogens and spoilage microorganisms in food mostly rely on the sequential steps of culture enrichment, selective and differential plating, identification, confirmation, and strain typing (Sperber et al. 2015; Holbrook 2000; Petran et al. 2015; Wehr and Frank 2004; Ercolini 2014).

4.1.1.4 Enrichment Methods

Enrichment may be necessary because the target microorganism may be present in food at very low initial levels (as low as 1 cell/100 g) and may be needed to grow a detectable population. Also, the target microorganism may be damaged by sub-lethal stresses or injury and may require resuscitation in the appropriate conditions so that cellular damage can be repaired, and metabolic pathways activated to allow the growth. Finally, enrichment may allow the proliferation of the target microorganism to detectable levels while repressing the growth of competing non-target microorganisms (Sperber et al. 2015). The enrichment methods are qualitative and are designed to indicate the presence or absence of the target microorganism, e.g. Salmonella, and not its numbers. They may be used in conjunction with direct plating and/or the most probable number (MPN) technique (Petran et al. 2015; Chandrapati and Williams 2014; Ercolini 2014; Dwivedi et al. 2014). Some popular pre-enrichment media used in food microbiology include Buffered peptone water (BPW), Tryptic Soy Broth (TSB), and Universal pre-enrichment broth (UPB) (Bailey and Cox 1992). Several sample concentration techniques such as centrifugation, filtration, and immuno-concentration have been applied prior to the primary enrichment in an effort to reduce the time required for pre-enrichment (Dwivedi et al. 2014).

Selective enrichment (Sperber et al. 2015; Dwivedi et al. 2014) is designed to selectively enhance the growth of target microorganism while simultaneously minimize the background microflora using specialized selective broth medium. The efficacy and length of secondary enrichment depends on several factors including the selectivity of the medium which depends on certain selective agents or conditions: temperature, antimicrobials, salts, acids, and metals; number of target cells expected in the primary enrichment during the transfer to secondary enrichment, transfer of nutrients from pre-enrichment media, and the growth rate of target bacteria in the selective broth. The incubation temperature for selective enrichment is another important factor influencing the efficacy of selective enrichment. Although several target- and food-specific enrichment strategies have been reported, it is not possible to recommend a single universal enrichment approach applicable to amplify the most common pathogens from diverse food matrices (Dwivedi et al. 2015; Dwivedi et al. 2014).

4.1.1.5 Selective and Differential Plating

Conventional methods for bacterial identification and characterization of pathogenic and spoilage microorganisms in food typically involve isolation of the target microorganism by plating an aliquot of enrichment medium on selective and differential agar media. The streak plate or spread plate method is used to obtain discrete, well-isolated colonies, which are then further "purified" by a streaking on a non-selective medium and used for subsequent morphological, physiological, and biochemical tests for further identification, characterizations, and confirmation of the target microorganism. Detailed discussion on selective and differential media recommended for isolation of foodborne pathogens and spoilage microorganisms can be found in literature (The FDA's Bacteriological Analytical Manual On line (BAM) 2018; Salfinger and Tortorello 2015; Food Safety and Inspection Service, USDA 2014; Atlas 2010; Corry et al. 2011; Difco and BBL Manual 2009). Chromogenic agar media are culture media used to isolate, identify, and differentiate a specific microorganism from a heterogeneous population (Perry and Freydiere 2007; Manafi 2000; Van Dijk et al. 2009). Several chromogenic media for the detection, isolation, and enumeration of foodborne pathogens such as Salmonella, Shiga toxin-producing, and non- Shiga toxin-producing *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* have been developed, evaluated, and many are made available commercially (Kase et al. 2015; Park et al. 2014; Hegde et al. 2007; Klachayanand et al. 2013; Galat et al. 2016).

4.1.1.6 Identification, Characterization, and Strain Typing

The phenotypic methods of identification based on morphological, physiological, and biochemical characteristics are well-known and easily performed. However, this approach is material and labor intensive, tedious, time consuming, and subjective.

Also, the identification tests are based on phenotypic characters that are influenced by physiological and environmental conditions in which the microorganism is cultured and may not always be stable. Several, miniaturized, biochemical kit/strip-based systems and semiautomatic or fully automatic commercial systems allowing a high throughput biochemical identification have been available for identification and characterization of isolated bacteria from food and are routinely used in industry and academia (Cox et al. 1987; Russell et al. 1997; Cox et al. 1984; Feng 1996; Mahon et al. 2015; Russell and Vasavada 2007). They yield identification accuracy exceeding 95%, if used with a standardized inoculum. However, they may be expensive and their accuracy may be subject to the origin - clinical vs industrial (food) - of the microorganisms in their databases.

Automated systems for microbial identification system based on substrate utilization, gas chromatographic (head-space analysis) of fatty acid methyl esters, and analysis of cellular fatty acids have been used for rapid microbiological identification for many years (Olson 1996). The Vitek Automicrobic System (AMS), and Vitek 2 (bioMérieux, Inc., Hazelwood, MO, USA) (Clontz 1996; Bailey et al. 1985; Graf et al. 2000; Crowley et al. 2012; Yibar et al. 2012) are well-established rapid and automated microbial identification systems used in clinical and industrial microbiology. The Biolog system (Hayward, CA., USA) is a miniaturized system using standardized 96-well microtiter plates in which comprehensive substrate utilization testing is performed (Fung 2002; Bochner 1996). The MIDI Sherlock Microbial Identification System (MIS) (Microbial Identification System, Newark, DE, USA) is a commercially available, fully automated gas chromatographic system for bacterial identification based on their unique fatty acid profiles (Olson 1996). The MIDI Sherlock MIS and FAME profiles of Campylobacter isolates were used to demonstrate the presence of *Campylobacter spp.* on poultry carcass and scald tank water sample (Hinton et al. 2004). Correct classification of *Helicobacter spp.* was made based on analysis of dendrogram of Campylobacter strains (Hinton et al. 2004; Lambert et al. 1987) In a recent study, 22 strains of *L. monocytogenes* detected by the MIS were statistically clustered into three major subgroups, which were highly relative to their food sources. Strains in the same cluster were isolated from the same food source, while strains in different clusters were isolated from different food sources (Guo et al. 2010).

Strain typing is used for differentiation of strains within the same species of pathogenic microorganisms, which is very important in clinical microbiology, epidemiological studies, studying antibiotic resistance, understanding the pathogenesis of infection, and in hospital infection control (Perry and Freydiere 2007; Lambert et al. 1987; Bopp et al. 2003; Webb and Brown 2013). Strain typing is also important in food microbiology, especially in epidemiological investigations of bacterial pathogens and source tracking of foodborne illness outbreak and can also be useful in characterization of starter culture and specific isolates used for industrial fermentation. Traditionally, typing was performed principally through phenotypic typing methods such as biotyping, phage typing, serological typing, bacteriocin typing, protein typing, and multi-locus enzyme electrophoresis (MLEE). Although immunological or molecular methods involving direct DNA-based analysis of

chromosomal or extrachromosomal genetic elements are being more popular typing methods, some of the phenotypic methods for further differentiation of bacterial species into sub species, variants, and strains are still being employed today. Fourier-transform infrared spectroscopy (FTIR) can also be used to analyze FTIR spectra of bacterial cells for the detection, differentiation, and taxonomic classification of bacteria from both cultures and food products (Davis and Mauer 2010). Phenotypic methods for typing are increasingly replaced by molecular typing methods involving the study of the microbial DNA, homology, presence or absence of specific genes, DNA sequencing, or DNA fingerprinting. These methods are discussed in Sect. 4.1.4 in this chapter.

The conventional microbiological methods have been used and trusted for years but they are time consuming, laborious, and often subjective. A variety of methods have been developed for rapid detection, identification, and characterization of foodborne pathogens, spoilage microorganisms, and contaminants in food plant environment. The emergence and acceptance of rapid methods including molecular methods, biosensors, and whole genome sequencing (WGS) have been increasing exponentially in the past decade (Brehm-Stecher and Tortorello 2015; Tortorello 2014; Petran et al. 2015; Li et al. 2009; Mandal et al. 2011).

4.1.2 Microscopic Methods

Qualitative analysis for detection, identification, and characterization as well as quantitative analysis for enumeration of microorganisms in food, ingredients, water, and food plant environment can be done using microscopes (Brehm-Stecher and Tortorello 2015; Tortorello 2014; Betts et al. 1989). Also, microscopic techniques are useful in differentiating live from dead microorganisms, and in cell viability studies (Petran et al. 2015; Betts et al. 1989). Several microscopic techniques using bright field, dark field, or phase contrast microscopy have been used for routine microbiological examination of foods. Others such as confocal laser scanning microscopy, electron microscopy, atomic force microscopy, flow cytometry, etc., involve the use of sophisticated microscopes, imaging tools, and combination of filtration, flow-through optical analysis and are used for clinical and biomedical research due to their applicability, cost, and technical expertise required (Brehm-Stecher and Tortorello 2015; Tortorello 2014). Microscopic techniques are especially useful in studying viable but non culturable (VBNC) and difficult to culture microorganisms (Tortorello 2014).

4.1.2.1 Bright Field, Dark Field, and Phase Contrast Microscopy

Bright field microscopy is the most common technique used in food microbiology. Routine microscopic analysis using simple stain such as methylene blue and differential staining techniques such as Gram staining or acid-fast staining is commonly

used for quick determinations of basic morphology (e.g., rods, cocci), visualization of cell structures (e.g., capsule, spores, flagella), and differentiation of fundamental groupings (e.g., Gram positive or Gram negative, or acid fast).

Dark field microscopy is a technique used to observe unstained samples causing them to appear brightly lit against a dark, almost purely black, background (Wittenbrink et al. 1994a; Ahamad et al. 2005; Xu et al. 2018; Wilson and Vigil 2013). While not suitable for routine food microbiology analysis, the dark field microscopy is often used in detection of spirochetes involved in periodontal diseases, Lyme borrelliosis (Wittenbrink et al. 1994a), and Leptopspirosis (Ahamad et al. 2005). Xu et al. (2018) described an ultrasensitive and on-site method for counting *E. coli* using magnetic nanoparticle (MNP) probe using a dark field microscope. They detected 6 CFU/μL of *E. coli* in 30 min (Xu et al. 2018). The phase contrast microscopy is useful in observing microorganisms without staining and in their natural viable state. The phase contrast microscope uses specialized optics to increase contrast of specimens for observation of microscopic details. In a typical application, a "wet mount" is prepared by placing a loopful of culture in liquid suspension under a coverslip on a glass microscope slide, and the cells are then observed using an objective lens providing an appropriate magnification (Brehm-Stecher and Tortorello 2015). Applications of phase contrast microscopy include observation of characteristic motility of Campylobacter isolates, bacterial structures such as endospores, and intracellular inclusions such as poly-β-hydroxybutyrate.

4.1.2.2 Fluorescence Microscopy

Fluorescence microscopy involves the use of a fluorescence microscope and a variety of fluorescent indicators, fluorochromes, or fluorophores for analyses of microorganisms (Harbottle and Pendrak 2013; Hohman 2007; Webb and Brown 2013). Epifluorescence microscopy is a method of fluorescence microscopy in which the arrangement of optical components of the microscope is designed to permit illumination from above the specimen. Epifluorescence microscopy applications in food microbiology include the direct viable count (DVC) which involves incubation of cells at an optimal temperature in the presence of yeast extract and nalidixic acid (Brehm-Stecher and Tortorello 2015; Tortorello 2014; Webb and Brown 2013). The sample is filtered and stained with a fluorescent dye, such as acridine orange or fluorescein isothiocyanate, and the membrane is analyzed by epifluorescence microscopy. Nalidixic acid inhibits DNA synthesis leading to viable cells elongation without division (Brehm-Stecher and Tortorello 2015). Another method for viability detection involves the use of specific dyes such as fluorescein diacetate (FDA) uptake through intact membranes. The dye is hydrolyzed to fluorescein by nonspecific intracellular esterases after uptake resulting into free fluorescein accumulation that results in measurable fluorescence (Tortorello 2014). Nonviable cells do not retain fluorescein due to damaged membrane, do not fluoresce and are considered nonviable. This method is useful for evaluating both membrane integrity

and intracellular enzyme activity. Carboxyfluorescein diacetate (cFDA), a derivative of FDA, is retained better in Gram-negative cells in which FDA may be cleaved by periplasmic enzymes. The use of inhibitors other than nalidixic acid has extended the technique to a variety of bacteria, including Gram-positive cells (Tortorello 2014; Webb and Brown 2013).

A dual staining procedure using SYTO®9 and propidium iodide stains allows detection of viable cells as fluoresce green cells, damaged cells fluoresce red. This method is commercially available as LIVE/DEAD® BacLight™ Bacterial Viability kits. The Live-dead staining kits has been combined with specific probes and have been widely used (Tortorello 2014; Berney et al. 2007; Boulos et al. 1999).

Direct Epiflourescent Filter technique (DEFT) combines membrane filtration with epifluorescence microbiology in which liquid samples of homogenates are filtered through membrane filter to concentrate the cells. The membrane filter is stained using fluorescent stain and observed using epifluorescent microscope (Brehm-Stecher and Tortorello 2015). Rapid methods for direct enumeration of microorganisms in foods by the DEFT have been reported (Frank et al. 1992; Hermida et al. 2000; Zwirglmaier 2005). The fluorescence *in situ* hybridization (FISH) technique involves the use of fluorescent oligonucleotides complementary to rRNA sequences for microbial cell detection (Brehm-Stecher and Tortorello 2015; Zwirglmaier 2005). Fluorescent antibodies and peptide nucleic acids (PNAs) probes have been applied for specific enumeration of microbial cells (Eden 2014b). The microcolony epifluorescence microscopy (MEM) technique involves collection of cells on a membrane filter, followed by a short incubation (3–6 h) to allow growth, fluorescence staining of cell membranes with a fluorescent dye, and enumerating micro colonies using epifluorescent microscope. Fluorescent antibodies or oligonucleotide probes have also been used to provide specificity to the staining in the antibody-MEM and fluorescence *in situ* hybridization (FISH)-MEM techniques, respectively (Angelidis et al. 2011; Tortorello et al. 1997; Perry-O'Keefe et al. 2001). Specific enumeration of microbial cells and hybrid membrane-filtration/microcolony approach for PNA-based in situ chemiluminescent detection of *E. coli*, Pseudomonas, *S. aureus*, and Salmonella has been described (Tortorello et al. 1997; Perry-O'Keefe et al. 2001; Almeida et al. 2010; Baumstummler et al. 2010). MEM correlated well with total plate counting on nutrient agar and has been used with selective media for microscopic enumeration of pseudomonads, coliforms, staphylococci, and streptococci in food samples (Perry-O'Keefe et al. 2001; Baumstummler et al. 2010).

4.1.2.3 Flow Cytometry

Flow cytometry (FCM) is a rapid method for flow-through optical analysis of single-cells for bacterial viability and physiology without the requirement for growth on agar plates (Comas-Riu and Rius 2009). Samples of bacteria suspended in a liquid are passed in a stream in front of a laser beam, where particles suspended in the liquid are illuminated one at a time, up to several thousand per second. Scattered light is detected using sensors in line with, and perpendicular to the laser beam,

indicating particle size and "granularity" (a measure of particle optical complexity), respectively. Aspects of bacterial physiology and viability can be analyzed by measuring particle fluorescence of samples stained with fluorescent dyes (Bridier et al. 2015). FSM is rapid, allowing detection of subpopulations present in a sample (e.g. numbers of live, dead, and injured bacteria) in minutes. It also allows analysis of not only culturable but also nonculturable, and thereby also VBNC (viable but nonculturable) bacteria (Comas-Riu and Rius 2009; Bridier et al. 2015; Nebe von Caron et al. 2000). FCM has been used successfully for the analysis of various microbial cell types, including bacterial or fungal spores, vegetative bacterial cells, yeasts, and protozoa. Application of FCM in food microbiology include fluorescent Gramstaining used to characterize the microbial flora of foods such as milk (Holm and Jespersen 2003). FCM can be used for the detection of specific pathogens through using labeling with fluorescently labeled antibodies, rRNA-targeted probes, or nucleic acid aptamers (Shrestha et al. 2011; Dwivedi et al. 2010). Specific fluorescent respiratory or enzyme substrates, intracellular redox indicators, and reporters of membrane integrity may be used to study cellular activity, exposure to applied stresses, or cell viability. Additional examples of FCM applications in food microbiology include monitoring of food fermentations, detection and quantification of food spoilage bacteria, evaluation of starter culture or probiotic activity, detection and enumeration of pathogens in foods, assessing the impact of antimicrobial treatments or common food processing stressors on the physiology and viability of foodborne microbes, analysis of drinking water, and differentiation of antibiotic- sensitive and antibiotic-resistant pathogens (Brehm-Stecher and Tortorello 2015; Holm and Jespersen 2003; Shrestha et al. 2011; Dwivedi et al. 2010; Anvarian et al. 2018).

4.1.2.4 Confocal Microscopy, Electron Microscopy, and Atomic Force Microscopy

The confocal laser scanning microscopy is designed to eliminate blurred image resulting from the widefield illumination of samples thicker than the focal plane in conventional fluorescence microscopy. The Confocal Laser Scanning Microscope (CLSM) allows detailed resolution of thicker specimens or those having complex surface topographies by eliminating blur resulting from out-of-focus or "stray" fluorescence in a conventional "widefield" fluorescence microscopy system. Many studies have involved the CLSM as a principal tool to study the localization and internalization of bacterial and protozoan foodborne pathogens in fruits and vegetables. Other diverse applications in food microbiology have included determinations of physiologically active foodborne pathogens in foods, removal of foodborne pathogens from fresh produce, distribution of bacterial populations in dairy products and other foods, permeabilization and lysis of starter cultures in Gouda cheese, in vitro attachment of foodborne pathogens to meat proteins, spatial and temporal determinations of foodborne pathogens in biofilms, and comparison, growth, and determination of fungal hyphae (Brehm-Stecher and Tortorello 2015). The use of

confocal laser scanning microscopy in food research has been reviewed (Auty et al. 2005; Vodovotz et al. 1996; Takeuchi and Frank 2001).

The electron microscopy (EM) and the atomic force microscopy (AFM) are two advanced methods that provide unique advantages over the light microscopy. e.g. an electron microscope can magnify the objects nearly 100,000 times and provide spatial resolution of 0.2 nm. The AFM is capable of resolving structures at the nanometer level and can provide high quality imaging of discrete topographical features of microbial cells, including crystalline S-layers and the peptidoglycan fibers that constitute the cell wall and provide resolution smaller than 50 pm (Erni et al. 2009). Both transmission electron microscopy (TEM) and scanning electron microscopy (SEM) have been used for high-resolution study of microbial attachment, colonization, and survival *in situ* in biofilms, in food matrices, on stainless steel surfaces, or on produce (Liao et al. 2010; Whitehead et al. 2010). AFM imaging and measurement may be carried out on living cells under aqueous conditions. AFM-based observations have revealed fundamentally new biological observations, such as the discovery of regular nanomechanical oscillations of the cell wall of *Saccharomyces cerevisiae*. These oscillations that can be translated into audible sound, allowing us to hear yeast cells "sing" (Pelling et al. 2004). The EM and AFM are elegant and sophisticated research methodologies with unique applications in clinical and industrial microbiology and require expensive instruments and reagents, analytical skills, and technical expertise and are not generally suitable for routine food microbiological analysis.

4.1.3 Rapid Methods

Bacteria, yeasts, and molds can grow on or in a nutrient rich substrate and since the introduction of agar media in the 1800s, it has allowed for the development of various methods to enumerate microorganisms from foods.

Microbiological agar medium generally involves the use of dehydrated powdered ingredients dissolved in water and the addition of supplements or antibiotics. The medium is then boiled or sterilized in a pressure vessel using steam, tempered approximately to 46 °C and dispensed into Petri dishes. The use of microbiological medium gave rise to colony count methods, such as total aerobic plate count, that are used widely to enumerate the number of viable microorganisms from foods. The addition of additional conditions such as anaerobic or modified atmosphere conditions in an enclosed container further allowed for the enumeration of microorganisms that would grow under specific environmental conditions.

These cultural growth methods are based on the assumption that each microbial cell in the food sample is dispersed homogenously and will form a separate, visible colony on the agar medium under optimum growth conditions. Microbiological plate culture methods remain the gold standard for the isolation, detection, and enumeration of microorganisms from food and used by many regulatory agencies to assess the hygiene of food and food manufacturing environments, there have been

innovations in the area that have led to improved convenience, speed of detection, selectivity, and specificity and more importantly they can be used in an operating environment with little or minimal equipment.

4.1.3.1 Modification of Conventional Methods

One significant development in the convenience and speed is the introduction of prepared shelf-stable media such as Petrifilm™ by 3M Company (Minneapolis, MN, USA) (Fig. 4.1). The medium is prepared in conveniently packaged shelf stable dry film with the incorporation of specific dyes or chromogens (Jordano and Medina 2000). The film is inoculated with the sample which rehydrates the medium, causing it to gel. The films are placed in racks and incubated in either a specific 3M incubator or a general incubator for colony development. These rehydratable films are available for conducting the standard plate count (Chain and Fung 1991), specific counts for coliforms, and *E. coli* (Brodie and de Boer 1992; Curiale et al. 1991; Priego et al. 2000; Blumberg et al. 1991; Taniwaki et al. 2001; Mach et al. 2000).

The performance of Petrifilm systems has been rigorously evaluated against conventional reference methods and received approval by numerous government authorities for inclusion in methods such as the US FDA Bacteriological Analytical Manual. The Petrifilm systems have several advantages such as they do not require media preparation, Petri dishes, or specific media preparation facility; simple and easy to use; and, compact with a small footprint.

Other examples of the dry film technology or modification to the conventional aerobic plate count media are the Compact Dry TC system developed by Nissui

Fig. 4.1 3M Coliform Count Petrifilm. (Reproduced with permission. © 3M 2020. All rights reserved)

Pharmaceutical Company (Tokyo, Japan) and widely available through distributors where the medium is incorporated into a modified Petri dish, and activated on addition of the sample inoculum (Ellis and Meldrum 2002). The Compact Dry TC system is comparable to both Petrifilm system and spiral plate method (Ellis and Meldrum 2002) and ISO convention methods (De Vaugelade et al. 2017).

A companion to the 3M Petrifilm is the 3M Redigel™ that eliminates the need for media preparation and autoclaving. The nutrient medium contains pectin, and is purchased in bottles as a sterile liquid reagent. Food sample, after homogenization and dilution, is added to this bottle, the contents, after thorough mixing, are poured into a specially treated Petri dish containing divalent cations (Ca^{2+}) that causes gelation of the pectin. The plates are then incubated and colonies counted as per conventional methods. Total plate count data obtained with 3M Redigel are comparable to those of conventional plating (Beuchat et al. 1998). Although 3M Redigel is now discontinued, Easygel media from Micrology Laboratories (Goshen, IN, USA) has filled the void and incorporates differentiation dyes in their products for the detection and differentiation of microorganisms from foods.

The development of chromogenic agar media over the years have led to the easy detection, differentiation, and identification of microorganisms and are commonly used with coliforms, *E. coli*, enterotoxigenic strains of *E. coli*, *L. monocytogenes*, *Salmonella*, *Bacillus cereus,* and *Clostridium perfringens*. These media are readily available under various commercial names such as Colilert, CHROM agar, Rainbow agar, and Rambach agar and utilizes specific enzymatic or metabolic activities of a particular microorganism or company specific dyes or reagents for detection and differentiation such as *o*-nitro-phenyl-β-D-galactopyranoside (ONPG) which is hydrolysed by β-galactosidase-producing coliforms to give a yellow *o*-nitrophenyl, fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronide (MUG) or its chromogenic counterpart 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-Gluc or BCIG) for *E. coli* detection.

4.1.3.2 Membrane Filter Techniques

Membrane filters have been used in food microbiology as a way of concentrating a large volume of sample or to process large liquid samples so that the microbiological quality can be assessed. One example of use is in water microbiology where large volumes of samples are commonly analyzed to provide colony counts or direct membrane plating after passing a specific volume of sample through the membrane. Membrane filtration technology offers a number of analytical advantages over conventional cultural methods. Large volumes can be filtered or processed thereby significantly increasing the sample size and improved limits of detection while removing potential inhibitors from a sample that may interfere with the enumeration process. However, depending on the filter pore size, not all inhibitors are removed.

Through the action of passing a liquid sample through a filter, microorganisms are immobilized onto the filter and can be easily transferred to various microbiological media including selective medium. The filters, with microbial cells immobilized on

the filter surface acts like a mould and can be "stamped" from one medium to another allowing for easy comparison or the entire membrane can be placed on a nutrient medium as a pre-incubation step to recover injured cells before culture on a selective medium and examined under a stereomicroscope. One example is the direct membrane plating method used for *E. coli* detection from large quantities of homogenized food or liquids passed through a filter and incubated onto non-selective media. Bacterial colonies on the filter can either be picked for further analyses or sub-cultured onto other microbiological media.

The traditional uses of membrane filters for the enumeration of microorganisms in foods or beverage samples are well documented (Sharpe 1994) with one disadvantage – fouling or clogging of the membrane and generation of back pressure has restricted its use to certain foods and liquid foods. Efficiency of membrane filters is enhanced through the use of larger pore size filters as pre-filtration screens, in combination with various detergents and enzymes (e,g, trypsin and pronase) (Entis et al. 1982).

The use of direct epifluorescent filter technique (DEFT) allows for the direct analysis of the colonies on the filter by staining with a fluorescent dye and examined with a microscope (Pettipher 1989). The fluorescent cells can be counted to provide numbers or concentration of bacteria in a sample, usually within 30 min. The incorporation of specific dyes such as acridine orange can be used for live and dead cells differentiation and automated (BactoScan by Foss, Hil-lerød, Danemark, COBRA by Biocom, Les Ulis Cedex, France) in the analysis of milk.

Hydrophobic grid membrane filters (HGMF) are square (60 × 60 mm) membrane filters of 0.45 μm pore size with a black hydrophobic grid, containing 40 × 40 small squares (1600 grid cells), printed on one side (Sharpe 1994). After inoculation by filtration, microbial growth is randomly confined within each of the grid cells or grid units due to the hydrophobic nature of the grids that prevents lateral spreading growth of the colonies. Interpretation and processing of the data follows the most probable number (MPN) principles where each grid unit behaves like one tube in a MPN count done at a single dilution and due to 1600 grid cells, there is a very high precision.

4.1.3.3 Electrical Methods

When microorganisms grow in media, they break down growth substrates such as protein, sugars, polysaccharides, which generally have little or no charge, to smaller products or metabolites that are charged and the changes in the ionic state of the medium affects the ability to transmit current i.e. conductance and resistance that is recorded by a probe inserted into the medium. The probe, consisting of a pair of electrodes, measures the flow of current between the electrodes resulting in a change of impedance defined as the resistance of current flow and a decrease in impedance results in an increase in conductance and increase in current flowing through the medium. Therefore, microbial growth in the medium translates into an increase in conductance and capacitance or a decrease in impedance.

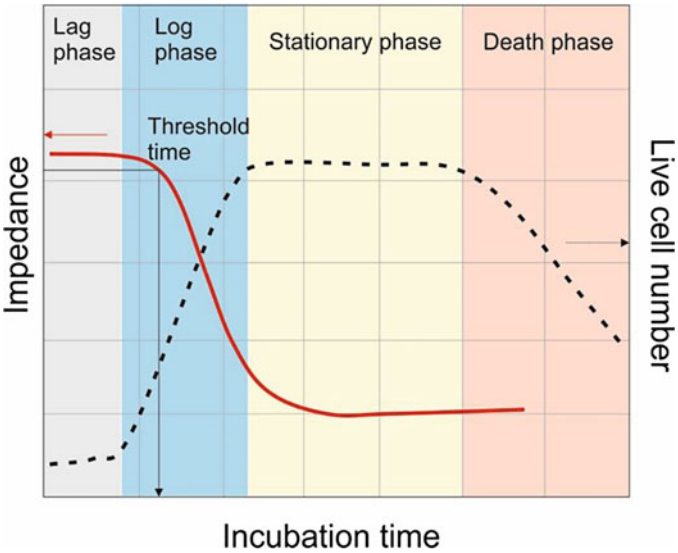

Fig. 4.2 Bacterial impedance growth curve showing changes in impedance and viable cell numbers in relation to the different stages of bacterial growth phases. (Reproduced from Brosel-Oliu et al. (2015))

Microorganisms need to grow to a threshold population in order to cause a detectable change in the conductance or impedance properties of the medium. This threshold population is usually about 6–7 log CFU/ml and the time required to initiate this change is defined as the detection time (DT) and is inversely related to the initial viable microbial population (Fig. 4.2). Samples with high microbial loads e.g. 6-log CFU/ml may only take a few hours for detection while low microbial load samples e.g. 2-log CFU/ml may take 10–15 hours for detection while samples with no viable microorganisms will result in no conductance or impedance change. Other factors that affect DT are lag phase (initial period where cellular metabolism is accelerated, cells increasing in size but not able to replicate) and the generation time of the microbial population (the time it takes for the population to double). Factors affecting the growth rate and metabolic activity of microorganisms that result in changes to generation time will influence the DT. Such factors include: composition of the medium used; incubation temperature; inoculum volume and concentration; presence of interfering substances e.g. salt, sugar, acids, ethanol from foods; and growth rate of microbial species in the food sample. Therefore, it is important to control the operating conditions, prepare reliable calibration curves for each matrix in order to obtain reliable and reproducible data.

The metabolites or breakdown products of growth medium can be measured using direct or indirect measurements. Direct measurements uses immersed impedance probes for detecting bacterial metabolism changes taking place in the bulk of the growth medium. Indirect measurements involve the detection of CO_2 produced by live bacteria reacting with potassium hydroxide producing carbonates leading to

Table 4.1 Characterization of bacterial growth in different medium using impedance

Microorganism	Medium	Detection limit (log cfu/ml)	Detection time (h)	Reference
Enterobacteriaceae	Brain Heart Infusion broth +0.1% yeast extract	4	8–9	Bülte and Reuter (1984)
	Brain Heart Infusion broth	5	2–6	Cady et al. (1978a)
E. coli	Trypticase Soy Broth	5	5–6	Cady et al. (1978b)
	Tris-Glycine buffer +dextrose	7–8	2	Gómez et al. (2002)
Listeria spp.	Tris-Glycine buffer +dextrose	5–7	2	Felice et al. (1999)
	Tris-Glycine buffer +dextrose	7–8	2	Gómez et al. (2002)
Coliforms (*E. coli*)	Specific Medium	4	5	Martins and Selby (1980)
Bacillus lactis	Specific Medium	8	6	Walker et al. (2005)

decreased conductivity (Owens et al. 1989) and utilized for measuring bacterial growth in complex food matrices by Johnson et al. (2014).

Commercially available instruments over the years that offer computer processing of DTs and capability of handling several hundred sample modules at one time include Bactometer (bioMérieux, Hazelwood, MO, USA), BacTrac™ (SY-Lab, Neupurkersdorf, Austria), Malthus systems (Malthus Instruments, Crawley, UK), and Rapid Automated Bacterial Impedance Technique (RABIT) (Don Whitley Scientific, Shipley, UK). Various attributes of the different instruments have been described and compared and they have been validated against other conventional methods such as MPN or microbial colony counts (Blivet 2000a, b; Bolton and Gibson 1994; Dupont et al. 1996; Priego et al. 2011; Wawerla et al. 1999). There are application of these systems for the detection of microorganisms e.g. *E. coli* and *Salmonella* in foods, characterization of bacterial strains in foods such as the performance of Lactobacillus strains in milk (Bancalari et al. 2016) or used to characterize the growth of microorganisms in different growth medium (Table 4.1).

The main advantages of impedance measurement may include automated processing of large numbers of samples with no labor handling provides greater confidence in results; labor costs savings (no dilutions, less media making, less clean-up); rapid results over conventional testing (data on total microbial load are obtained in less than 24 h and presence of specific microorganisms detected in less than 24–48 h); and potential to perform a complete range of microbiological tests on one system. A major disadvantage is the high capital cost of the

equipment and may not be entirely an 'off-the-shelf' technology due to the calibration required for each growth medium or food matrix.

4.1.3.4 Immunological Methods

Immunoassay technology involves the specific interaction of antigens (Ag) with antibodies (Ab) to form a detectable end product, the antigen-antibody complex (Ag-Ab). The specific antibody, manufactured or produced as monoclonal or polyclonal antibodies in a cell culture system or animal system, is designed to specifically interact with the antigen of interest to form the Ag-Ab complex. The detection of this Ag-Ab complex indicates the presence of antigen or a specific target in a matrix. The detection of the Ag-Ab complex could be through visible precipitation or clumping or agglutination such as those used in serotyping assays (Radcliffe and Holbrook 2000).

Over the last few decades, there have been advances in immunoassay technology that enable the use of the technology not only for detection of a specific target but also for isolation and extraction of a specific target from a very complex matrix such as foods. Some of the major advances were seen in detection technologies used for the detection of the Ag-Ab complex and development of specific tags or labels, chemically labelled antibodies that enhances specificity. Labels such as radioactive labels, chemiluminescent and bioluminescent reagents, enzymes, bacteriophages and avidin can be coupled to antibodies. Enzymes can also be used as labels because it is relatively stable, easily handled, and can be detected by simple colorimetric assays with readily available substrates. Horseradish peroxidase and alkaline phosphatase are the most widely used enzyme tags although others such as β-galactosidase, urease, glucose, oxidase, and acetyl-cholinesterase have been used (Radcliffe and Holbrook 2000).

The development of supporting materials to attach or immobilize either the antibodies or antigens had significant advances as well. Materials such as polystyrene, polyvinyl, polyacrylamide, glass, silica, nylon, magnetic beads, and nitrocellulose have been developed and used in various applications to immobilize and support either the antigen or antibody to localize the site of the Ag-Ab complex allowing for various application configurations to be developed such as polystyrene dipstick, plastic multiwell microtitre plates, latex beads, magnetic resin beads, and charged porous membranes. The theory and advances of immunoassay technologies including the development of antibodies and enzymatic reporting technologies for detecting Ag-Ab complexes have been reviewed extensively and available in review articles and books (Barbour and Tice 1997; Deshpande 1994; Hall et al. 1993; Hefle 1995; Wild 1994). The applications of immunoassays are now widely used. Enzyme-linked immunosorbent assays (ELISA), agglutination assays, and immunocapture and immunomagnetic separation formats have been used for the isolation and detection of microorganisms or specific targets from foods.

4.1.3.4.1 Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA uses a solid support to immobilize the antigens or antibodies, commonly in a microtitre plate, and using antibody-enzyme conjugates to detect the formation of the Ag-Ab complex. There are many different ELISA formats including competitive assays that allow quantitative measurements of the antigen, and non-competitive assays that allow simple presence or absence detection of the antigen. The non-competitive assays can be conducted as direct, indirect, and "sandwich" formats with the "sandwich" (Fig. 4.3) being the common format for the detection of microorganisms in foods (Barbour and Tice 1997; Sharma 2000).

ELISAs have been developed and packaged into kits for the detection of a wide range of microorganisms, toxins, and allergens that are significant in foods and commercially available since the mid 1980s. ELISA has several advantages over the conventional, culture based approaches to microbiological testing that include the availability in a convenient, easy-to-use kit form that allows the processing of single or multiple samples to rapidly detect microbial species, toxins, and specific food allergens with high degree of specificity, reliability, and sensitivity. When coupled to a calibration curve or standards such as those used for the detection of *Clostridium botulinum* neurotoxins (Sharma et al. 2006), ELISA can be used to detect low amounts (nanogram or picogram range) neurotoxins in foods and offers a good screening tool prior to confirmatory assays using the more expensive gold standard mouse bioassay.

Although ELISAs can detect microgram and even picogram amounts of microbial toxins e.g. *C. botulinum* neurotoxins (Sharma et al. 2006; Bartlett et al. 1996), they cannot reliably detect less than 10^4–10^5 CFU/ml because the antigens to be detected are located on the cell wall or flagella of the cells in small amounts. Since there may be a need to detect certain microorganisms e.g. *Salmonella*, *Listeria*, *E. coli* at 1 cell

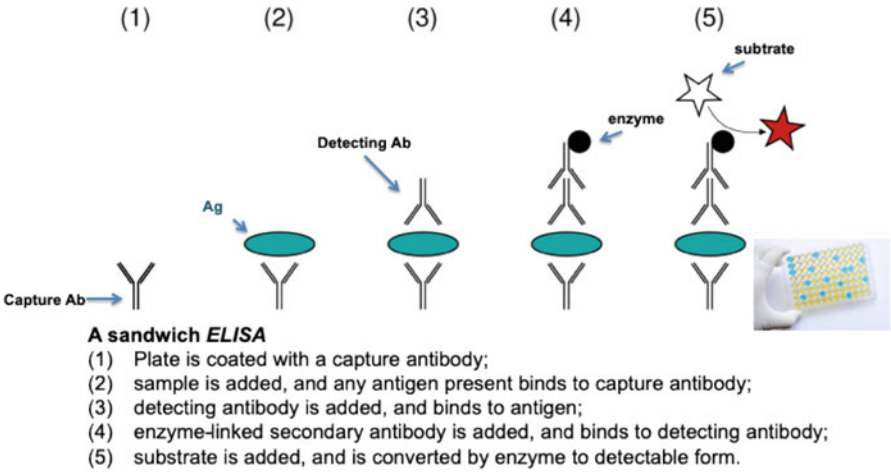

Fig. 4.3 A schematic representation of a sandwich ELISA format

in 25 g samples, it is necessary to amplify the initial microbial population through culture enrichment before applying the ELISA method. The enrichment step can add additional time for detection, typically 12–24 h.

ELISAs can sometimes be labor intensive as the various reagents are manually applied to each microtitre well and washed with a buffer after incubation to ensure the reagents do not carry over into subsequent steps to avoid the possibility of false positive or false negative results (Notermans 1992). False negative results occur when the specific antibody fails to bind to the antigen (toxin or microorganism) under test and can occur when substances or compounds in the test sample mask the antigen, prevent or destroy the formation of the Ag-Ab complex or inactivate the reporting enzyme or label on the detecting antibody. False negative results can also occur when the target microorganism does not grow to a minimum threshold or fail to grow during enrichment. False positive results arise when an antigen other than that of the target species or toxin reacts with the specific antibody being used in the assay. Therefore, the purity and specificity of the antibody and purity of the reagents are critical factors. Non-specific binding of the various ELISA components to the solid support is crucial and should be avoided to avoid false positive and therefore, optimization assays to determine the optimal concentrations of each of the ELISA reagent or components are conducted before any analytical assays to avoid false positives and false negatives.

4.1.3.4.2 Immunocapture Technology

This method has undergone significant development especially with immunomagnetic separation beads and their application to isolate microorganisms from complex food matrices. The beads generally range between 1–5 μm in size and can be made of various materials such as polystyrene. Typically, the beads are uniform in size and incorporated with iron oxide to magnetize them. Specific antibodies can be linked to the surface of the magnetized beads and then used for the capture of the corresponding target such as microorganisms. The beads can be applied to either pre-enrichment or early enrichment cultures to isolate and concentrate specific microorganism when placed in a magnetic field. The beads with the bound target are washed and separated from the original matrix and resuspended in a medium that is compatible with downstream processes such as molecular methods or selective or differential culture medium, cutting down the time for detection. One example is the Pathatrix™ Auto Instrument marketed by ThermoFisher Scientific (ThermoFisher Scientific, Waltham, MA, USA)) is a fully automated, multi sample system that quickly concentrates pathogens and spoilage microorganisms from various food and environmental samples. The miniaturization and modular configuration of systems such as microtitre plate format allows for the processing of multiple samples offering large throughput with computer processing of data and examples of such systems in use within the food industry and testing laboratories are bioMérieux VIDAS® Automated Food Pathogen Detection Solution and the VITEK system (bioMérieux, Hazelwood, MO, USA).

These systems have been rigorously evaluated and give data comparable to other ELISAs and cultural methods. The advantages of the technology are the specific concentration of a target that enables shorter incubation times during the enrichment step, removal of inhibitory substances or partial purification of the target for greater accuracy and can be automated to reduced labor and times for detection.

4.1.3.5 Bioluminescence/ATP Methods

Bioluminescence, either by way of measurement of ATP or lux genes, can be used estimate the total viable microorganismsin in foods.

4.1.3.5.1 ATP-Bioluminescence

This method involves the measurement of ATP from viable bacterial cells since ATP are present in living cells and generally rapidly broken down during cell death. The biochemical reaction involves a specific reaction between the enzyme luciferase and its substrate luciferin and in the presence of ATP and Mg^{++}, a luciferase-luciferin-AMP complex is formed which becomes oxidized in the presence of oxygen. The oxidized complex is an electronically excited state and returns to its unexcited state with the emission of a photon of light, measured by a photometer or luminometer. The reaction is rapid, very sensitive (detection at picogram levels) and highly specific for ATP with a linear relationship between ATP concentration and light output. The linear reaction can be translated to microbial cell concentration due to the relatively stable levels of ATP (generally 1 femtogram) in bacterial cells and can detect within the range of 3–6 log CFU/ml of bacterial cells (Stanley 1989). Some modifications of the assay have occurred over time such as modifications to measure the total pool of adenine nucleotides, i.e. AMP, and ADP, different enzymes such as adenylate kinase, pyruvate kinase and myokinase to improve sensitivity and applicability are added to the sample to convert AMP and ADP to ATP, which is then measured by the standard bioluminescence assay (Champiat et al. 2001; Corbitt et al. 2000; Murphy and Squirrell 2000).

There are limitations with ATP bioluminescence technology especially when applied to foods and beverages because plant and animal cells, generally having higher ATP content, when present in foods can significantly impact the assay and when ATP is measured, it may not differentiate the source of ATP thus lacking specificity. Food constituents, low food pH, or cleanser/sanitizer residues may inhibit the luciferase-luciferin reaction by ‘quenching’ of the reaction and light output leading to variation. Variation can be controlled by running calibration curves and control assay (Calvert et al. 2000).

4.1.3.5.2 Bacterial Bioluminescence

This method utilizes the natural phenomenon of certain species of bacteria within the genera Photobacterium and Vibrio that are naturally bioluminescent. The reaction requires the enzyme, luciferase, the reduced form of flavin mononucleotide ($FMNH_2$), oxygen, and a long chain aliphatic aldehyde, which is converted to a corresponding fatty acid. The reduced flavin mononucleotide is a product of the electron transport chain occurring only in living cells with a functional electron transport system will produce light and the production of long chain aldehydes from fatty acids are essential for the reaction as natural substrate. The genes, *lux* genes, responsible for bacterial bioluminescence have been identified and extensively studied (Hastings et al. 1985; Meighen 1991).

Since not all microorganisms possess the lux genes and hence, the lux genes of bioluminescent bacteria have been cloned and can be incorporated into the DNA of bacteriophages, that will specifically infect certain bacteria making them bioluminescent was developed with applications in food microbiology that are rapid and with specificity (Baker et al. 1992; Stewart and Williams 1992). The detection of targeted bacterial species including indicator microorganisms relies on the availability of bacteriophages with either very narrow or broad host specificities. A recombinant phage, carrying the lux gene, is used to infect its host, which then acquires the ability to bioluminess and, be detected by measurement of light output. Such assay systems have been developed for the detection of enteric indicator microorganisms such as *E. coli* (Kodikara et al. 1991; Loessner et al. 1996; Kaniga et al. 1992; Chen et al. 1996; Chen and Griffiths 1996; Turpin et al. 1993) and have been successfully applied to the detection of the target species in food homogenates or enrichment cultures with claimed sensitivity as low as 1–10 cells/g within 24 h including time allowed for the bacteriophage to infect the host.

4.1.3.6 Biosensors

The demand for accurate and rapid detection of microorganisms has fueled the development of biosensors technologies by enhancing existing technologies for sensitive and rapid detection and where possible, detection of the target without enrichment. The biosensor is an analytical device incorporating biological component e.g. antibody, nucleic acids, enzymes or aptamers with a physiochemical transducer to generate a signal e.g. electrical current or optical that is measurable when the target binds or interacts with the biological component.

One of the early microbial cell-based sensor involved the use of *Acetobacter xylinum* in combination with an oxygen probe for the measurement of ethanol concentrations up to 0.4 mM at pH 2.5–7 (Diviés 1975). By utilizing certain characteristics of microorganisms, biosensors can be configured for the detection of microorganisms in food. By measuring pH variation cause by ammonia produced by interaction of urease and *E. coli* antibodies. The system called potentiometric alternating biosensing had the capacity to detect 10 CFU/ml with an assay time of

approximately 1.5 h when applied to vegetable washing (Ercole et al. 2003). Similarly, the use of an antibody, peptide or aptamer have been explored as electrochemical, optical or mass based biosensors for the detection of *Staphylococcus aureus* from various foods (Rubab et al. 2018). A review by Law et al. (2014) explored the use of optical, electrochemical and mass-based biosensors for use with various enteric pathogens in various food matrices with good detection sensitivities e.g. 53 CFU/ml of *E. coli* O157:H7 in milk within 4 h, 10–50 cells/ml of *E. coli*, *L. monocytogenes* and *Campylobacter jejuni* from milk and chicken extracts within 30 min (Law et al. 2014; Chemburu et al. 2005; Shen et al. 2011).

Over the last decade, the use of nano-technology has enabled miniaturization of the technology, making them more portable and sensitive than ever. Biosensors development have enhanced existing technologies such as antibodies for immunosensors with high affinity to antigens, DNA biosensors or coupled to magnetic functional micro- or nanoparticles for use isolation, detection and characterization of specific low concentration targets or antigens from complex matrices without having the need for enrichment. These nano-materials provide greater surface area for more robust attachment to its target to improve selectivity, sensitivity, time efficiency and cost effectiveness and applied to food and agriculture to detect a range of targets from pesticides, viruses, insects and pathogenic microorganisms (Sekhon 2014; Pashazadeh et al. 2017; Sharma et al. 2015). Another area of interest is the incorporation of microfluidics with biosensors that allows for high throughput, smaller sampler size and reagent volume with increased detection sensitivity. These microfluidics devices are portable, disposable, offering real time detection and simultaneous multiplex analysis of different targets (Luka et al. 2015; Kim et al. 2015). One example is the use of microfluidic nano-biosensor to rapidly detect Salmonella from borate buffer and chicken extract with a detection sensitivity of 3 log CFU/ml (Kim et al. 2015).

Although biosensors have played a major role in food microbiology, there are still challenges to overcome such as improved sample preparation methods that efficiently removes inhibitors and contaminants which could interfere biosensors functionality may improve overall robustness, selectivity and sensitivity. The combination of different types of biosensors in a single, easy to use platform could offer real time monitoring of various parameters during food manufacturing.

4.1.4 Molecular Methods

In the context of this chapter, molecular methods are defined as those that are based on the analysis of microbial nucleic acid, either DNA or RNA. Such methods will offer certain great advantages over conventional and other rapid methods, such as a greater specificity and lower limits of detection, or ability to identify pathogenicity determinants that are coded by specific genes. There may however also be disadvantages, the lower limit of detection may also allow detection of dead

microorganisms, and the ability to detect pathogenicity determinants, may cause some degree of confusion when it comes to the interpretation of results and how those results relate to risk to consumers.

Molecular methods offer the ability to undertake testing and get results in very short time periods, they enable the relatively simple testing for non-culturable microorganisms. In epidemiology, molecular typing methods can help to match human isolates with strains originating from foods or the environment, thus tracing where outbreaks originate quickly and effectively. There is also the initiation of a whole new range of testing methods, that look not at individual microorganisms, but a whole populations present in particular samples, these new "ecological" tests give a quantum leap forwards in how we can use microbiology in the future, ranging from better shelf life determination, to authenticity testing in cultured products.

4.1.4.1 Nucleic Acid Based Methods

Nucleic acids form the genetic code that is the blueprint for every living microorganism. As such the genetic code for each microorganism is distinct and different and it is this difference between the codes for different Genera, Species, Strain and cell, that makes Molecular tools so powerful in microbiological analysis. Nucleic acid based methods use the specific sequences of the bases in either DNA or RNA to detect the presence of a specific microorganism and they can be made very sensitive achieving low limits of detection, by use of various biochemical tools and reactions. In this section, the main types of molecular method that can be used for detection of specific microorganisms in foods will be covered.

4.1.4.1.1 Nucleic Acid Hybridization Probes

DNA hybridization is based on the very high specificity of base pairing between complementary bases in two strands of DNA. In is natural state within cells, the DNA molecule is double stranded, the strands being held together by bonding between the complementary bases on each strand. It is possible to break these strands apart by applying heat, creating two single strands of DNA. At this stage, any DNA molecule that has a complementary matching base sequence to the single strand of DNA, will be able to bind to it. If that molecule carries some form of marker or label, then it will be possible to measure that attachment has occurred. This is the basis of the simplest and earliest form of nucleic acid-based method, which is the direct hybridization probe.

The early uses of DNA probes utilized radioactive labels, and these were of little use in routine testing laboratories due to safety concerns, however soon enzyme based color based markers were adopted, opening up routine use of DNA probes to a wide range of laboratories. The limits of detection for non-radio labelled probes, initially fell far below that of those with radio-labels, requiring amplification of the target, before detection. Early probe-based methods amplified target microbial cells

using growth of the target in suitable enrichment broths, followed by release of DNA, separation of the DNA strands and addition of a complementary labelled DNA probe. Various washing steps removed unbound probe. Therefore, if the label was detected, the target microorganism must have been present in the sample. It has been estimated that a probe of only 20 nucleotide bases in length could identify an microorganism uniquely (Gutteridge and Arnott 1989).

The earliest commercial hybridization probe-based kits for the analysis of microorganisms in foods were produced by Gene Trak (Framingham, MA) in the 1980's for Salmonella detection (Fitts 1985). These tests used DNA probes directed towards chromosomal DNA to detect Salmonella in enriched food samples, the total test time was around 48 hours. In order to decrease the limit of detection and make the method more sensitive, the company soon moved to direct probes at ribosomal RNA (rRNA). As there are many more molecules of rRNA within a single cell, this effectively amplifies the signal from the label. Comparisons of the results obtained from those methods to those of conventional microbiology indicated good equivalency of results for Salmonella and Listeria methods (Mozola et al. 1991).

A number of commercial manufacturers went on to produce kits based on hybridization probes for the detection or confirmation of various foodborne pathogens (e.g. (Bobbitt and Betts 1991)). Looking towards the future, the development and use of hybridization probes in the food industry has advanced little in recent years, few are being used in laboratories and even fewer developed into commercially available formats. This is undoubtable due to the development and rise in use of techniques based on nucleic acid amplification, such as the polymerase chain reaction (PCR), as these offer significant improvements in test method sensitivity, thus lowering limits of detection.

4.1.4.1.2 Nucleic Acid Amplification-Based Methods

The nature of nucleic acids, either DNA or RNA, is that they can be made a part of simple biochemical reactions. The use of heat to denature double stranded DNA into two single strands, the use of enzymes that can create new complementary strands of DNA, all lead towards methods by which DNA can be amplified biochemically. In contrast to any biological amplification, via enrichment and cell growth, biochemical amplifications is very fast, with million to billion ford amplifications possible with a few hours (Betts and Blackburn 2002). Such a rate of increase means we can have detectable levels of a target DNA molecule from a microorganism in a much shorter time, bringing down test times significantly. There are a large number of nucleic acid amplification techniques that have been developed. The next sections will describe the most common types.

Polymerase Chain Reaction (PCR)

This was first reported in 1985 as a method for the specific amplification of DNA (Mukkis and Faloona 1987). Classic PCR utilizes two short oligonucleotide primers

that can hybridize to opposite strands of a DNA molecule and flank the region of interest in the target DNA. PCR proceeds via a series of repeated cycles involving target DNA denaturation, annealing of the specific primers, followed by primer extension. Extension is achieved by including a mixture of nucleotide triphosphates and a DNA polymerase enzyme within the reaction mix. The polymerase simply adds successive nucleotides that are complementary to those on the single DNA stand, onto the end of the annealed primer. This creates a copy of the DNA in double strand format. Once one cycle of replication is over, the procedure is repeated, each repeat doubling the number of copies of the target. The three stages of the cycle are simply controlled by changing the temperature of the reaction, as each stage will only occur at a defined temperature. The temperature changes are controlled within a thermocycler instrument.

PCR has developed into a fairly routine laboratory tool, with numerous companies now producing commercial kits for the detection of foodborne pathogens based on the PCR reaction. In its role as a method for pathogen detection PCR kits usually utilize a period of enrichment before employing amplification. This effectively does two things: ensures that if very low levels of microorganism are present (e.g. <10 in 25 g of food), these are increased in number to a point that it is known that the small volume used in a PCR reaction will contain at least one target cell, which is amplified and detected; secondly it gets over one much quoted negative of PCR, the detection of dead cells- if enrichment is required to be able to detect an microorganism, then that microorganism has to be able to divide.

Commercially available PCR kits have effectively reduced the time to detect many common foodborne pathogens down to 24–30 h and made a major contribution to the ability to obtain rapid results. There have been several modifications to standard PCR that are worthy of mention, one being Multiplex-PCR. This allows the simultaneous detection of a number of targets from the same PCR reaction. It is achieved by included multiple primers aimed at different target DNA sequenced within the same PCR reaction. Multiplex PCR can be used to detect a number of different pathogen types within a single reaction, or to detect a number of different targets within one pathogen (e.g. presence or toxin genes or other pathogenicity determinants). It expands the usefulness of a single PCR reaction and can reduce time and costs of analysis. A good example of the power of multiplexing was been the development of a universal PCR protocol for the simultaneous detection of 13 foodborne pathogens (Wang et al. 1997).

Reverse Transcriptase PCR (RT-PCR)

RT-PCR utilizes RNA as the target for the PCR reaction. As traditional PCR will only work with DNA, RT-PCR must first convert the target RNA into DNA utilizing the enzyme reverse transcriptase (RT). This produces a copy of the RNA but in DNA format and is known as cDNA. Once cDNA is obtained the PCR reaction progresses as it would for traditional PCR.

RT-PCR has been used in two areas. Firstly, when there are concerns that the PCR reaction may pick up dead microorganisms. rRNA is believed to degrade very

rapidly after cell death, so it is unlikely that dead microorganisms would be detected using an RT-PCR protocol. The second reason is when using PCR to detect RNA viruses. Most virus testing is now done using PCR. For RNA viruses such as human Norovirus (HuNV), the only route is to use RT-PCR to convert viral RNA into DNA that can then be amplified using PCR (Bustin and Mueller 2005).

Real Time PCR

This is also known as quantitative PCR (qPCR). Whilst standard PCR proceeds via two steps; amplification, followed by detection of the amplified product; real time PCR can combine those two steps in one reaction giving a "real time view of the development of amplified product. This technique requires the use of specific fluorescent dyes which intercalate into the PCR product during amplification. As the dyes are incorporated into the PCR products and they become fluorescent for the detection. The intensity of fluorescence being proportionate to the amount of amplified product. Therefore, this makes Real-Time PCR is a quantitative method (Bustin and Mueller 2005). Various commercial companies have different chemistries for these reactions, but all offer similar advantages and a majority of the currently available kits for detection of foodborne pathogens are now based on Real-Time PCR chemistry.

Isothermal Amplification

Standard PCR techniques all operate on the original protocol based around using varying temperatures to enable the reaction to progress. The ability to make these temperature changes quickly and effectively has improved significantly with instrument improvement, but they still have to be made.

Over many years there has been research into a wide variety of alternative nucleic acid amplification techniques many utilizing an isothermal approach, excellent reviews of the area including that many different types of reaction used, are given in (Karami et al. 2011; Gill 2008; Zhao et al. 2015; Li and McDonald 2014). The advantage of isothermal amplification is the lack of any requirement to do temperature changes to enable the reaction to progress, therefore in many cases amplification can proceed far more quickly, giving a more rapid result.

4.1.4.2 Molecular Methods for Typing and Fingerprinting Microorganisms

In the event of an microorganism being isolated from a food product, a decision must be made as to what happens next? In many cases the result is enough and will result in some form of action to clean an area (if the microorganism has a hygienic or food quality significance) or recall the product (if the microorganism may harm health). However, in some cases there may be great interest in knowing much more about the isolate, questions such as "where did it come from", "is it the same as a similar

microorganism", or "is it the cause of the illness" may arise. In these cases, we move into the area of microbiological typing or sub-typing (it can also be called fingerprinting or characterization).

In the past, we could only type a few of the better-known pathogens, serotyping and phage typing for Salmonella are good examples. The methods were based on serology and could not be applied to every different isolate.

The onset of the use of molecular microbiology brought with it a range of new methods that were capable of being used to type isolates, at first these were confined to use by public health or enforcement organization, now however many food producers make use of typing to confirm the root cause of problems and permanently eliminate them.

Microbiological typing methods can be broadly separated into two different forms: (1) DNA fragment length analysis, and (2) DNA sequencing-based techniques. Fragment length techniques are based on the use of restriction enzymes that cut DNA at defined sets of base pair sequences. These generate various lengths of DNA fragments which can be separated by electrophoresis according to their size. Because the restriction enzymes always cut the DNA in the same place, the fragments produced for one strain will always be identical, therefore the electrophoresis patterns will be the same and this will "type" the microorganism. Different strains of the same microorganism are likely to have different lengths of restriction fragment, therefore will be shown on electrophoresis to be a different "type". Sequencing based techniques are based on establishing the base sequence of the DNA of an microorganism, as every strain of microorganism has a different DNA base sequence, they can be differentiated using this technique.

The details of the various types of typing techniques have been reviewed on many occasions, and these publications should be consulted if needed (Wenjun et al. 2009; Adzitey et al. 2013). Some of the main techniques are described below.

4.1.4.2.1 Pulsed Field Electrophoresis (PFGE)

In PFGE, DNA is separated from a pure culture of an microorganism. It is then treated with a restriction enzyme that is designed to cut the DNA into larger fragments (the enzymes are often known as rare cutting enzymes). The resultant mix of fragments are then separated according to their size using electrophoresis. The patterns of fragments obtained from different microorganisms can then be compared to establish if they are different. In normal constant electric field electrophoresis, DNA fragments greater that about 20 kb, tend to show the same mobility, making it difficult to differentiate between these molecules. In traditional PFGE the electric field is applied at different angles allowing these larger fragments to be separated. Before the development of faster more inexpensive DNA sequencing systems, PFGE is considered by many to be the gold standard for typing many bacteria and has been used by public health, regulatory and enforcement bodies throughout the world. In order to do this the analytical procedure had to become highly standardized (so that different laboratories would obtain exactly the same

results from the same strain), and there had to be a system for sharing and comparing PFGE results, so that strains tested in different locations can be matched. There are excellent detailed reviews of PFGE that can be consulted for more information on the operation of the method (Goering 2010).

4.1.4.2.2 Ribotyping

Ribotyping utilizes frequently cutting restriction enzymes that can produce a larger number of varying size DNA fragments. These fragments are then treated with labelled probes to the bacterial rDNA genes, marking fragments that contain these genes. The resultant mix is then separated by electrophoresis. The banding patterns obtained are called ribotypes. An advantage of ribotyping is that it enables analysis without any need for prior knowledge of the genomic DNA sequence because the rDNA genes are universal amongst bacteria. Additionally, the results of ribotyping are easier to interpret as fewer fragments are produced. Ribotyping is one of the only typing systems to have been fully automated (from DNA extraction through to results interpretation). The RiboPrinter (Hygiena, Camarillo, CA, USA), accepts a suspension of a pure culture, and undertake all of the extraction, probing, electrophoresis, blotting, and comparison of results to a database, giving the user a final result as a series of DNA bands on a membrane. This end point is known as a RiboPrint Pattern™. The pattern for an isolate is automatically compared to a database in the instrument that allows it to be identified to a sub-species level. . Overall Ribotyping, and particularly automated ribotyping can provide an excellent system for the typing of some foodborne pathogens (e.g. Salmonella and Listeria), giving a very rapid results (approximately 8 h from colony to final result) that are highly reproducible (Pavlic and Griffiths 2009).

4.1.4.2.3 Amplified Fragment Length Polymorphism (ALFP)

AFLP (Blears et al. 1998) uses two restriction enzymes, one of average cutting frequency and one of higher cutting frequency, to digest total genomic DNA. After digestion, adapters are linked to the sticky ends of the fragments and amplification of a subset of those fragments is done. Gel electrophoresis is used to identify DNA fragments to identify polymorphisms. It is reported to have good discriminatory power and good reproducibility but can be a lengthy test to perform.

4.1.4.2.4 Multi-Locus Sequence Typing (MLST)

MLST uses sequencing to discover variations in the sequences of a number (usually seven) housekeeping genes in bacteria. The sequences themselves are constrained because of the essential function of the proteins that they encode. Approximately 450 to 500 base pair fragments are sequenced and most bacteria have enough

variation within those housekeeping genes to provide many profiles. Each different sequence for a gene is allocated a number, and each strain is therefore assigned a seven digit profile designated as a sequence type. MLST has been used successfully to differentiate bacterial isolates at levels below that of species (i.e. sub-typing), however it may not be as discriminatory as other forms of typing due to the conserved nature of the genes used.

4.1.4.2.5 Whole Genome Sequencing (WGS)

WGS has become a more widely used typing tool as the costs of sequencing has reduced and the ease of performing it has increased. It is becoming widely used by many public health bodies for monitoring microorganisms originating from food sources (Ronholm et al. 2016). The basis of WGS is the sequencing of the complete genome of an microorganism, and then comparing sequences between different strains to observe nucleotide polymorphisms (basically differences in the base nucleotide sequence). If the strains are the same, then the number of polymorphisms will be low; however, if they are different, then the number will be much higher.

The operation of WGS required the use of an instrument known as a sequencer. This will not be able to sequence the whole of a bacterial genome, but instead produces short sections of sequence, a few hundred base pairs in length. A bioinformatic program must them be used to take the short sequences, and build a whole genome sequence up, looking for overlaps in the short sections. This process would be virtually impossible to do without the help of the computer program.

Once the sequence has been obtained, it must them be compared to others to decide if strains are different. There has been some controversy as to how many single nucleotide polymorphisms (SNP) are required to "make" a strain different from another. Current ideas are that 5 to 10 SNP's difference would make a strain different.

Over recent years, WGS has become the method of choice for public health epidemiology, it has been aided by the building of large databases (e.g. GenomeTrakR) to hold WGS information and make comparisons of strains easier. Of course, the ability to have and hold WGS data, allows other analyses to be done, so databases can be searched for the presence of particular genes (pathogenicity determinants, toxin genes, antimicrobial resistance genes, stress response genes) making these systems a great resource for the future.

4.1.4.3 Culture Independent Methods

Culture has always formed a key part in the microbiological analysis of foods. It is an amplification technique that increases the number of viable microorganisms to a level that can be detected, and because growth occurs, the microbiologist knows that they are dealing with viable cells and not those rendered non-viable due to processing.

Culture does, however, have disadvantages, it always selects for microorganisms capable of growing under the culture conditions used, therefore it introduces bias into analysis. The use of method that can provide the data, but without the need for culture can eliminate the bias and may give a very different view of the microorganisms in our foods as we would see all organisms that were present, not just those capable of growth in artificial growth media. Perhaps the biggest move forwards in culture independent analysis, has been the development of metagenomic analysis. This is a form of DNA sequencing but does not attempt to sequence a whole genome.

In food analysis, metagenomic analysis usually proceed with the extraction of total DNA from a food (there is no enrichment), this DNA is then treated with PCR primers for bacterial 16S rDNA genes. These are amplified and sequenced and compared to a large database. This will allow the identification of every type of microorganism present within the food sample, whether it will grow on media or not.

The level of identification will not approach that given by WGS, it will enable the differentiation of Families, and Genera, and in some cases species, but it will show up populations present in samples. This is a powerful tool in examining what occurs during shelf life, which microorganisms grow, which do not; it can be used in forms of authenticity testing in cultured products which will have highly conserved microbial populations; it can be used in hygiene assessment, which populations develop after cleaning, and do different cleaning regimes result in different populations. This approach can revolutionize food microbiology (Jones 2017).

4.1.4.4 Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectroscopy(MALDI-ToF)

This is not strictly a molecular method by the definition given at the beginning of this section, however, it does require the analysis of a chemical signal from a pure microbial culture to give an identification of that microorganism (Murray 2012).

Bacterial colonies are removed from isolation plates, mixed with a UV-absorbing matrix, and dried on steel target plates. The dried preparations are placed into the MALDI instrument and then illuminated by pulses of a laser. This results in energy transfer from the matrix to the nonvolatile analyte molecules in the microbial cells, with removal of analyte into the gas phase. The ionized molecules are accelerated by electric charge, through a flight tube to the mass spectrometer. The molecules being separated according to their mass and charge. The profile of these markers is detected by the mass spectrophotometer, and the pattern compared to a database that allow the microorganism to be identified. The technique is fast and simple to use, and can result in the identification of an unknown microorganism in a few minutes. As MALDI ToF develops, there are indications that it can be used for typing as well as identification.

4.1.5 Method Validation and Verification

The one question everyone must raise before using a new method is, how do I know it will work? This usually means will the new method give at least equivalent results to those given by the standard reference method, and will it give those results in my laboratory. These are actually two separate questions and are answered by the practices of method validation and method verification.

4.1.5.1 Method Validation

Validation is the independent testing of a method by a third party that will report on whether it meets the key requirement of giving equivalent results to a reference method. There are a number of different Validation schemes that will do this: AOAC Official Method Program, the AOAC Research Institute Performance tested methods program (PTM), AFNOR, MicroVal, and Nordval all operate validation/certifications schemes, the latter three all operate to the ISO 16140: 2016 part 2 method validation standard (ISO 16140; Part 2 2016).

Usually method validation proceeds in two parts, an expert laboratory study done in one laboratory (usually known as the expert laboratory) and a collaborative study in which samples are tested in between 8 and 12 laboratories.

There are a number of key points to look out for in any validation study to ensure it meets users' requirements; what standard reference method was used as the comparator method and is this accepted in the country in which I operate?; what is the scope of the validation, which microorganisms was it designed to detect, and which foods was it designed to work with; are there any major exclusions noted in the validation study that would indicate areas in which the method would not work. Users should never take the validation certificate as proof that a method works, without reading the validation report and understanding what was done within the validation, and what statements were made about methods operation.

Only when the potential user is convinced that the method will give at least equivalent results to the reference method, when used with foods sample types that the laboratory is going to use, should consideration be given to adopting the method.

4.1.5.2 Method Verification

This is the work done by the user laboratory to prove that a method will work in their hands, on the sample types that they will be testing. At present, there is no consensus on how much work is required to undertake method verification, however a new ISO standard, ISO 16140 part 3, will be published in 2020/21 that will cover method verification (ISO 16140, Part 3 (Draft under development) n.d.), and this will give some assurance that this important process is standardized across testing laboratories.

4.1.6 Perspective and Selection Criteria

The significance of microorganisms in food production, processing, and fermentation is well recognized. Microbial contamination of food and food ingredients with foodborne pathogens presents an important food safety concern. Pathogenic microorganisms such as Salmonella, Listeria, Campylobacter, and *E. coli* O157:H7, and toxin producing microorganism such as *Staphylococcus aureus*, *Clostridium botulinum*, etc. have been linked to several large foodborne outbreaks, some involving fatality. Also, intentional contamination of food, water, and food ingredients is recognized as a significant threat to our food supply.

Microbiological examination of ingredients and raw materials, food, and food processing environment allows assessment of quality, shelf life, safety, and stability of food as well as compliance with industry and regulatory standards, specifications, and guidelines regarding numbers and types of microbial contamination. Microbiological examination of a food plant environment helps determine the level of hygienic operation and sanitation used during processing, handling, and storage, as well as possible sources of a specific microbial type in a food. Finally, microbiological examination can also help in assessing success of processing and preservation methods used to kill and prevent the growth of microorganisms, and degree of abuse leading to microbial growth and toxin production.

Many conventional methods used for qualitative and quantitative examination are so-called "standard" methods that are recommended by professional organizations or regulatory agencies and are given in the Compendium of Methods for the Microbiological Examination of Food (Salfinger and Tortorello 2015), the Standard Methods for the Examination of Dairy Products (Wehr and Frank 2004), the FDA Bacteriological Analytical Manual (BAM) (The FDA's Bacteriological Analytical Manual On line (BAM) 2018), and the USDA-FSIS (Food Safety and Inspection Service, USDA 2014).

Conventional methods for food microbiology testing are considered as "gold standard" for their simplicity, specificity, reliability, universal recognition as recommended or approved methods, and relatively low cost. These are important in international trade and compliance testing worldwide. Complex supply chain for procuring raw material and ingredients, global nature of the food industry, extended distribution network, and emphasis on proactive food quality and safety assurance such as the Hazard Analysis and Critical Control Points(HACCP), the International Organization for Standardization (ISO) 9000 series (e.g. ISO 22000) – all have underscored the need for microbiological surveillance of products, process, and environment. In addition, the food industry programs such as the Global Food Safety Initiative (GFSI) auditing schemes and implementation of the Food Safety Modernization (FSMA) Preventive Control require microbiological testing for monitoring effectiveness of control of microbiological hazard throughout the global supply chain.

The increasing scope, significance, and need for microbiological testing in food industry have only served to reveal further the limitations and drawbacks of

conventional methods and has prompted the development of rapid methods in food microbiology. Interest in rapid methods and automation in microbiology has been growing steadily in the past several decades and many rapid methods for the detection, enumeration, identification and characterization of foodborne microorganisms have been developed to overcome the limitations of their conventional counterparts (Fung 2002; Vasavada 2001; Vasavada 1993a; Foong-Cunningham et al. 2006; Vasavada et al. 1993; Vasavada 1993b).

There are several labor and material saving methods for enumeration and detection of microorganisms that essentially involve modification, miniaturization, or mechanization of traditional methods (Fung 2002; Vasavada 2001; Vasavada 1993a; Gutteridge and Arnott 1989). These alternative methods are convenient, labor and material saving, provide large samples throughput, and are commercially available. However, they require enrichment and the same incubation period (2–3 days or longer) as the conventional methods, and hence are not truly "rapid" methods. Advances in immunology, molecular biology, computation science, and instrumentation have led to development of elegant immunology-based methods, nucleic-based methods, and biosensors-based methods for the detection of pathogens, toxins, and biomarkers of pathogenicity and virulence. While these methods are elegant and offer unique advantages depending on the target pathogen and the food sample, they are complicated, require expensive instruments, reagents, and trained technical staff. Undoubtedly, they are useful in food microbiology research, but they are not practical for routine daily use in a food microbiology laboratory.

Recently, new and advanced technologies, such as whole genome sequencing (WGS) and Culture Independent diagnostic techniques (CIFT)s including metagenomics have been developed for rapid pathogen detection and surveillance of the food supply for pathogens and source tracking. These methods have been successfully used by the CDC and the FDA for investigation of foodborne illness of outbreaks caused by *Salmonella, Campylobacter*, and *Listeria monocytogenes*. These methods are rapidly becoming the gold standard as the primary molecular subtyping method for foodborne outbreak characterization in the USA (Harbottle 2018; Li et al. 2009).

Many novel rapid and automated methods for microbiological testing of food are available commercially and new methods are being introduced regularly. However, their acceptance by the industry depends on several factors such as speed, reliability, versatility, ease of use, acceptance by vendor, and recognition by a pertinent regulatory agency or competent body. Selection criteria for rapid and automated methods include the time to results, performance characteristics, and method parameters viz. specificity, selectivity, reproducibility, repeatability, relative accuracy, detection limit, quantification limit, and linearity of rapid methods. Additional criteria for method selection and adoption include availability, quality, and cost of reagents and consumables as well as need for training and technical support. The decision to adopt a rapid method is not made lightly and should include considerations for the customer, vendor, and regulatory acceptance and recommendations from industry colleagues who have used the method for similar analysis and food

matrices. The selection criteria and important consideration for selection and adoption of rapid and automated methods have been discussed (Fung 2002; Vasavada 1993b; Jasson et al. 2010).

4.1.7 Conclusion and Future Trends

Recent outbreaks of foodborne illnesses associated with the consumption of food that had been contaminated with pathogenic microorganisms or toxins and large scale recalls of a wide variety of foods, including RTE foods, fruits and vegetables have underscored the significance of microbial contamination. The significance of spoilage and pathogenic microorganisms is also emphasized by increasing use of RTE foods, exotic raw materials, ingredients, global sourcing, and supply chain. Undesirable microorganisms constitute the primary hazard to safety, quality, shelf life, and wholesomeness of foods. Consequently, increased emphasis has been placed on the microbiological analysis of food, ingredients, and food plant environment designed to evaluate quality and to ensure safety and regulatory compliance. The focus of food microbiology, however, remains largely on conventional methods, which are slow, tedious, material and labor intensive, and often not suitable for assessing the quality and shelf-life of foods. Routine microbiological analysis in the food industry seldom involves isolation and characterization of various microorganisms occurring in food. However, increased demand for microbiological (pathogen) surveillance of products, process, and food plant environment have increased the need for the detection, isolation, enumeration, and identification of pathogens. Also, epidemiological surveillance, source tracking, detection, characterization, and identification of known pathogens as well as emerging, less recognized and novel microorganisms, pathogenic determinants and certain genes etc., has underscored the need for unique methods that can provide rapid results with a high degree of selectivity and specificity.

Over the past 30 years, interest in rapid methods and automation in microbiology has increased and there is a trend for increased adoption of rapid methods in food microbiology laboratory worldwide. A Delphi survey conducted in 1980s to predict the future development in rapid and automated methods in food microbiology predicted that the traditional methods for enumeration of microorganisms in foods would be superseded by automated and mechanized methods for colony count by the end of the century (Hefle 1995; Vasavada 1993b). Molecular methods, biosensors, whole genome sequencing (WGS), and Culture Independent Diagnostic techniques (CIDTs) including metagenomics provide novel, unique, and reliable, state-of-the-art methods that can be useful in the protection of our food supply from accidental or intentional contamination.

Recent food microbiology testing market reports indicate that microbiology testing in the food sector has grown at a rate of >7% Compound Annual Growth Rate (CAGR) over the last 15 years (Ferguson 2018) and the total market for industrial microbiology tests is projected to be 2.5 billion tests, with the food safety

microbiology testing is approaching 1 Billion tests worldwide. (SCI 8th) (Weschler 2014). Adoption of rapid and automated methods is relatively high in North America and Europe but the use of rapid methods is increasing in Asia and South America. Also, there is a shift in where the testing is done. Many food companies do not have fully integrated programs, facilities, trained staff, and specialized equipment for microbiological testing of food samples for "in-house" quality and safety testing done in laboratories located at the food plant. Food safety and quality programs such as HACCP and regulations such as Food Safety Modernization Act (FSMA) Preventive Control for Human Food have increased the process, documentation and accreditation requirements for food quality and safety programs and laboratories. Also, the third party independent contract laboratories specializing in microbiological testing for food quality, safety, and logistics, and availability of transportation especially over-night delivery of samples made it possible for food companies to obtain accurate test results in a timely manner. Consequently, Food companies around the world are increasingly outsourcing microbiological testing, especially pathogen testing to a third-party contract testing laboratories and the volume of food testing conducted at contract laboratories is growing at a faster rate than the food testing market as a whole.

The advances in computerization, immunology, microscopy, instrumentation, sensor technologies, data acquisition, analysis, and diagnostics have led to the introduction of miniaturized, automated or semi-automated methods for rapid detection, characterization and enumeration of microorganisms and their activity. Molecular methods, biosensors, whole genome sequencing (WGS), and Culture Independent Diagnostic techniques (CIDTs) including metagenomics provide novel, unique, and reliable, state-of-the-art methods that can be useful in the protection of our food supply from accidental or intentional contamination. Molecular methods have substantially revolutionized microbiological examination. They are generally faster, more specific, precise, more convenient than conventional methods, and often do not require enrichment and growth of microorganisms. These methods allow the detection, identification, and characterization of unculturable and slow-growing pathogens. However, interpretation of results with respect to food safety may be challenging to food microbiologists. As molecular methods for microbiological examination of food become more common, a question arises: can the molecular methods replace conventional techniques completely? A perfect diagnostic method must be sensitive, specific, rapid, easy to perform and interpret, but also cost-effective and provide high-throughput. While the conventional culture-based methods may not be able to adequately meet the challenges of rapid detection of foodborne pathogens in timely manner, they do serve an useful function in assessing the food quality and safety. Several modified, miniaturized, automated, and semi-automated methods are adequate for food microbiology testing. There is no perfect microbial testing method as all methods have both advantages and limitations. It may be better to use a combination of conventional and molecular techniques depending upon the purpose and need for speed in testing. It may be advisable to use molecular methods for generating rapid and reliable results and culture-based assays for confirmation. It is likely that the use of conventional

methods will continue for general food microbiology testing while the molecular methods are being adopted by the regulatory agencies and food industry. Will these methods completely replace the conventional, culture-based methods? Only time will tell.

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Chapter 5
Interactions of Foodborne Pathogens with the Food Matrix

Jennifer Acuff and Monica Ponder

5.1 Introduction

Microorganisms inhabit a range of environments (soil, water, plants) where they have key roles in the recycling of nutrients. On occasion, these microorganisms are transferred to products that are designated for human or animal consumption, aka, food. Foods provide the energy and nutrients essential for growth and good health. They provide important nutrients such as proteins, carbohydrates, and fats, which are essential for human and animal health. These nutrients are also metabolized by microorganisms. Therefore, the battle to preserve these vital nutrients from the saccharolytic, proteolytic, and lipolytic activities of foodborne microorganisms has been the primary driver of food processing. Assuring a safe and good quality food is not only dependent on preventing the growth of spoilage microorganisms, but also on reducing numbers of microorganisms that may cause human illnesses. Food processing strategies that reduce pathogens in foods are discussed in other chapters in this book. In this chapter, the effect of the food itself as a habitat on the survival and growth of foodborne pathogens will be considered.

Foods are dynamic environments and the interactions of foodborne pathogens with other microorganisms in the matrix can have an important role in food preservation. Fermented foods offer excellent examples of foods where the role of microorganisms to convert the raw product to a product with new acceptable sensory and textural properties create an environment that can be challenging to human pathogens. Fermentative metabolic activities can produce organic acids that lower the pH of the food; if the pH drops below 4.6, it reduces the concerns associated with growth of many human pathogens, including spore-formers. Microbial fermentation may also produce other antimicrobial compounds including alcohols, mainly ethanol, and

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bacteriocins that can help to reduce growth and persistence of foodborne pathogens in the food. However, in the cases of some spoiled food products, nutrients may potentially be used by the pathogenic microorganisms for growth.

A particular challenge associated with studying microbial interactions in foods is due not only to the dynamic aspects of food where the composition changes over time, but also the physical structure of food. The physical structure of foods may vary greatly from liquids, to gels, to solids, which affects nutrient and microorganism distribution. Solid foods may restrict movement of microorganisms and nutrients throughout the medium, which alters the behavior and growth patterns of the microorganisms. In gels and solid foods, micro-niches develop as colonies metabolize available nutrients. If these microorganisms are non-motile, they may quickly exhaust available nutrients and growth can be inhibited due to the build-up of by-products. Processing, including mincing, extruding and grinding has been shown to change the properties of intact meats allowing, for increased bacterial growth compared to intact muscle due to enhanced nutrient availability and ability of motile microbes to change location (Bohnsack and Hoepke 1990). Location within an intact product or within a package can also have important consequences for bacterial growth. Bacteria localized away from antimicrobial gases, or within products where diffusion is limited, may be protected. It has been observed that bacterial counts of turkey burgers packaged under high-CO_2 gas conditions are lowest on the top of the patty, but portions of the patty below the top and outer 1 cm have much higher bacterial counts (Dhananjayan et al. 2006). While these examples illustrate fabricated meats, the environment of the intact carcass up to this point prior to fabrication is very dynamic. Nutrient availability, temperature, and pH affect the growth of bacteria on carcasses. Immediately after slaughter when the carcass is still warm, mesophilic bacteria can proliferate. During the fabrication and cooling stages of production, the growth rates and bacterial load shifts to reflect the lower temperatures that favor growth of psychrophilic microorganisms (Liu et al. 2016; Nortjé and Naudé 1980). It can be a challenge to model these conditions within the confines of a laboratory experiment, so researchers often rely on growth media and standard growth conditions to study microorganisms associated with foods. Interpretations from these models should be applied with caution as the interactions and microenvironments may not be true to the foods themselves. In addition, the physiological state of the bacteria in foods may be difficult to recreate in a lab environment. Bacteria in the majority of solid and gel foods are likely to be in colony form or encased within a biofilm. This may result in different growth rates, morphology, and responses to stress (Jeanson et al. 2015).

Many laboratory experiments are conducted in liquid media, but predictions solely based on planktonic cells could possibly deviate from realistic bacterial responses under stress (Skandamis and Jeanson 2015). In this chapter, the intrinsic and extrinsic factors that influence microbial growth within the food matrix, including physiological state and interaction with other microorganisms in the food matrix are briefly discussed in terms of food matrix. More details for the intrinsic and extrinsic factors can be found in another chapter of this book.

5.2 Intrinsic and Extrinsic Factors Influencing Microbial Growth and Persistence within the Food Matrix

The complexities of a food microenvironment dictate the relationship and interactions between microorganisms and the food matrix. Often times, this microenvironment highly influences and determines whether a microbial cell opts for growth, survival, or persistence in an environment, each of which require different metabolic actions. Water activity, nutrient availability, pH, antimicrobial constituents, oxygen concentration, and redox potential are inherent to the food matrix and thus known as intrinsic factors that influence the response of bacteria in the matrix. Extrinsic factors are external and can be changed to alter and prevent growth of bacterial pathogens. Food processors routinely manipulate extrinsic factors such as pH, temperature, atmospheric gas levels, and addition of antimicrobials to control growth of pathogens. While many aspects of food processing are focused on preventing growth of human pathogens in foods, the prolonged survival of pathogens in foods is a continuing challenge to public health. For bacterial pathogens with moderate to high infectious doses, preventing growth in the food product is an essential step. For other pathogenic microorganisms with low infectious doses, including *Escherichia coli* O157:H7 and many parasites that contaminate, but do not grow, in the food matrix, the challenge is to develop control strategies that reduce these microbes without negatively harming the sensory properties of the food. To accomplish this, intrinsic and extrinsic factors can be carefully manipulated and controlled. The main intrinsic and extrinsic factors of foods and their impact on the growth and persistence of the major bacterial human pathogens are summarized below.

5.2.1 Nutrient and Antimicrobial Presence in Foods

Nutrient availability is an intrinsic factor that heavily determines bacterial growth, as macronutrients are necessary for energy generation, while micronutrients can be essential for key enzymatic activities in bacterial growth or survival. Without the production of ATP, the cell cannot proliferate or maintain vital metabolism. The types and amounts of nutrients available often dictate the success of microorganisms in a food matrix. Raw meats, for example, have high concentrations of simple proteins and carbohydrates that may support the growth of the majority of human bacterial pathogens. Fresh vegetables, on the other hand, have higher levels of starches that require further breakdown, which can prevent or slow the growth of foodborne pathogenic bacteria (Jay 2009). Human pathogenic bacteria such as *E. coli* and *Salmonella* lack the enzymes necessary for penetrating the thick cell walls of plants to obtain the simple sugars needed for their growth. These pathogenic bacteria can enter plants through mechanical damage or naturally through the leaves of plants through the stomata or the fruit through the stem, stem scar or calyx where

they are capable of growth at low levels (Lim et al. 2014; Wright et al. 2017). This low level of growth may be due to the exhaustion of nutrients in the tissue and reduced ability of the human pathogen to distribute through the plant. There are, however, examples of pathogens taking advantage of the efforts of other microorganisms to make nutrients available. Concentrations of *Salmonella enterica* ser. Typhimurium on carrot, pepper, and fresh potato were increased ten-fold when co-inoculated with the bacterial soft rot pathogen, *Erwinia carotovora*, compared to *Salmonella* alone, likely due to the increased amount of nutrients released through the action of the soft rot bacteria (Wells and Butterfield 1997). Other plant phytopathogens, including downy mildew oomycetes, *Bremia lactucae,* similarly promote the colonization of romaine lettuce by *E. coli* O157:H7 and *Salmonella* (Simko et al. 2015). Co-inoculation of lettuce seeds with *Wausteria paucula* was associated with increased growth of *E. coli* O157:H7 on lettuce foliage, while co-inoculation with *Enterobacter asburiae* was associated with decreased (20–30 fold) growth of *E. coli* O157:H7 due to competition for the same carbon and nitrogen sources (Cooley et al. 2006). These are key examples of how interaction with other microorganisms in the environment alter fitness of human pathogens in the food matrix.

In solid foods, certain nutrients may be localized rather than homogenously spread. Food processing strategies that result in damage to food cells are associated with growth of human pathogens on vegetables, including *E. coli* O157:H7 on peeled carrots, and greater levels of various pathogens on shredded or damaged lettuce leaves compared to intact leaves (Dharmarha et al. 2018; Aruscavage et al. 2008). Breads with inclusions, like raisins, will have higher sugar content specifically where the fruit resides. The effects of this localization are easily observed, as the yeast are more active during fermentation around the elevated sugar levels, and larger pockets of carbon dioxide are formed, impacting the texture of the final product. Processing may provide pathogens with access to microenvironments and nutrients. An outbreak of *Listeria monocytogenes* was likely due to the ability of *L. monocytogenes* to grow in the microenvironment between the low-a_w caramel coating and low pH environment of the apple. Insertion of a wooden stick into the apples released enough nutrients to enable growth in the interface of the apple and caramel coating (Glass et al. 2015). Intermediate moisture foods, especially those hydroscopic in nature, may be in a non-equilibrium state of thermodynamic stability that would influence diffusion rates of nutrients and water, potentially influencing localized microbial growth. Highly viscous foods may also offer reduced diffusion of nutrients that may reduce microbial growth, however there is limited evidence in foods themselves (Chirife and del Pilar Buera 1994).

Ability to shift energy metabolism, so called metabolic shifts, may be a key indicator of the success of a foodborne pathogen to persist within a food. Some microorganisms are more flexible than others when carbon sources and other essential nutrients are limited, which can enable them to survive in diverse food matrices. *Salmonella* is very flexible in its metabolic strategies when only certain nutrients are available and can use metabolic shifts to resist the disturbances to its metabolism (Bumann and Schothorst 2017). *Salmonella* metabolism is intricate and diverse with capabilities of processing of more than 50 different types of nutrients

and has even been shown to degrade its own rRNA as a source of nutrients in an act of autophagy (Finn et al. 2013). Increased metabolic flexibility may also be associated with persistence of foodborne pathogens in foods with diverse microbial communities. Some human pathogens possess rapid growth rates in the presence of dilute nutrients and may have strategies to acquire nutrients that are not readily available. For example, in eggs, ovotransferrin sequesters free iron, preventing growth of most bacteria, especially those with high iron requirements. *Salmonella* spp. have high iron requirements and are able survive and multiply in eggs due to its production of ferritin and other specialized enzymes for iron acquisition and storage (Kang et al. 2006). Alternatively, some foodborne pathogens, including *Yersinia entercolitica* are poor competitors with native microbiota in foods. Therefore its more common to see *Y. entercolitica* outbreaks associated with post-processing contamination in foods where the native microbiota have been reduced by pasteurization or other processing (Divya and Varadaraj 2011).

Fostering or creating competition for resources in foods can be an important strategy for food processors creating a value-added product. For instance, addition of starter cultures whose presence outnumbers the naturally occurring bacteria is common. Processors may also exploit the naturally occurring, competitive activities of other bacteria to control foodborne pathogens in foods. Inclusion of lactic acid bacteria starter cultures that produce antimicrobial compounds, mainly bacteriocins, are of special interest in control of human pathogens in fermented foods. *Leuconostoc carnosum* 4010, a bacteriocin producer, was found to be more effective in preventing growth of *L. monocytogenes* in sliced, gas-packed meat products compared to purified bacteriocins (Jacobsen et al. 2003). This likely reflects both the competition for nutrients and bacteriocin production, as well as a slight acidification of the environment due to the lactic acid produced by its competitor. Nisin and nisin-producing strains of *Lactobacillus lactis,* are added to cheese spreads and other processed foods to inhibit toxin production by *Clostridium botulinum*. It is important to note that the diffusion of these inhibitors may be hampered, especially in solid or gel type foods. Inhibition has been shown to increase in a model cheese system when the distance between a pathogen and lactic acid bacteria decrease, likely due to poor diffusion through the casein gel (Jeanson et al. 2015).

Natural components of certain foods, chiefly enzymes, essential oils, and plant-based bio-actives influence the microbial ecology of the food matrix. For instance, compounds in garlic and ginger have been shown to provide some lethality to *E. coli* O157:H7 (Gupta and Ravishankar 2005). Growth of foodborne pathogens was reduced with increasing concentrations of garlic. Gram-negative pathogens *E. coli* and *Salmonella* were more sensitive to garlic compared to *L. monocytogenes* (Kumar and Berwal 1998). The effectiveness of these compounds are unclear when used as seasonings in foods, as outbreaks of human illness have been attributed to products containing garlic and other spices. Despite these examples of weak bacteriostatic compounds, there are examples of antimicrobials that in their naturally occurring concentrations are bacteriostatic against various foodborne pathogenic bacteria. The α-acids in beers made with hops, including alcohol-free beer, inhibit growth of foodborne pathogens such as *Staphylococcus aureus, L. monocytogenes, Salmonella*

spp. and *E. coli* (Karabín et al. 2016; Menz et al. 2011). Nevertheless, *Sal. enterica* ser. Paratyphi was shown to survive in beer for 63 days, leading to questions regarding its mode of resistance to ingredients that are often considered natural antimicrobials (Menz et al. 2011).

Dispersal of antimicrobials within the product to assure contact with the pathogens of interest is an important consideration for its effectiveness. For instance, solubilized essential oils are more effective in assuring reduction of foodborne pathogens compared to the dried spices from which they are sourced. Thyme oil applied to lettuce leaves and carrots resulted in reduction of *E. coli* O157:H7, particularly when followed by sequential washes of aqueous chlorine dioxide or ozonated water (Singh et al. 2002). Thyme oil also showed activity against *L. monocytogenes* when mixed thoroughly within minced pork (Aureli et al. 1990). Oregano contains essential oils and phenolic compounds that are antimicrobial against most Gram-positive and -negative microorganisms and has even been suggested as an alternative to chlorine for lettuce-washing (Beaubrun et al. 2016; Gündüz et al. 2010). Carvacrol, a monoterpene phenol responsible for the characteristic odor of oregano has been shown to reduce growth of different clinically relevant Shiga toxin-producing *E. coli* and *Salmonella* in growth media, but few trials have investigated the effectiveness of the antimicrobial in foods (Stratakos et al. 2018; Beaubrun et al. 2018). *Bacillus cereus* inoculated on the surface of rice was inhibited when carvacrol was added, though in concentrations that exceeded the dose that might be obtained from typical seasoning (Ultee et al. 2000). Nevertheless, foods containing oregano and thyme, sources of carvacrol, have been associated with recalls due to the detection of viable *Salmonella,* indicating that natural concentrations in seasonings are not necessarily inhibitory (Zhang et al. 2017). Oils of black peppercorns are also characterized as having broad antibacterial activity. However, based on the ability of *Salmonella* to survive on dried black peppercorns and ground pepper, this activity must be due to specific contact of the oil with the pathogen (Karsha and Lakshmi 2010; Keller et al. 2013). *Salmonella* contamination of several dried spices in the United States has been associated with both ground and whole products including black pepper, curry powder, garlic, oregano, paprika, and red pepper (Zhang et al. 2017). The concentration of the natural antimicrobials and contact with the pathogens of interest may explain some of the discrepancies in terms of the effectiveness of natural antimicrobial ingredients in the foods. Therefore, additional research should consider the interaction of the pathogens, but also the compounds, within the matrix.

5.2.2 Water Activity: Osmotic and Desiccation Stress

Presence of unbound or free water is a key indicator of microbial growth in foods. Water activity (a_w), or the ratio of free, unbound water compared to pure water is an important indicator of microbial safety. During desiccation or drying processes, energy is transferred from the environment to the food, in the form of heat, and in

turn, moisture from the food is evaporated into the environment (Raponi et al. 2017). The removal of water limits the microorganisms' abilities to carry out normal metabolic functions and enzymatic reactions for growth forcing the cell to funnel energy into pathways that promote survival and persistence (Burgess et al. 2016; Deng et al. 2017; Cronan 2002). Pathogenic bacteria cannot grow at a_w <0.86, though the a_w-limiting growth of Gram-negative pathogens is 0.95 (Maserati et al. 2017). Other Gram-positive microorganisms, such as *St. aureus* and *B. cereus* can grow at lower a_w of 0.86 and 0.92, respectively (Sperber 1983). Molds have a much larger a_w range for growth, as low as 0.60, indicating a food safety risk of dried foods associated with mycotoxins. Reduced water activity has historically been used to prevent proliferation of pathogens, but growth is not necessary for persistence of foodborne pathogens. This can make low water activity foods (LWAF) vehicles for disease, despite the stress placed on bacterial cells, and provide incentives to understand how osmotic and desiccation stress impact microorganisms when water activity in food is manipulated. For some microorganisms, exposure to low water activity will trigger sporulation, as in the case of foodborne pathogens *B. cereus*, *St. aureus*, *C. botulinum*, and *C. perfringens* (Montville et al. 2012). Although some microorganisms have mechanisms of resistance against osmotic stress, most microorganisms are susceptible to high concentrations, which can be exploited through manipulating water activity to prevent pathogen growth in foods.

Water activity can be reduced either through the introduction of solutes such as sugars or salts, or by removal of water through drying or desiccation, both of which increase localized osmolyte concentrations and decrease water available for biological use. Salt (sodium chloride or potassium chloride), one of the oldest preservatives, is effective in reducing or inhibiting microbial growth by placing microorganisms under osmotic stress. The addition of salt or other osmolytes to food causes water to be drawn out of the cells, ultimately to the surface of the food, thus lowering the amount of water available for biological growth within the food. Salt is commonly used to lower the a_w of Gouda cheese, and combined with lactic acid production, inhibits growth of *L. monocytogenes* (Wemmenhove et al. 2016). Addition of salt, while reducing Listerial counts, does not eliminate the risk of listeriosis. *L. monocytogenes* has been observed to form filaments, where the cell is elongating but not dividing, on the surface of vacuum packaged hams with 2.35% NaCl (0.99 a_w) (Liu et al. 2014). These filaments form due to the downregulation of genes involved in cell division on the surface of ham. Other foodborne bacteria including strains of pathogenic *E. coli* and *Salmonella enterica* have been shown to form filaments under osmotic stress typically at the lower threshold associated with microbial growth. Filament formation in hyperosmotic and high-a_w environments can confer protection if the cells are later faced with certain desiccation stresses (Stackhouse et al. 2012). Upon rehydration, the filaments may dissociate and this increased population could result in an increased risk of foodborne illness. Capsule formation, primarily associated with bacterial virulence, has also been linked to desiccation resistance. Many clinical strains of *Cronobacter* spp. are found to possess capsules, and some isolates from dried powdered infant formula also produced capsules (Umeda et al. 2017).

The type of solute used for reduction of water activity may also influence the reduction of microbial growth. For example, when the water activity of a laboratory medium was adjusted with glycerol or NaCl, the minimum a_w allowing for growth of *C. botulinum* E was 0.943 and 0.966, respectively (Chirife et al. 1996). Glycerol has been shown to be less inhibitory of growth of several kinds of molds compared to mixtures of fructose and glucose (Chirife and del Pilar Buera 1994). Glycerol was also less inhibitory of *B. cereus* as indicated by the onset of sporogenesis triggered at a_w 0.94, 0.95, and 0.91 for NaCl, glucose and glycerol, respectively (Jakobsen and Murrell 1977). It has been suggested that reformulating ingredients to replace glucose with fructose has led to increased spoilage, however this has been refuted in other studies due to inaccuries in measuring the a_w in some foods (Slade et al. 1991; Chirife and del Pilar Buera 1994).

In foods themselves, bacterial responses to osmotic stress may be influenced by certain components within the food. Betaine and L-carnitine, two molecules essential for the human diet and found in a variety of plant and animal-based foods, are transported by *Listeria* spp. in response to osmotic stress. It is hypothesized that they may form a shell surrounding protein enzymes that may partially explain the increased baro-tolerance of *Listeria* at elevated osmolarity (Smiddy et al. 2004; Sleater and Hill 2002). The presence of other solutes, including trehalose, sucrose, and other disaccharides added to foods to maintain texture in LWAF and frozen foods may also play a role in survival of human pathogens. Trehalose, glucose and other compatible solutes may take the place of the lost water and become a glass-like solid within the cytoplasmic membrane that stabilizes the cell membrane (García 2011). Glasses form to prevent the destruction of cell layers and maintain the membrane's structure, as well as physically block chemical reactions from occurring that could lead to cell degradation, thus promoting a state of dormancy (Burgess et al. 2016; Koster 1991). Some of these disaccharides are undoubtedly used by the pathogens as energy sources. Intriguingly, increased use of trehalose as a food additive has been proposed to select for hypervirulent strains of *C. difficile* that have evolved to use low concentrations of the sugar, potentially explaining the rise in human infections (Collins et al. 2018). Typically, trehalose levels that occur naturally in foods would be expected to be absorbed in the small intestine. When large concentrations are added, low concentrations of trehalose may pass into the large intestine where the *C. difficile* spores germinate and begin making toxin.

Comparisons of low and intermediate water activity foods provide insight into pathogens' abilities to survive in desiccated states. Following inoculations of peanut butter (intermediate water activity) and nonfat dry milk powder (low water activity), greater survival was observed on peanut butter (Li et al. 2014). The rate of a_w reduction also plays an important role in both altering the food environment and damaging pathogens during desiccation. Slower drying rates are associated with increased lethality compared to faster drying rates (Raponi et al. 2017). For example, *Salmonella* survival on inoculated ginger root was increased when drying was performed at a high rate (a_w reduction from 0.99 to 0.1 in 4 hours) compared to a slower reduction in a_w (0.99 to 0.4) over a longer period of time (Gradl et al. 2015). During storage of the dried ginger at ambient temperature (25 °C), it was noted that

Salmonella survived longer (365 days) when the relative humidity and a_w were kept low (33% and 0.35 a_w, respectively), as opposed to 25 days when the relative humidity and a_w were higher (97% and 0.85 a_w). Similar trends of higher levels of survivability were noted in loose-leaf teas that were stored at lower relative humidity (<30%) than higher relative humidity (>90%). *Salmonella* was shown to survive as high as 7–8 log CFU/g on peppermint, chamomile, and green tea leaves for over 6 months at low relative humidity (Keller et al. 2015). It may be inferred that certain cellular processes are arrested due to the low water activity responses, leading to heightened use of survival strategies. Clearly, temperature, relative humidity, and rates of desiccation widely vary between food-drying processes, and understanding how each parameter affects microbial survival and resistance to inactivation is vital for the food industry.

5.2.3 Temperature

Temperature is one of the most researched and commonly manipulated factors imposed on food that provides a full array of examples for diverse interactions between microorganisms and food matrices. The temperature of a food has great impacts on the rates of growth and inactivation of foodborne bacterial pathogens. Foodborne pathogens are typically characterized as mesophilic, growing between 20–45 °C; however, maximum growth temperatures can be as high as 50 and 55 °C for *C. perfringens* and *B. cereus*. Psychrotrophs, like *L. monocytogenes, St. aureus* and *Y. enterocolitica*, grow below 10 °C (Hudson et al. 2011; Montville et al. 2012; Jay 2009). Additionally, wide ranges for optimum growth temperature complicate temperature control strategies. For example, *L. monocytogenes* grows well at body temperature (35–37 °C) and at refrigeration conditions, albeit more slowly, making it a concern in a variety of foods. The ability of *L. monocytogenes* to grow in refrigeration and freezing temperatures is due in part to their production of cold shock proteins and cold adaptation proteins (Poimenidou et al. 2016). Post-pasteurization contamination with *L. monocytogenes* or *B. cereus* spores that easily survive at lower storage temperatures has been associated with foodborne illnesses in foods that were previously considered unlikely culprits, such as humus, frozen fruits, and ice cream. Increased shelf life achieved by refrigeration may allow the pathogens adequate time to grow and in the case of spore-formers produces toxins that result in illnesses. For spores of *B. cereus,* a minor temperature abuse from 2 to 8 °C can cause a massive outgrowth of up to 10^3 *B. cereus*/mL in about 9 days in milk (Andersson et al. 1995). This subtle increase in temperature may occur during transport of refrigerated products, and may reflect the temperature of some home refrigerators.

Although the abovementioned temperature ranges promote active growth, many microorganisms *survive* outside these ranges while maintaining virulence, even if not proliferating. As cooling takes place, growth rates typically slow as enzyme reaction rates slow. Additional absorption of osmolytes and oligopeptides changes

balance in osmotic pressure, while cold shock proteins are produced to stabilize proteins (McMeechan et al. 2007; Poimenidou et al. 2016). One of the most drastic events that a cell must adjust to during cooling is the lipid phase transition, when the fatty acyl chains in the membrane in the lipid bilayer form a crystalline, hexagonal structure that decreases the fluidity (de Mendoza and Cronan 2002). In order to maintain membrane fluidity and combat the stress of lipid phase transition, unsaturated fatty acids with lower melting points are integrated, known as homeoviscous adaptation (Berry and Foegeding 1997; Capozzi et al. 2009). Cooling rates of different foods can determine whether microorganisms require aid from cold shock proteins or can acclimate to the changes of the microenvironment. Improper cooling times can allow proliferation of microorganisms, particularly those whose spores survived the cooking processes (Schaffner et al. 2016). Spore-formers, such as *C. botulinum*, *C. perfringens*, and *B. cereus* can germinate and reproduce quickly during improper cooling of foods, leading to foodborne illness (Taormina and Dorsa 2004). An observational study of restaurant cooling practices, while not determining the cooling rates, saw that certain foods such as thick meat cuts and viscous stews took longer to cool from 135 °F to 41 °F compared to other foods like pastas, posing greater challenges for food safety (Schaffner et al. 2016).

In some situations, a food may act as an incubator for microorganisms, especially at dangerous, but common food service holding temperatures (40–140 °F). Temperature abuse can lead to proliferation of foodborne pathogens, thus heightening the risk of foodborne illnesses. These incubation temperatures encourage growth of most foodborne pathogens, but further increasing the temperature can place stress on the microorganisms. Cooking, baking, or some other heat treatment can result in the damage of cellular components, such as membranes, and denaturation of proteins, DNA and RNA. However, the composition of the food matrix can also affect the survival of the foodborne pathogens at elevated temperatures. Increased fat content is associated with increased survival of *Salmonella* when exposed to thermal processing. In two studies, salmonellae were shown to have significantly different *D*-values at 55 °C in muffin batter and bread dough (Channaiah et al. 2016; Channaiah et al. 2017). Muffin batter contained much higher percentages of fat than bread dough (8.88 vs. 3.79%) and resulted in larger D_{55}-values (62.2 vs. 28.6 min). It is important to note the protective effect of fat is not universal but does vary with bacteria and the food composition. Nevertheless, responses to temperature varies between strains, emphasizing the need for more applied research that elucidates gene expression in response to particular temperatures commonly used for storage or treatment of foods.

5.2.4 pH

Acidity levels of a food matrix are one of the key intrinsic factors that determine safety, as well as quality of foods. Controlling acidity has long been used as a tool for preservation, increasing food safety and extending shelf life of foods. Acidified

foods are those in which an acid, such as acetic acid, is added to lower the pH to 4.6 or below (FDA 2018). Foods in which microorganisms produce acid during cellular processes, such as fermentation, also qualify as acidified foods. Low-acid foods with a pH between 4.6–7.0, are most commonly associated with outbreaks of human illnesses. Foodborne human pathogens grow at a narrow pH range, between 6.6–7.5, but they can survive in environments with a pH as low as 2.0, by adjusting to changes of pH using specific mechanisms and stress responses. The relationship between microorganisms and the pH of their microenvironment within the food matrix leads to complex intracellular interactions and reactions that may increase or decrease food safety or quality.

In most cases, acids in food matrices are considered weak acids with smaller dissociation constants, typically these acids are not completely dissociated within the water of the food matrix. The molecules may pass through the bacterial membranes and dissociate within the cell, thus leading to stress responses. Immediate response of a microorganism to a sudden change in pH is often gauged and handled by the F-ATPase system within the cell, or by passive diffusion to remove the H^+ (Burgess et al. 2016). Foodborne pathogens can often withstand small changes to the pH and maintain metabolic activity through acid adaptation and may persist until conditions again allow for growth. Microorganisms may not only implement stress responses to the changing pH of their environment immediately, but develop long-term resistance, as well. Compatible solutes as protectants may play a role in this response, as they can promote normal cellular function during exposure to stress (McMeekin et al. 1997). One study demonstrated that *E. coli* O157:H7 up-regulated genes that are used to gather the osmoprotectant glycine betaine, which likely contributed to the microorganisms' ability to grow in apple juice at a pH of 3.5 (Bergholz et al. 2009).

Interactions of foodborne pathogens with other members of the environment may be impacted by the pH. In fermented products, lactic acid produced by starter cultures is often relied on to prevent the growth of human pathogens by reducing the pH. Lactic acid bacteria and bacteriocin-like inhibitory substances in Sicilian cheeses can prevent growth of *L. monocytogenes* (Scatassa et al. 2017). Lactic acid bacteria have also been shown to inhibit the growth of *S. enterica* ser. Gallinarum and *S. aureus* in milk products and cheddar cheese (Gilliland and Speck 1972). However, it is important to note that the presence of other bacteria can alter the microenvironment and allow for the growth of pathogens in a fermented product. Acid-tolerant yeasts and fungi, especially on cheeses, have been shown to de-acidify the local microenvironment and increase the surface pH. In blue cheese, the growth of lactic acid bacteria in early stages of cheese production dropped the pH to 5.0, resulting in a significant reduction, but not elimination of *L. monocytogenes.* During the ripening period, *L. monocytogenes* may grow and survive for as long as 120 days because the growth of *Penicillium roqueforti* results in localized increases of pH (Papageorgiou and Marth 1988). This phenomenon is observed in other foods as well. Botulism outbreaks have resulted from consumption of spoiled tomato juice where growth of the acid-tolerant *Cladosporium* mold on the surface of tomato juice altered the pH (Huhtanen et al. 1976). Typically, the low pH (approximately 4.2) of

tomato juice would prevent *C. botulinum* spore outgrowth, but the mold formed a mat where the pH increased to 5.78 along the underside, allowing for growth and toxin production.

Fat content has been previously discussed as providing some protection for pathogens in certain circumstances, such as thermal treatments, and it may also provide protection in acidic food environments. Survival of *S. enterica* ser. Typhimurium in acidified lactose broth (LB) was greater in high-fat ground beef (28% fat) than in rice (0% fat). However, exposure to increased fat content in ground beef was not protective for *Campylobacter jejuni* or *Vibrio cholerae* in media at pH 2.5. Protein may also provide pathogens with tools to survive an inhospitable pH. Increased survival was observed for boiled egg white (33%) with low-fat content, suggesting that protein content may be concomitantly protective for *Salmonella* (Waterman and Small 1998).

Understanding how differences in pH impact the microorganisms that may contaminate or reside in various foods carries great importance for food safety. Cells that are adapted to a lower pH could survive and colonize in the human intestine, thus causing disease (Álvarez-Ordoñez et al. 2009). Experiments may establish presumptive growth and death kinetics of microorganisms in acidified foods, but there can be significant variation between strains.

5.2.5 Gases

Atmospheric conditions, especially in terms of oxygen availability, is crucial to the growth of foodborne pathogens in food matrices (Jeanson et al. 2015). There is a diverse spread of requirements for gaseous requirements among foodborne bacterial pathogens from facultative anaerobes (pathogenic *E. coli, Salmonella, Shigella, Vibrio, Staphylococcus,* and *Bacillus* spp.) to obligate anaerobes (*C. botulinum* and *C. perfringens*). Microaerophilic microorganisms require only a very small, controlled amount of oxygen. *Campylobacter* spp., for example, prefers 5–10% oxygen, 8–10% carbon dioxide, and 85% nitrogen to flourish (Hofreuter 2014; Bolton and Coates 1983). Facultative anaerobes are more flexible microorganisms, growing with or without oxygen, which may be an important strategy for continued metabolic flexibility in oxygen depleted microenvironment in food.

The ability of oxygen to diffuse through a food matrix depends on the state of matter. Oxygen more easily diffuses through a liquid medium, as opposed to a solid or more viscous liquid, which may create a gradient of oxygen concentration and a more anaerobic microenvironment at the bottom of a food, possibly allowing for the proliferation of many foodborne pathogens (Bhunia et al. 2016; Chaix et al. 2016). It is important to note that not only the composition but also the processing of food can alter the available oxygen and the resulting redox potential of a food system. Cooking meat broth can reduce the redox potential to −200 mV and drive out all oxygen from the product. Canned or vacuum packaged vegetables and meats are of larger concern because the oxygen within the food is depleted. Similarly, packaging

in air-tight containers can result in depletion of available oxygen and increase in CO_2. In a particularly unique outbreak, foil used to wrap baked potatoes prevented oxygen diffusion, allowing for the growth of the obligate anaerobe *C. botulinum* and unfortunately resulting in several cases of botulism (Angulo et al. 1998). An alternative to vacuum packaged foods is modified atmosphere packaging. A vacuum is used to remove air from the package and then specific concentrations of oxygen, CO_2, and nitrogen are injected into the package before sealing (Jay 2009). High levels of oxygen (70%) in packaging for meats and fruits are associated with lower levels of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* (Vermeulen et al. 2013). Modified atmosphere packaging can be used to control microbial growth by considering growth requirements of certain pathogens.

5.2.6 Multiple Stresses in Food Leads to Cross-Protection

When microorganisms are exposed to certain stressors, they can alter their gene expression in response to improve their ability to grow or survive. Bacterial responses to many types of single stressors, such as osmotic or acid stress, are associated with resistance to other types of stresses, like heat or starvation, for example. This method of acquired resistance is called cross-tolerance or cross-protection. When changes to the food or surrounding environment do not induce lethality, the microorganisms may adapt in a variety of ways. When changes are subtle or gradual, the cells may be able to maintain homeostasis by adjusting cellular components and metabolic functions that make up for the new stress (Burgess et al. 2016). Cross-protection is observed when the same protein is used to respond to multiple stresses (Begley and Hill 2015).

Temperature and osmotic stress events are well studied and linked as cross-protectants for many microorganisms under certain circumstances. For example, the cold shock proteins expressed by *L. monocytogenes* during cold stress are the same as those expressed under osmotic stress (Capozzi et al. 2009). Another study noticed that *L. monocytogenes* developed significant heat resistance after exposure to osmotic stress (Burgess et al. 2016). A connection between osmotic stress and temperature was observed when *L. monocytogenes* on fresh produce displayed resistance to osmotic stress when stored for five days at low temperatures and deprived of nutrients (Poimenidou et al. 2016). Adaptation to osmotic stress by *L. monocytogenes* was also seen to promote resistance to bile stress, which could suggest that exposure to certain stresses may consequently increase the pathogenicity of some food pathogens (Begley and Hill 2015).

A reduction in pH, either through biological activity or through addition of acid is a commonly encountered stress for pathogens. While human bacterial pathogens do not actively grow at low pH, they express proteins that promote survival. Acid-adapted cells of *S. enterica* ser. Typhimurium (6 N HCl for 4 hours) resulted in much higher levels of survival in milk fermented with lactic acid bacteria, as opposed to non-acid adapted cells. In addition to acid resistance, the pathogen also displayed

resistance to the cold stress and antimicrobial compounds inherent to the milk and those made by the lactic acid bacteria, such as bacteriocins, peroxidases, ethanol, and diacetyl (Shen et al. 2007). Another study noted connections of acid resistance and cold stress when *S. enterica* ser. Typhimurium cells displayed resistance to acid stress following exposure to low temperatures (Shah et al. 2013). In some cases, acid-adaptation can also lead to cross-protection resulting in thermal resistance. For example, acid-adapted *E. coli* O157:H7 in brined ground beef developed thermal resistance during pan broiling, requiring additional cooking time to result in adequate reductions (Shen et al. 2011). Additionally, acid-adapted *Salmonella* and *E. coli* O157:H7 exhibited thermal tolerance in cantaloupe and watermelon juice (56 and 57 °C), but cross-protection was not noted for *L. monocytogenes* (Sharma et al. 2005).

Some adaptations to desiccation lead to resistance and cross-protection from other challenges posed by the environment. For example, after having been in a desiccated state, various serovars of *Salmonella* showed resistance to high salt concentrations, bile salts, UV irradiation, dry heat, ethanol, bleach, dodecyl dimethyl ammonium chloride, and hydrogen peroxide. However, the bacteria showed some vulnerabilities to organic acids (Begley and Hill 2015; Burgess et al. 2016; Gruzdev et al. 2012). Desiccation of foods is often used as a processing control or preservation method, but there are many observed accounts of increased thermal resistance of *Salmonella* serovars in low water activity foods, such as dried milk powder, flour, cocoa, etc. (Burgess et al. 2016). These particular findings lead to concerns about low water activity foods that are minimally processed by low heat treatments, since desiccation and osmotic stress are often used to control foodborne pathogens (Capozzi et al. 2009).

Understanding these cross-protection events is essential to food processing and safety. To preserve certain sensory qualities of foods, minimal processing has become a popular trend. However, the use of multiple, less severe interventions (multi-hurdle approach) could allow pathogens to develop cross-protection from sublethal treatments, diminishing treatment effectiveness and allowing pathogens to persist due to acquired resistance. The responses of pathogens to sub-lethal conditions and cross-protection strategies should be considered and further researched.

5.3 Physiological States of Foodborne Bacteria in Foods

Within the food matrix, it is expected that foodborne pathogenic bacteria will be in physiologic states associated with adaptation. Presence of planktonic bacteria is unlikely except in foods with very high nutrient contents and absence of additional stressors. Planktonic cells are potentially more common in liquids. In contrast, on solid foods, bacteria are likely attached and present in colonies of various sizes. This is visualized when mold grows on bread in nonuniform patterns. Similarly, many pathogens are opportunistic and colonize where nutrients are more prevalent, such as the cut stems of produce as discussed above. Bacterial colony growth is determined

by localized concentrations of carbon sources, and diffusion of substrates, which in turn affects growth rates (Jeanson et al. 2015). Micro-gradients of pH, oxygen, and redox potential may additionally influence the growth of attached bacteria in foods. Bacteria in food may also respond by persistence within adaptive physiological states that vary from spores, to biofilms, or a persistent state, referred to as viable but not culturable (VBNC).

5.3.1 Attachment: Surface Type and Mechanisms

Contamination of microorganisms, such as bacteria, yeasts, and molds including those that are pathogenic, usually occurs on the surfaces of foods. To grow or survive on solid food matrices, microorganisms must make efforts to adhere and attach. The first step involves particle forces between the cell surface and the surface of the food, which are nonspecific, reversible interactions. These early, weak attractions, such as van der Waals forces and weak hydrophobic interfaces encourage noncommittal attachment (Burgain et al. 2014; Goulter et al. 2009). Initially, this attachment is based on the characteristics of the cell surface (as influenced by surface proteins, polysaccharides, teichoic acids, etc.) and the physical attraction to a food surface, which is also influenced by temperature, pH, and the food composition. As a second step, committed and stronger covalent and hydrogen bonds follow as irreversible interactions that further the bacteria's ability to attach to a surface (Tan et al. 2016b). This attachment may be followed by the development of a biofilm, as discussed in Sect. 5.3.2.

The surfaces of produce are hostile with low nutrient availability and microorganisms must heavily adapt to survive starvation in this environment. Nevertheless, fresh produce is one of the most commonly implicated foods for foodborne illness globally (Tan et al. 2016b). Microorganisms must strategically attach to the surface, as well as interact with the native microbiota and attain the proper nutrients to survive in that particular environment (Poimenidou et al. 2016; Tan et al. 2016b). Some microorganisms have been observed to adhere to plant surfaces, but damaged or cut tissues offer even greater chances of bacterial attachment, because the cells can more easily bypass the waxy cuticle of the plant surface (Frank 2001). Plant cell walls exposed during minimal processing provide microorganisms with an opportunity to attach and persist on produce. Some carbohydrates that comprise plant cell walls might provide the structure and nutrients for microorganisms to persist in the microenvironment. For example, the combination of pectin, celluloses, and hemicelluloses may be balanced in such a way that enhances attachment. Pectin, for example, can reduce the porosity of plant cell walls by encasing the cellulose-hemicellulose structure, which can then promote attachment and harborage of pathogens like *Salmonella* (Tan et al. 2016b). The highly hydrophobic nature of *B. cereus* promotes stronger binding to lettuce surfaces compared to cabbage surfaces, and adherence is further strengthened by the length of attachment (Elhariry 2011). Attachment of *Salmonella* to cabbage and lettuce leaves also increases with

time (Patel and Sharma 2010). For *E. coli* O157:H7, the overall hydrophobicity and charge of the cell wall did not influence the ability to adhere to iceberg lettuce, but hydrophobicity and charge were seen to impact adherence of *E. coli* (O157:H7 and non-O157:H7), *Salmonella*, and *L. monocytogenes* on cantaloupe rinds (Boyer et al. 2007; Ukuku and Fett 2002). These examples highlight how differences in cell properties such as hydrophobicity, charge and roughness of a surface may impact the interaction between bacteria and food surfaces.

Meats also present a unique surface for attachment and survival. Contamination usually occurs when microorganisms pass from the carcass to the freshly fabricated meat. Bacterial attachment to meat surfaces may also occur within a thin water film that exists on the surface of meats, especially on poultry skin. The cells are generally free-floating, but they may attach more permanently if they are not washed off with a surface treatment (Frank 2001). Through microscopy, cells have been observed to gather in the ridges of poultry skin after dunking in water by overcoming the hydrophobic repelling forces of the surface using van der Waals attractions (Frank 2001). Many bacteria express membrane binding proteins or surface structures that could aid in attachment to the muscle, fat, or skin (Selgas et al. 1993). There is some uncertainty, however, as to whether or not the microorganisms are more or less attached or are entrapped by the fibrous networks of the meat tissue (Lillard 1984). Whether pathogens are either attached or entrapped, they can be extremely difficult to remove (Frank 2001).

Inanimate surfaces, specifically food contact surfaces, also pose unique attachment challenges for microorganisms. Microorganisms interact with both the chemical and physical properties of the abiotic surfaces. For example, less colonization of *E. coli* cells was seen on copper surfaces than stainless steel. This was somewhat due to the antimicrobial properties of copper, but also due to enhanced attachment to hydrophilic surfaces like rubber and plastic than stainless steel (Goulter et al. 2009). Additionally, the time bacteria remain attached to a food-contact surface may impact their abilities to survive on actual foods if presented with a contamination opportunity. *L. monocytogenes* is known to easily attach to stainless steel food contact surfaces and be transferred to food surfaces where the attachment is mediated because the charged teichoic acids in the peptidoglycan cell wall create a hydrophobic cell surface that promotes binding to hydrophilic surfaces. The presence of lactic acid, a common organic acid often found in food environments or in foods, has even been shown to strengthen this attachment (Briandet et al. 1999; Bridier et al. 2015).

The physical interaction between microorganisms and solid food matrices depends on the abilities of the microorganisms to adhere to the surfaces mediated in part by non-specific interactions, but bacteria also produce specialized appendages that can be used for attachment. Fimbriae, pili, and flagella are examples of structures known to promote binding to solid surfaces. These structures play key roles in cell-to-cell interactions. Pili and fimbriae use adhesin proteins to attach to surfaces, as well as to one another for conjugation. Thin aggregative fimbriae have been studied in *S. enterica* ser. Enteritidis, and it has been noted that several types are involved in cell clumping and attachment to surfaces like stainless steel, a common food-contact surface (Austin et al. 1998). Other surface structures, such as curli,

have been studied for their involvement in cell-to-cell interactions and surface attachments. When compared directly to motile flagella, it was shown that curli played a more vital role in attachment to swarm plates than the flagella in *E. coli* K12 (Prigent-Combaret et al. 2000). Further, curli were crucial for cells to form layered clusters on the abiotic surfaces. On intact lettuce surfaces, however, curli were not seen to be a significant factor in the attachment of *E. coli* O157:H7 (Boyer et al. 2007).

Flagella can be used by microorganisms for motility, but also to interact with other cells and surfaces (Bridier et al. 2015). For some time, it was believed that motile cells attached to surfaces at higher rates and efficiencies, however, other studies indicated that attachment was not correlated with motility appendages, like flagella. Rather, these surface appendages play a role in attachment but are not necessarily the most important factor in surface attachment (Selgas et al. 1993; Tan et al. 2016a). In one study, flagellated and non-flagellated microorganisms (*S. enterica* ser. Typhimurium and Gallinarum, *Proteus vulgaris, Pseudomonas fluorescens, C. perfringens, S. aureus* and *Micrococcus*) displayed no differences in adherence capabilities to poultry skin (Lillard 1984). Still, one study examining *S. enterica* ser. Typhimurium determined flagella to be a more important surface structure involved in attachment to plant cell walls compared to fimbriae (Tan et al. 2016a).

While there are a variety of studies that investigate whether or not the surface structures or motility appendages of all microorganisms always aid in attachment to food and food-contact surfaces, these appendages have been correlated in a number of laboratory experiments, and the theories of attachment and persistence on food matrix surfaces, as well as food preparation surfaces, warrant further investigation.

5.3.2 Biofilms

Biofilms, or "microbial communities encased in a self-produced protective extracellular matrix composed of polysaccharides, proteins, and/or extracellular DNA" present food processing plants with many challenges from safety and quality standpoints (Fagerlund et al. 2017). Essentially a safe haven for microorganisms, biofilms provide protection, nutrients, and opportunities for genetic material transfer. The biofilm is constructed of biopolymers and water and provide a matrix onto which microorganisms may adhere and resist various types of interventions and treatments (Bridier et al. 2015). The first step in biofilm development is attachment to a surface, where the bacteria begin secreting exopolysaccharides that strengthen the attachment and encase the cells within a protective matrix. Foodborne pathogens are often recruited into biofilms in the maturation stage of development as opposed to initiating the biofilm formation. Biofilms can enhance survival and may be formed in response to a stressor. In one example, it was shown that *B. cereus* biofilm formation was heightened by contrived starvation of nutrients in an *in vitro* lab experiment (Elhariry 2011).

Many different species typically make up a biofilm, and it has been surmised that multispecies interactions within a biofilm increase survivability and increase foodborne pathogens' abilities to contaminate the food system (Srey et al. 2013). Biofilms provide arenas for cooperative growth and survival of pathogens. A number of foodborne pathogenic bacteria have been demonstrated to form biofilms including members of the genera *Bacillus, Campylobacter, Escherichia*, *Salmonella*, *Shigella*, *Staphylococcus, Yersinia* (Bridier et al. 2015). Others, including *Listeria* and *Vibrio* spp., have been isolated from biofilms within food processing plants. Biofilms containing foodborne pathogens have been detected in facilities used for dairy, egg, meat, seafood, and vegetable processing. It is suspected that *L. monocytogenes* biofilm on equipment used for washing cantaloupes provided the inoculum leading to internalization in cantaloupes associated with a large multistate outbreak (Srey et al. 2013). The majority of studies investigate biofilm formation in food processing environments and on food preparation surfaces; however, there are a number of examples that indicate the same activities on the actual surfaces of foods, and some postulate that biofilms on the food surfaces may be the culprits of some outbreaks (Sapers et al. 2000; Fransisca et al. 2011). *E. coli* O157:H7 within biofilms have been detected on radish sprout tissues even after a chlorine wash (Fransisca et al. 2011). Similarly, *S. enterica* serovars Tennessee and Thompson formed biofilms on cabbage and lettuce leaves (Patel and Sharma 2010).

Bacteria within biofilms may be more resistant to stresses encountered in the food processing environment including desiccation, exposure to antimicrobial compounds, and disinfectants. Several studies have noted that biofilm persistence and resistance to chemical treatments increase as the number of species within the biofilm increases, possibly due to the exchange of DNA and proteins through appendages (Bridier et al. 2015; Dubey and Ben-Yehuda 2011; Burmølle et al. 2006; Srey et al. 2013). This interaction between bacterial species within a biofilm was noted in one study where *E. coli* and *S. enterica* ser. Typhimurium shared proteins that are known for forming curli, an appendage known to aid attachment (Zhou et al. 2012). Resistance to disinfectants increased for *L. monocytogenes* and *S.* Typhimurium when biofilms developed on cantaloupe rinds after 2 and 12 hours, respectively. In this biofilm state, researchers were unable to achieve even a 1-log reduction of *L. monocytogenes* at the highest concentration (2000 μg/mL) of disinfectant using a biofilm formed in 2 hours (Fu et al. 2017). Furthermore, *Salmonella* present in biofilms on dried black peppercorns were more resistant to vacuum-steam pasteurization, requiring longer treatment times that resulted in a 5-log reduction (Newkirk et al. 2018). In dried milk powder, larger numbers of *S. enterica* ser. Tennessee were recovered by plate count analyses when cells were in a biofilm state compared to planktonic cells (Aviles et al. 2013). This enhanced survival was correlated with increased expression of stress response genes. There is increasing evidence that cross-protection-associated stress response genes are expressed in *Salmonella* and *E. coli*, which could be due to shared information if the organisms coexist in a biofilm (Hamilton et al. 2009; Schembri et al. 2003). Several studies have also noticed increased expression of genes in biofilms that are associated with

virulence (Aviles et al. 2013; di Ciccio et al. 2015). This suggests mitigating biofilm formation on foods and food contact surfaces could be important for reducing overall number of illnesses.

5.3.3 *Viable but Nonculturable and Spores*

Physiological state plays an important role in growth and survival within food matrices. Adaptive stress responses often include the transition of vegetative cells into other physiological states in which all metabolic activities are diverted to necessary processes for survival rather than further growth. Viable but nonculturable (VBNC) states and formation of spores are two of the most common and effective physiological states for extended survival in stressful environments. In the spore state, the bacteria can remain dormant for many years, and upon encountering favorable conditions, germinates to form a vegetative cell and can then produce toxins that lead to illness. Examples of foodborne bacterial spore-formers that cause disease are *B. cereus*, *C. perfringens* and *C. botulinum*, which survive process interventions due to the hardiness of the endospore (Ryu and Beuchat 2005; Logan 2011). Spores are regularly detected in milk, dried spices, rice, and multi-step prepared foods (Soni et al. 2016). VBNC cells have been observed across many different bacteria, both Gram-positive and -negative. This state is characterized as cells that are in a dormant physiological state, as well as those that are metabolically active but unable to be cultured. In this state, the bacteria are very much alive, typically meeting the minimum metabolic needs for survival, but may experience morphological changes. *Campylobacter* spp., for example, has been well characterized to enter the VBNC state, changing its morphology from its famous spiral shape (Thomas et al. 2002) to coccoid when entering a VBNC state as a response to stress (Burgess et al. 2016; Baker et al. 2016).

Exposure to stresses common in food processing environments, including desiccation, high pressure, high temperature, disinfectants, and low temperature storage has been demonstrated to induce VBNC in several foodborne pathogens (Zhao et al. 2017). Many foodborne bacteria such as *Salmonella*, *Campylobacter, Vibrio* and *E. coli* have been shown to enter VBNC states, which indicates metabolic activity without the ability to be cultured, or extensive injury that prevents metabolic activity, and thus culturability. In either case, certain metabolic functions are redirected, focusing on those necessary for survival. VBNC foodborne pathogens have been detected in grapefruit juice (Nicolò et al. 2011), milk (Gunasekera et al. 2002), infant formula (Barron and Forsythe 2007), salted squid (Morishige et al. 2017) and vegetables (Dinu and Bach 2011). It has been suggested that *E. coli* O157 at low infectious doses classified as VBNC was also recovered from salted salmon roe (eggs) that was associated with an outbreak in Japan, and it has been hypothesized that some of the cells responsible for an outbreak of *E. coli* O104:H4 in fenugreek sprouts in Germany were VBNC from copper ion or tap water stress (Makino et al. 2000; Eriksson de Rezende et al. 2001; Zhao et al. 2017; Aurass et al. 2011). Low

infectious doses of *S. enterica* ser. Oranienburg associated with consumption of dried squid are also hypothesized to be due to low culturability from squid due to VBNC state. Investigators confirmed that the strain could enter to a VBNC state in the lab at a salinity comparable to dried squid (Asakura et al. 2002). Induction of a VBNC state in *S. enterica* ser. Tennessee by prolonged exposure to desiccation (a_w=0.5) in dried milk powder stored for 28 days was recently described (Aviles et al. 2013). Culturability decreased, however molecular analysis indicated no changes to the log copies of 16S rDNA and stress response genes, indicating the cells were still metabolically active. Moreover, the culturability of the cells returned after challenge with simulated gastric fluid and log CFU/g increased within simulated intestinal fluid, indicating these cells remained virulent. Other studies have noted the ability of VBNC cells to maintain virulence. Infectivity of VBNC cells of *L. monocytogenes* and *Vibrio* spp. have been demonstrated within animal model systems (Oliver 2010). The true burden of VBNC cells associated with foodborne illnesses is unclear but the growing body of evidence of their presence warrants research to examine the interactions of these VBNC cells with the matrix and development of new resuscitation mechanisms.

Some foodborne Gram-positive bacteria such as *Clostridium* and *Bacillus* spp. pose threats to the food industry due to their abilities to form endospores, a dormant non-reproductive structure, that may survive pasteurization processes and persist until conditions become favorable for germination and growth in the food (Logan 2011). Pathogenic spore-formers have been detected in a variety of foods including dairy, freeze-dried meats, spices, fruits, and vegetables (Chitrakar et al. 2018). The spores are likely transferred to the food ingredients through contact with soil or air. Members of the *Bacillus* and *Clostridium* genera are common soil bacteria, where spore formation in response to low nutrient, desiccation, and other stresses enhance fitness. Initial contamination of raw milk and survival during pasteurization has been reported as a major factor for the occurrence of *Bacillus* spores in dairy products (milk, cheese) (Burgess et al. 2010). *B. cereus* spores from the environment may survive in dried milk and germinate when the milk powder is rehydrated, creating a highly nutritious environment for the new vegetative cells to survive and produce enterotoxin that may lead to foodborne illness. Spore persistence within food processing facilities is well documented, where they may become embedded in biofilms, increasing the resistance of the spores to disinfectants (Ryu and Beuchat 2005). It may also be possible that stresses experienced in foods may lead to sporulation. Enhanced thermotolerance has been observed in strains of *B. cereus* subjected to sublethal salt stress due in part to induction of spore formation at the end of the growth phase. These spores resulted in small subpopulations that were highly resistant to the thermal treatment (den Besten et al. 2006). *C. perfringens*, another sporeformer, sporulates within the gastrointestinal tract when contaminated food is consumed, thus causing disease through the production of enterotoxin (Li et al. 2016). *C. perfringens* spores may form in the environment prior to contaminating the food, or within the food if the microenvironment is hostile.

As microorganisms experience stress in their immediate environment, many make adjustments that may include entering a different physiological state. These physiological states, such as VBNC and sporulated cells, may increase the chances for survival. Both of the discussed physiological states should be better understood, as they can pose great threat to the food industry by overcoming what may be assumed to be a lethal treatment or environment.

5.4 Conclusions and Future Trends

The interactions between foodborne pathogens and the food matrix are exceedingly complex. The physiological state of bacteria and its responses to the stresses within the food matrix are variable, dependent on the physical nature of food, diffusion of nutrients, and metabolic end-products. Diffusion limitations and micro-gradients within colonies may influence microbial growth in foods (Jeanson et al. 2015). Furthermore, interactions between bacteria in foods may be impacted by metabolic capabilities of the microbes, population density, and diffusibility in foods. There are many examples of both collaborative and competitive actions that take place between different species, and these relationships add to the intricacies of the observed responses of microorganisms to stress, as well as the effects seen on food matrices. The responses and reactions of microorganisms to their food matrix might be a generic strategy that many microorganisms possess or strategies that may be specialized to a particular species.

Although many laboratory studies examine bacterial growth and stress responses, the majority utilize simplified matrixes of growth media that do not always fully represent food. Future research examining bacterial growth and stress responses of foodborne pathogens within model food systems are essential for predictive modeling of bacterial growth in complex food systems. Development of microelectrodes that can measure pH, osmotic pressure, and redox potential will be key tools to understanding the dynamic environment of foods. Metagenomics and metabolomics are additional tools that can be used to determine how the microbial community members and the metabolic activity of bacteria in foods develop over time. Technologies including qPCR and microarrays allow for monitoring responses of specific foodborne pathogens. However, the quality of nucleic acids extracted from food systems can be affected by the natural constituents of the food (Ercolani 2013). Poor quality nucleic acids due to presence of lipids, proteins, salts, and some carbohydrates, all key constituents of foods, may inhibit PCR amplifications prompting the necessity of optimizing nucleic acid extraction protocols based on the food matrix and target microorganisms (Quigley et al. 2012). Future advancements in technologies, bioinformatics pipelines, and cost-reductions will allow for broader applications of metagenomics in food systems. An increased awareness of food matrix and pathogen interactions and the use of new technologies will contribute to the characterization of the dynamic nature of food as an environment.

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Part II
Preventive Practices

Chapter 6
Good Manufacturing Practices and Other Programs in Support of the Food Safety System

Martin Bucknavage and Jonathan A. Campbell

6.1 Introduction

In order to produce safe, wholesome food, a food company must develop and enact several programs, policies, and procedures. These programs, policies and procedures become integral components of the company's food safety system. Good manufacturing practices or GMPs set the foundation for all types of food safety systems. GMPs are broad, general statements that describe activities that develop the basis for the minimum regulatory requirements of food safety. These types of statements often lead to more focused procedures that describe an activity in more detail. Figure 6.1 shows an example of a broad set of GMPs for employees to be trained on.

GMPs and these detailed programs are a major component of the entire food safety system. Although GMPs encompass the requirements that government regulatory agencies can enforce, a food processing facility's food safety program must rise above the limited scope of GMPs and include broader aspects of control.

An important component of GMPs is controlling the process, which is covered by a company's HACCP program, or Hazard Analysis Critical Control Point, which will be covered in the next chapters. Along with this system are several other programs that focus on various elements of an operation including the food processing environment and personnel. While the company's HACCP program will focus on hazards with greatest risk, these other programs focus on hazards that pose lower risk, but still can make food unsafe if these programs are not properly developed and implemented.

When discussing programs regarding HACCP, these programs are termed prerequisite programs. The term prerequisite program refers to the fact that they must be

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Establishment Name
Establishment Address
Establishment City, State, Zip Code

Date
Establishment Number

Good Manufacturing Practices
For Personnel

- Personnel should not be or become sources of food contamination.
- Proper personal hygiene is required.
- Employees will not be allowed in areas where food is handled if they are found to show evidence of communicable disease.
- Hands are to be thoroughly washed with soap and water, dried, and disinfected when handling food products. If handling other items such as pallets, boxes, etc., hands must be washed and sanitized again before handling food products. If wearing gloves, these must be removed and disposed of and hands must be washed and sanitized again before wearing a new pair of gloves.
- Nail polish and unclean fingernails are prohibited.
- Work clothing must be maintained in a clean, orderly, and sanitized condition. Employees must wear a clean frock or apron every day. Frocks and aprons should be changed as often as necessary if soiled. Frocks and aprons are to be worn in food processing areas only; they are not to be worn in toilet areas, eating areas, or any other non-processing areas.
- Employees must wear appropriate hair restraints (i.e. hair net, beard nets) to prevent hair from contaminating the food products.
- When manufacturing processed products, it is prohibited to wear earrings, badges, rings, watches, bracelets, and/or other jewelry that may fall into the food product. Single post pierced earrings are an exception with tape (only 30 days after piercing). Plain rings are allowed if clean disposable plastic gloves are worn.
- Smoking is prohibited in all areas.
- Spitting is strictly prohibited in all areas.
- Eating is prohibited in all food processing areas.
- Rubber boots and footwear must be kept clean.

Date: _____ Approved by: _____

Fig. 6.1 Good manufacturing practices for personnel

operational as a pre-condition for HACCP. In essence, these programs serve as a building block or foundation for higher level of control. This phrase "prerequisite program" was developed by Agriculture and Agri-Food Canada (AAFC) during the development and implementation of HACCP in the mid 1990's and is now commonly used in the food manufacturing industry to describe the basic structure of the procedures and programs that support food safety during every aspect of the production. Many of the prerequisite programs established in the United States are

created as a foundation to support not only the basic regulatory requirements for the safe food production but also to support the quality of the food produced.

6.2 cGMPs as Part of the Food Safety System

One of the key components of these programs are the current Good Manufacturing Practices, or cGMPs. GMP's are covered under US federal regulations (21 CFR 117 Subpart B) and form the minimum food safety standards that must be in place within a food operation. The GMPs cover the major facets of a food operation including personnel, plant and grounds, sanitary operations and facilities, equipment and utensils, basic processing controls, and warehouse and distribution. The word 'current' is included within the official title and reflects the importance that the companies need to use current technologies and systems to meet the regulations.

The GMPs are not prescriptive in terms of how the company must meet the standard, so it is up to the company to develop the specific programs and procedures in order to meet the established standards. Often a company's programs and procedures will go beyond what is required within the regulation. A company's procedures will incorporate particulars of their own facility or may address higher standards imposed on them by a customer or a third-party auditor. As an example, the GMP section on Personnel (21CFR117Subpart B Sec 117.10) requires hair restraint that could include the use of hair nets, headbands, caps, and beard covers. Facilities will determine where in their facility, hair restraints must be worn and the type of hair restraint that must be used.

6.3 Requirements for Prerequisite Programs

The minimum requirements for prerequisite programs are established within the federal regulations for food processing companies in the United States. Companies must, at a minimum, meet these regulations. For higher levels of control, companies seek to adopt a best practices approach, often found within third party audit standards.

The primary regulatory requirement for the prerequisite programs are the *cGMPs* or the "current Good Manufacturing Practices", which require sanitary standards and controls for personnel, equipment, and facilities, as well as for processing operations and warehousing (21 CFR 117). Good Manufacturing Practices are codified within the federal regulations in Title 21, Part 117 subpart B and when written, provide a broad and general overview for items that could impact food safety. GMPs are often written in very plain language that is easy to understand.

In general, federal regulations require that food production facilities meet the minimum standards, but with few exceptions, there is no explicit requirement to have formalized programs with written procedures and documentation. With this

stated, it is important to note that documentation would be needed to demonstrate compliance, and therefore most companies have these procedures and processes in written or printed form. One stated requirement within the regulation is that employees are trained in those aspects of GMPs that would be required for their work within a food processing facility. Having written programs may assist in meeting the goal of training personnel.

More explicit requirements for documentation are found within USDA's *Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems; Final Rule* (USDA-FSIS 1996). In the final ruling, companies regulated by the Food Safety & Inspection Service (FSIS) are required to have written standard operating procedures for items related to sanitation. These written programs are often referred to as "SSOPs". The details of HACCP and SSOPs are covered in other chapters in this book. Another example requiring more formalized procedures at FSIS regulated establishments is known as "The Listeria Rule". This regulation requires that written procedures be implemented to control *Listeria monocytogenes* as a hazard in ready-to-eat (RTE) foods. This is especially true for foods exposed to the food processing environment after a thermal lethality process (9 CFR 430.4).

As part of the Food Safety Modernization Act (FSMA), companies must address the hazards being controlled by prerequisite programs and determine if elements of those programs, specifically sanitation, allergen control, or supplier control, need to be identified as preventive controls. In those cases, a higher level of control with written procedures and documentation will be required, similar to what is required with a critical control point or process preventive control.

Best practices, often identified within third-party audit standards such as those administered through the Global Food Safety Initiative (GFSI), will provide more detailed requirements for prerequisite programs with the need to have written procedures coupled with documented monitoring, corrective action, and verification.

6.4 Establishing and Maintaining Programs

Since many of the food companies wish to be able to show compliance with regulatory and/or audit standards, most would have detailed, written procedures for GMPs, SOPs and SSOPs. This would include not only very broad and general language about personnel, equipment, and facilities, but also very specific procedures to address maintenance and sanitation of the production environment, tools, and equipment, and any other item that could impact the safe production of food. Although writing and implementing prerequisites to meet both the needs of the operation and regulatory statutes are important, being able to verify that the described procedures took place and making a permanent record of the event is paramount for regulatory and audit compliance.

6.4.1 Verification

Verification assists the food processing companies in answering the following question: Are the observed or performed procedures being executed in the correct manner? Verification should be performed at a set interval described in the written procedures to confirm that an activity took place and that it was manifested in the correct way. The frequency of verification is specific to the operation being performed and is typically managed and overseen by an individual other than the employee carrying out the prerequisite procedure. New companies may have an initial frequency of verification that is higher or more frequent than an operation that has a stellar performance record historically or with low employee turnover. Again, what works and is necessary at one manufacturing facility may not be a good fit at another manufacturing facility. A good example of a verification activity is environmental sampling of food processing equipment and surfaces. Results from microbiological sampling of the environment provides the food manufacturer with evidence of whether or not the prescribed sanitation procedures are being performed correctly. Depending on test results and observations by employees, the sanitation procedures may need to be reviewed and/or revised.

6.4.2 Records

Having a written record of any procedure provides evidence that may be used for regulatory compliance and audit standards. Records may also be used as historical in-plant evidence to notice trends related to variations in production over time. The written procedure is part of the formal record and should be written by an individual that has great knowledge of the products and processes. In addition to the list of procedures and activities, many establishments also utilize a written log for activities that took place. For example, a written SSOP will describe in detail the procedure utilized to effectively clean a specific area or a piece of equipment step-by-step, including mixing of chemicals and procedures pertinent to the sanitation activity. This procedure becomes part of the written record. A company may also have a written checklist or log to record the result of the sanitation activity. Records must be filled out completely in ink and should be signed and/or initialed and then dated by the individual responsible for the action that took place. The record should provide a real-time assessment of the activity and data should never recorded before or too long after the observation or activity took place. Figure 6.2 shows an example of a food product testing record for the presence of *E. coli* O157:H7. This log form becomes a pertinent evidence for both regulators and purchase partners in the event of a product recall.

As technology becomes more user friendly and cost-effective, companies may utilize electronic means to write and record procedures related to prerequisite

Establishment Name
Establishment Address
Establishment City, State, Zip Code

Date
Establishment Number

***E. coli* O157:H7 Testing Log For Raw, Ground Beef Products**

Products available for testing: Bulk ground beef, ground beef patties

Sample Date	Name of Product Tested	Lot Number	Name of Supplier	Supplier's Lot Number	Location of Held Product	Test Results	Verified By

Date: _____

Approved by: _____

Fig. 6.2 Example *E. coli* O157:H7 test results log

programs. Regulatory requirements and audit standards describe specific actions and operations that must be followed if e-records are utilized. Regulatory agencies will often provide compliance guidelines for use of technology to write, record, keep, and store evidence electronically (USDA-FSIS 2011).

6.5 Establishing Facility Specific Programs

Once broad and general cGMPs have been established, more specific language is utilized to describe various step-by-step procedures that can be used as evidence of regulatory compliance, a means to help train personnel on specific tasks and procedures, or to meet customer requirements as a requisite for trade. The following provides insight into several facility specific programs that are often incorporated into a food production environment.

6.5.1 Personnel, Equipment, and Utensils

Many food companies employ numerous personnel and have a wide array of production equipment and utensils used to make products. It is important for facilities to recognize that both people and equipment may be potential sources of food contaminants, and after considering these risks, develop procedures to ensure that employees and the equipment used to make the food products do not become a source of pathogens or other hazards that may render the food unsafe.

Addressing personal hygiene expectations and training employees are essential to ensure that personnel do not serve as a source for contamination. Many establishments require their employees to wear a minimum outer covering (e.g. hair restraint and lab coat) over clothing, as well as require hand washing before entering production areas. Having a written procedure of what is expected of employees is paramount to success. Of course, employees should receive this information appropriately through training so that the expectation of the employer can be achieved.

Production equipment can be very large and complex, with numerous moving parts and electronics. When purchasing new equipment or repairing or replacing older devices, it is important to consider the design of the equipment and construction materials to ensure that the device can be cleaned and sanitized effectively. Sanitation frequency, as well as preventive maintenance schedules, should be developed in order to maximize the life of the equipment and to ensure that food does not become contaminated through the production process.

6.5.2 Chemical Control

Food operations use a wide variety of different chemicals, everything from lubricants and sanitizers to ingredients, that can pose a food safety risk if they inadvertently contaminate food through improper use, handling, or addition. Establishments must understand which chemicals they use are hazards and how to control the risk. All chemicals used in a facility should be identified and evaluated as part of the Chemical Control program.

Controls for chemicals vary depending upon their use and storage location. Limiting access to chemicals is often accomplished by locking chemicals in storage areas and only allowing access to qualified individuals. Ensuring proper labeling of chemicals especially when chemicals are transferred to other containers or when marking on original containers may not be readily visible. Written procedures for use of chemicals must be in place detailing appropriate handling, application, and removal as well as means of verification that these proper procedures are followed.

6.5.3 Allergen Control

Allergens are food proteins that illicit an abnormal immune response in people who are allergic to them. While people can have allergies to any of a wide number of food proteins, there are eight specific groups where control is required. In 2004, the United States enacted the Food Allergen Labeling and Consumer Protection Act (FALPCA) to ensure that milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, and soybeans were adequately labeled if in the food product (FDA 2005). These allergens become a hazard when the foods have the wrong label or improperly designed label not accurately listing the allergen, or when the product is mis-formulated or is subject to cross contact, inadvertently adding the undeclared allergen to the food. Figure 6.3 shows an example of the major allergens for consideration in the United States. This type of program should encompass ways

Identify Allergens & Other Ingredients of Public Health Concern

The U.S. Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS) and the Food and Drug Administration (FDA) believe there is scientific consensus that the following eight major foods/allergy-related substances can cause serious adverse immunological or allergic reactions in sensitive individuals, due to the proteins in these foods:

- Milk
- Eggs
- Fish (such as bass, flounder, or cod)
- Crustacean shellfish (such as crab, lobster, or shrimp)
- Peanuts
- Tree nuts (such as almonds, pecans, or walnuts)
- Wheat
- Soybeans

Other ingredients that may cause potential adverse reactions in sensitive individuals were identified as monosodium glutamate (MSG), sulfites, lactose, carmine, and Yellow 5 (tartrazine).

Cleaning/Sanitation

Cleaning and sanitizing occurs at the establishment at the end of every production day or as needed during production days as part of a robust food safety program. Not only is this done for food safety purposes, but it is also done for control of allergen ingredients used during the production of some products.

This establishment's cleaning procedures are part of the sanitation procedures and are geared towards "allergen clean." Utensils and containers, if not dedicated to specific allergen products, are thoroughly cleaned between allergen-containing and non-allergen containing products. Equipment is cleaned when changing from an allergen-containing product to non-allergen containing products (when the production schedule requires) and is followed by a thorough visual inspection to ensure all parts of the equipment are accessible to confirm that it is free of contamination. Some equipment may need to be dismantled and manually cleaned to ensure all parts are free of allergen residue.

Date: _____ Approved by: _____

Fig. 6.3 Example allergen control program

to identify allergens entering the company, as well as how to segregate these ingredients in storage. Another important consideration is to declare them appropriately on the product label in plain language that clearly identifies the allergen component.

Establishments handling foods and ingredients should develop an allergen control program to address all facets where allergen misuse can occur. In designing labels, the product formulation must match the listed ingredients, including listing all allergens in simplified terms. At the receipt of the labels from the supplier or printer, allergen listing must again be checked for accuracy. Ingredients containing allergens must also have labels checked at the point of receipt. Establishments often highlight or color code allergens with placards to assist warehouse personnel with proper storage. Within the warehouse, storage of ingredients is done such that any leakage does not get onto different ingredients where that allergen is not a part of.

When allergens are moved from storage areas to processing areas for batching, cross checking of the ingredients against the formulation should be completed to ensure improper ingredients are not used. When rework is produced, or in-process product is held for later use, a labeling system may be utilized to designate the allergens incorporated into the product. A full sanitation process utilizing detergents that can remove allergens is the only way to prevent allergen residuals on equipment. As an establishment changes from one allergen grouping to another allergen grouping or no allergens, a full sanitation process must be completed. Production scheduling can be set to switch from a product not containing an allergen to a similar product that contains an allergen, but not the reverse of this. As finished product is labeled, the formulation of the product within the container must match the applied label.

Establishments verify the control of allergens using a test kit. To test a cleaned surface for residual allergens, swabs are rubbed across the surface and then tested for the allergen using a commercial allergen testing kit. Presence of allergens indicates that the surface was not properly cleaned.

6.5.4 Supply Chain

Food establishments rely on their suppliers to provide them with raw materials that meet their specifications for safety and quality. A company's supply chain program encompasses those policies and procedures that establish the requirements for supplier approval, or which suppliers can be used, and the requirements to monitor that suppliers ongoing ability to supply those materials. The program evaluates the performance of the supplier in meeting regulatory and company issued standards for raw materials, processing procedures, and delivery.

The supply chain process begins with conducting a hazard analysis of the material purchased from the given supplier. The requirements applied to a given supplier are

often adjusted to the risks associated with the material and the status of the supplier. For items with higher risk or for suppliers with an unknown history or a suspect history, there may be more requirements than those with lesser risk. Every item purchased should have a specification in place, and it is important that the specification reflect any hazard where the supplier is expected to control the hazard. For example, if a company is purchasing a cooked meat product and the supplier's process is expected to eliminate pathogens such as pathogenic *Escherichia coli* or *Salmonella*, then that should be appropriately reflected in the specification.

One important requirement may be an audit of the supplier. An audit of a supplier may be performed by an accredited third party. With higher risk items or suppliers, the company may want to perform their own audit in leu of, or in addition to a third-party audit. It is important that trained individuals performing the audit are knowledgeable about the process in which they are auditing and that they can ascertain whether those controls are properly applied.

Enhanced inspection and/or testing of the first lot of material or a trial lot of material can be part of the supplier approval process or for ongoing monitoring. Testing can be done by the supplier if the supplier has appropriate means to conduct the testing and there is an acceptable level of trust of that supplier. Sampling and/or testing can be conducted by a third party. The results of testing by the supplier or by a third party is communicated to the company via a COA (Certificate of Analysis). Whether or not a COA is requested from the supplier, the receiving company may conduct their own sampling and testing. The frequency of testing can be changed overtime as a supplier's material are judged as consistent.

In addition to testing product upon receipt, inspection procedures for evaluating the incoming load of material should be considered. The inspection procedures should assess each shipment for acceptability of lot numbers; any appropriate quality or safety specifications; the condition of boxes or cases; and the physical conditions of the trailer or box truck, including temperature, and other predetermined parameters. If materials arrive in conditions where the shipment is determined to be out of compliance, corrective actions must be taken depending upon the situation. Incoming materials can be refused, quarantined, or accepted depending upon investigation by qualified employees.

Other documentation may be required as part of the supplier approval process or ongoing compliance. The food company should investigate whether their suppliers have had past issues with government compliance (FDA, USDA, or State inspection). The company should also determine the following: Has the supplier had any past recalls? Or Have their operations been suspended at any point? A food company can also request that their supplier furnish any relevant processing records. HACCP plans are other forms of documentation involving the safety of the product. Attention should be given to whether the supplier is actively controlling a specific hazard identified in the hazard analysis.

6.5.5 Receiving

Regardless of the size of the operation, shipping and receiving areas can be very hectic during various moments of operations. Inevitably, scheduling may not go as planned and you have multiple deliveries, shipments or both that need attention at the same time. Therefore, it is important that the flow of traffic, people, and routes to storage and production areas are closely evaluated and have a procedure implemented to avoid a catastrophic break down of the food safety system. The environment for both receiving and storage areas for raw materials must be taken into consideration when planning, implementing, and potentially monitoring the area in consideration. Are raw materials temperature or moisture sensitive? These types of questions will describe the program necessary to be implemented for these specialized areas of the facility.

6.5.6 Sanitation Standard Operating Procedures (SSOPs)

Standard operating procedures describe, in detail, specific tasks that must be performed. For example, how to calibrate a thermometer or break down a piece of equipment for maintenance purposes. Sanitation Standard Operating Procedures, or SSOPs, communicates the detailed procedure that should be performed at a described frequency in specific areas and for specialized equipment in the food production facility. Without sanitation and SSOPs, a sanitary production environment cannot be achieved. More details about SSOPs can be found in another chapter of this book.

Sanitation is the act of both cleaning visible debris from the area or equipment, as well as applying sanitizer to reduce the presence of microorganisms. Food companies should work closely with both sanitation personnel and the chemical suppliers to ensure that areas to be cleaned and sanitized will receive the necessary care and attention that is needed for food safety. Although they are related, SSOPs differ greatly from cGMPs. GMPs may provide a basis or guide to build SSOPs from, but they do not provide the specific details that are needed to describe the sanitation activity in detail, nor the frequency, monitoring, and verification that occurs in order to provide evidence of a sanitary production environment.

6.5.7 Pest Control

Having rodents, flies, or other pests in a food processing environment is not generally accepted in the twenty-first Century because they can potentially impact food safety. Therefore, companies should work very closely with pest control specialists to have the appropriate controls in place for pests specific to a geographic

location and season. It would be inappropriate for a facility to assume that a pest control company knows the difference between food contact and non-food contact surfaces and the type of chemicals allowed for these specific areas of the establishment. Therefore, it is necessary to discuss the pest control company's capabilities and be able to marry those capabilities to the needs of the various areas in the processing environment, along with the suggested frequency of pest mitigation by the pest control company.

The exterior of the facility should also be considered when designing a pest control program. For example, frequently mowing grass and trimming brush, shrubbery, and trees reduces the likelihood that rodents and other pests have an environment that is conducive to being in the area. A company should also consider how often to monitor how well windows and doors are sealed and functioning properly. The frequency and monitoring location and process should be described in detail in the written program.

6.5.8 Food Defense

While most of the prerequisite programs focus on controlling hazards that can be expected in a food operation and unintentionally contaminate food, food defense programs focus on controlling the intentional contamination of food. The reasons why someone may want to commit intentional contamination can vary, from terrorism to acts performed by disgruntled employees, consumers, or competitors.

Food Defense programs focus on vulnerabilities rather than specific types of hazards. Establishments must assess their establishment including employees, facilities, processes, raw materials, and finished product. In many cases, broad based controls may already be in place or can be easily added. These include perimeter fencing and lighting, limited access to facilities or processes, personnel identification systems, and/or supervisory presence.

Taking these broad-based controls into account, the establishment then evaluates its operations for points of vulnerability where a person can contaminate product leading to wide scale harm. Specific operations that have been identified as Key Activity Types are bulk liquid receiving and loading, liquid storage and handling, secondary ingredient handling, mixing, and similar activities. At these steps, establishments would incorporate mitigation strategies, what FDA defines as "risk-based, reasonably appropriate measures that a person knowledgeable about food defense would employ to significantly minimize or prevent significant vulnerabilities identified at actionable process steps, and that are consistent with the current scientific understanding of food defense at the time of the analysis."

Mitigation strategy measures include determining and implementing a means of monitoring for those areas judged as a significant vulnerability, but also procedures for verifying the control is working, documenting the control, and completing corrective action when the measure is not working as intended.

6.5.9 Label Controls

Mislabeling of food products continues to be a major reason for food recalls, most of which are allergen related. In some cases, labels are not properly printed with the requested information, and in other cases, the wrong label is inadvertently applied to the package. Labels should go through a formalized label design process to ensure that the formulation matches the ingredients listed on the label, especially for allergens. Upon receipt of the labels, they must be rechecked to ensure accuracy to what was designed and ordered. At the point of application, appropriate checks should be in place to ensure that the correct label is being applied. This must be done at each label change and each restocking of the labeling apparatus. Creating a record of these activities may help a company with important information about the event during a potential recall event.

6.5.10 Consumer Complaints

Feedback from consumers is an important information source for issues relating to the product. Food establishments need a formalized system for receiving and responding to consumer issues. This included trained employees who know which questions to ask and how to properly respond. A Consumer Complaint Program must also track consumer complaints to identify trends that may be occurring with a given product. With this, the establishment must conduct root cause analysis to determine source of issues to put corrections in place to prevent reoccurrence. Verification that the correction is working along with documentation must also be a part of the system.

6.5.11 Other SOPs Impacting Food Safety

Ultimately, each food operation should identify a team of individuals to write, review, and implement prerequisite programs that support the production of a safe and wholesome food product that is of high-quality standards and is made with consistency and process control. Although the topics mentioned in this section can be applied to most situations, it is important to tailor the needs of each specific prerequisite to the needs of the operation, their customers, and ultimately, the consumers of the product. The goal is to reduce the risks of reasonable and foreseen hazards along the way to promote the production of safe food, while allowing for the entire food safety system to function as intended.

6.6 Integrating Prerequisite Programs

As stated previously, prerequisite programs provide a strong foundation to support the entire food safety system. It is, in fact, the basis for the entire systematic approach to food safety and product quality. During the establishment and implementation of HACCP systems and/or food safety plans (e.g. FSMA Preventive Controls for Human Foods), it is imperative that these foundational programs be written, implemented, and have an adequate training scenario for employees. These topics are covered in detail in the other chapters of this book. In many operations, especially FSIS regulated facilities, prerequisites may be referenced within the hazard decision making process of developing and implementing a HACCP system. If there is not a verifiable record of the prerequisite program that is referenced in the HACCP plan, then appropriate integration of the prerequisite program has not occurred. These basic and important prerequisites to food safety must look very similar in record design and truly serve as an integrated part of the entire food safety plan or system. The processing establishment is not only complying with regulation, but also may be required to integrate these prerequisites as part of a greater certification for global trade.

6.7 Concluding Remarks and Future Trends

Good manufacturing Practices set a strong foundation for food safety, as well as a basis from which to develop more specific systems that a food processing establishment may incorporate into their entire food safety system. As regulatory requirements evolve and new information about food safety hazards are identified, company's food safety plan should be updated. This helps to ensure that all potential hazards have been appropriately identified and controlled, as well as to assist with regulatory compliance. GMPs are just the basis of which many other company specific programs may be developed.

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Chapter 7
Sanitation Standard Operating Procedures (SSOPs)

Kai-Lai Grace Ho and Alex Sandoval

7.1 Introduction

Environmental pathogens such as *Salmonella* spp. and *Listeria monocytogenes* are major food safety hazards for many ready-to-eat (RTE) products and minimally processed foods that are exposed to the processing environment prior to packaging (FDA 2017a; NFI 2018). Sanitary facilities and equipment are essential to significantly minimize hazards from contaminating foods (Tompkin et al. 1999; NFI 2018).

Foodborne pathogens can enter a facility on raw materials. Clean and sanitary equipment can minimize the transfer of these pathogens from raw ingredients to finished products. Improper sanitation of equipment can potentially introduce hazardous contamination to food and enhance pathogens harborage in the food-processing environment. Lack of effective sanitation has contributed to major recalls (NFI 2018; Grasso et al. 2015; FDA 2017b, 4,5). The *Preventive Controls for Human Food* regulation issued by the Food and Drug Administration (FDA) in 2011 (USGPO 2011) listed sanitation as one of the preventive controls for food processors to implement in their facility to significantly minimize and prevent hazards such as environmental pathogens, biological hazards due to employees handling, and food allergen hazards (DHHS FDA 2019). Sanitation must be monitored. The term *monitor* is defined as "to conduct a planned sequence of observations or measurements to assess whether controls are operating as intended" in the *Preventive Controls for Human Food* regulation (DHHS FDA 2019; USGPO 2011).

Sanitation Standard Operating Procedures (SSOPs) are detailed procedures specifying what to clean, how to clean, how frequently to clean, and what records to use for monitoring the cleaning procedures. The objective of having SSOPs is to ensure

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consistency in the cleaning procedures thus preventing problems and possible issues that might affect food safety during sanitation. This is a requirement of Good Manufacturing Practices (GMP), which is covered in Chap. 6. SSOPs can be written for the cleaning process of direct food-contact surfaces such as utensils, conveyors, and equipment, and non-food contact surfaces such as doors, walls, floors, drains, evaporators, ceilings, and light covers. In this chapter we are going to describe in details with illustrated examples the steps for developing a SSOP, the essential elements of a generic SSOP specifically for fresh produce operations, master sanitation schedule, and monitoring records like forms for daily sanitation log, chemical titration log, and pre-operational inspection.

7.2 Steps for Developing a SSOP

Sanitation Standard Operating Procedures (SSOPs) as defined by the United States Department of Agriculture (USDA) are written procedures that an establishment develops and implements to prevent direct contamination or adulteration of products (FSIS 1997). A SSOP is a step-by-step document and a systematic approach shall be adopted in developing a SSOP thus ensuring that the goal of the procedure is clearly identified. Conducting interviews with employees that are completing the procedures daily and observing them in action are normal parts of any SSOPs creating process. This team approach shall aid in identifying possible operational barriers to the procedure. Poorly written or inaccurate SSOPs would lead to organizational failures, accidents, and have adverse impacts on the employees, equipment, and the environment.

Use clear and concise language in writing SSOPs to ensure that the final document is easy to read and follow. Simple and direct description of terms and procedures are the most effective way of bringing the instructions across to the end users. Ambiguous directions or long instructions can be difficult to follow correctly thus impeding the effectiveness of the SSOPs. In general, demonstrating critical steps or steps that are hard to describe with diagrams, flow charts, and/or photographs make it easier to read, understand, and grasp. Often the sanitation employees may not be native English speakers. Translation of the SSOPs into their first speaking language facilitates their training and thus enhancing their understanding of the procedures and proper usage of chemicals. This will in turn promotes employee's safety and reduces food safety risks.

After the completion of the draft of the SSOP, it is best to test the draft for accuracy. Initial step is to involve the employees that are implementing the procedure daily to review the draft as they know best about the procedure and could provide great ideas for any improvement opportunities. It is critical to let employees that have NOT performed the procedure before to test out the final draft. This is to avoid employees with prior knowledge to rely on their past experience and knowledge to get through the procedure and not the draft, thus defeating the purpose of testing the clarity and accuracy of the SSOP.

Advisor needs to review and approve the tested and final draft of the SSOP. Prior to implementing the SSOP, a formal training for the affected personnel must be conducted.

7.3 Essential Elements of a SSOP

The essential elements of a SSOP contains, but not limited to, the following sections:

7.3.1 Heading Format of a SSOP

Similar to other SOPs, the heading of a SSOP shall have the title of the SSOP, SSOP identity number, date issue, version, supersedes, approved by personnel name and title, address and logo of the company (DHHS FDA 2019) as illustrated in Table 7.1.

7.3.2 Purpose and Concerns

The reason of performing the procedure is stated clearly in this section to ensure that the operator understands the importance of the cleaning procedure, the consequence of not following the SSOP, and its negative impacts on food safety. Some examples of negative impacts include building up of product, rust, calcium, and other potential contaminants such as bacteria and mold.

7.3.3 Scope

Scope describes where the SSOP will be used and applied. For example, the SSOP will be used for all the drains of the facility or specifically used for the cleaning of the drains in the processing room.

Table 7.1 Heading of a SSOP

Company LOGO	Name of Procedure		Version #:	XXX
			Date Issued:	XXX
	Document ID #:	XXXXXXX	Supersedes:	XXX
	Approved By:	Name, Title	Company Address	

Table 7.2 Chemicals concentration for sanitation procedures

	Example: ABC solution	1–10% by volume
Chemicals Used:	**Sanitizer plus**	1.0–6.1 oz./gallon of water
		Target concentration 82-500 ppm
	Shine plus	3–8% by volume
	Wipes with Sanitizer	Ready for use

7.3.4 Chemicals and Chemical Concentrations

All chemicals required to perform the SSOP with effective concentrations and steps to obtain the optimal application concentration ***MUST*** be clearly stated in the section (Table 7.2) to ensure effective cleaning and employees' safety. Safety Data Sheet (SDS) for each chemical shall be listed in the reference section or attached as an appendix for easy access by employees.

7.3.5 Sanitation Equipment

Sanitation equipment and tools required to perform the SSOP such as high-pressure hose, water nozzle, portable foamer, scouring pads, brushes, squeegees, shovels, plastic liners, etc. need to be listed in the procedure. It is critical to identify the color of the tools that is designated for food contact surfaces and non-food contact surfaces in this section of the SSOP.

7.3.6 Personal Protection Equipment (PPE)

Personal protection equipment (PPE) is required to keep the safety of employees. Minimum PPE requirement follows instructions of SDS. Nonetheless more stringent practices may be implemented by individual companies according to their safety policy. Some examples of PPE are hard hat, hair net, beard net, chemical resistant gloves and sleeves, safety goggles, rain suit, chemical resistant steel-toed boots, and ear protection. Photos like Fig. 7.1 is helpful in illustrating the correct usage of PPE to employees.

7.3.7 Procedures and Photographs Illustration

The procedural instructions to accomplish the task, including identification of tools, chemicals, and specific steps are listed in this section. Photo illustration (Fig. 7.2) especially when disassembling of equipment is required would be very helpful.

Fig. 7.1 Sanitor with personal protection equipment (PPE)

Fig. 7.2 Photo illustrating foaming of wall with detergent and bagging of machines that cannot be wet

7.3.8 Frequency

The frequency (daily, weekly, monthly, semi-annual, and annual) or when the procedure needs to be performed is required in order to keep the equipment and environment sanitary.

7.3.9 Assigned Personnel

This refers to who is responsible for performing the steps listed in the SSOP. For example, the sanitation employees, the sanitation lead, sanitation supervisor, and/or maintenance crew.

7.3.10 Verification

Steps for visual verification by sanitation employees and Quality Assurance shall be listed in this section. Other verification via Adenosine Triphosphate (ATP) and microbial sampling of direct food-contact and non-food contact surfaces shall be briefly mentioned in the SSOP as the detailed procedures are generally described in the Environmental Monitoring Program.

7.3.11 Correction and Corrective Action

Steps to address inadequate cleaning of equipment and the environment (floor, drain, wall, ceiling, evaporators, etc.) as determined by visual inspection, ATP test, and microbiological swabbing verification procedures need to be listed.

7.3.12 References

In the event when other procedures need to be referenced in order to perform the tasks listed in the SSOP then reference shall be added to the SSOP.

7.3.13 History of Review and Modification of SSOP

This section documents all the issued dates and the history of changes and review of the SSOP (Table 7.3).

Table 7.3 Revision History of a SSOP

Revision History			
Version	Reason	Date	Revised by
1	New	XXXX	Name, Title
2	Updated chemical information	YYYY	Name, Title

7.4 Generic Checklist for Creating a New SSOP

Below is a generic checklist and related forms for generating a SSOP. It provides guidelines for writing a new SSOP for the seven steps of sanitation as listed by the United State Department of Agriculture (FSIS 1997).

7.4.1 Generic Checklist for Header and Content

The checklist includes information that needs to be on the header of the new SSOP as described in the Sect. 7.3.1 and the general items to be incorporated in the body of the SSOP (Table 7.4).

7.4.2 Generic Checklist for Step-1 Dry Cleaning (Table 7.5)

In the dry cleaning step, ensure to list the procedures for disassembling and reassembling of equipment. Often locked out equipment may require repowering. For example, a conveyor may need to be re-energized in order to clean the opposite side or a cover may need to be re-installed in order for the machine to run. Ensure that the specific instructions for disassembling and/or Logout Tag Out (LOTO) process (FSIS 1997) are referenced and listed in the SSOP.

7.4.3 Generic Checklist for Step-2 Pre-rinse

The pre-rinse step will be much smoother and efficient if operation is listed in the checklist for housekeeping at the end of the shift. It is critical to ensure that the standard practice of rinsing from the top down and in the direction of product flow is clearly stated in the SSOP of this step (Table 7.6).

Table 7.4 Generic checklist for header and content

	Description of Items	Yes	No
1	Name of company with company's logo.		
2	Name of facility and facility's address.		
3	Equipment, line, or item for the SSOP.		
4	Procedure approved by.		
5	SSOP number, version number, and page number.		
6	Department responsible for performing the tasks.		
7	Responsible personnel required to complete the task.		
8	Frequency.		
9	Chemicals and concentration of each chemical.		
10	Safety precautions or procedures required.		
11	Personal protection equipment (PPE) required to complete the task.		
12	Sanitation equipment required to complete the task.		
13	Procedures (refer to checklist tables for each step)		
14	Forms to be used and record keeping.		
15	Reference(s) and revision history.		

Table 7.5 Generic Checklist for Step 1 Dry Cleaning

	Description of Items	Yes	No
1	List of tools that will be required for the dry-cleaning process (air nozzles, brooms, shovels, etc.).		
2	Will any equipment need to be covered to prevent water damage from Step 2 (electrical panels, motors etc.)? Any need to dry clean or wipe down prior to covering?		
3	Will any equipment need to be disassembled/LOTO during this time? Who is responsible for the disassembling/LOTO process?		
4	Any specific color code you must follow for tools and debris containers?		
5	List steps for dry cleaning. Be specific when writing the procedures and ensure that tools, color code, etc. are included.		
6	Take any picture(s) that may be helpful to describe the dry-cleaning procedures.		

Table 7.6 Generic Checklist for Step-2 Pre-rinse

	Description of Items	Yes	No
1	List of tools that will be required for the pre-rinse process.		
2	Does water need to be set at a specific temperature and pressure?		
3	Is picking up of debris required during pre -rinse?		
4	List steps for the pre-rinse process. Ensure that the standard practice of rinsing from the top down and in the direction of product flow is followed.		
5	Take any picture(s) that may be helpful to describe the pre-rinse procedures.		

7.4.4 Generic Checklist for Step-3 Washing

Compatibility of the chemical with contact surfaces, concentration of chemical, and contact time are critical parameters to be on the checklist for ensuring effective cleaning. In some cases, mechanical force like scrubbing is required for dirt removal and need to mentioned in the SSOP (Table 7.7).

Special note must be included in the SSOP for using new scour pads each time and ensure all cleaning tools are sanitized after the sanitation process to prevent microbiological and allergens cross contamination. Last but not least, ensure that the standard practice of foaming from the bottom up is on the check list.

7.4.5 Generic Checklist for Step-4 Post Rinse (Table 7.8)

Similar to the pre-rinse step, it is critical to ensure that the standard practice of rinsing from the top down and in the direction of product flow is listed on the checklist. In addition, also mention post rinsing the non-food contact surfaces like the walls, floors, and drains prior to the direct food contact surfaces like the conveyors, shaker tables, and dryer baskets to prevent re-contamination through accidental splashing.

Table 7.7 Generic Checklist for Step-3 Washing

	Description of Items	Yes	No
1	List of tools that will be required for the washing process (scrub brushes, scrub pads, scrapers etc.).		
2	List of chemicals and chemical concentration.		
3	Time, location and record keeping of chemical titrations.		
4	Chemical contact time for each location.		
5	Methodology of applying various chemicals (central foaming system, portable foamers, clean in-place (CIP) system etc.).		
6	Does water need to be at a specific temperature and pressure?		
7	List steps for the washing process. Ensure that the standard practice of foaming from the bottom up is followed.		
8	Take any picture(s) that may be helpful to describe the washing procedures.		

Table 7.8 Generic Checklist for Step-4 Post rinse

	Description of Items	Yes	No
1	List of tools that will be required for the post rinse process.		
2	Does water need to be set at a specific temperature and pressure?		
3	Will condensation need to be removed?		
4	List steps for post rinse. Be specific when writing the procedures and ensure that tools, color code, etc. are included.		
5	Take any picture(s) that may be helpful to describe the post rinse procedures.		

7.4.6 Generic Checklist for Step-5 Inspection and Reassembling (Table 7.9)

This step needs to be performed by the sanitation supervisor after the post rinse step is completed. Workers should change uniform for reassembling of equipment and inspection. Checklist needs to include the usage of flashlight for inspection and most importantly listing in details the corrections and corrective actions for tailgating findings of non-conformance.

7.4.7 Generic Checklist for Step-6 Pre-operational (Pre-Op) Inspection (Table 7.10)

This step is performed by the Quality Assurance (QA) after the sanitation lead/supervisor have completed their own inspection.

Checklist needs to include the usage of flashlight for inspection and most importantly listing in details the corrections and correction actions for tailgating findings of non-conformance and failures of ATP verification

Table 7.9 Generic Checklist for Step-5 Inspection

	Description of Items	Yes	No
1	Inspection performs by sanitation supervisor with flashlight.		
2	Reassembling of any equipment, guards or parts that have been disassembled prior to the sanitation process.		
3	Record and report damaged equipment incidents.		
4	List steps for inspection and reassembling of equipment.		
5	Correction for any visual findings (e.g. re-clean, re-rinse)		
6	Repeated regular findings may cause a site to be placed into an area of concern. Update SSOP and provide training to sanitors.		
7	Take any picture(s) for the inspection and reassembling steps.		

Table 7.10 Generic Checklist for Step 6- Pre-Op inspectional

	Description of Items	Yes	No
1	Pre-Op inspection performs by Quality Assurance (QA)		
2	Use flashlight and document visual non-conformance.		
3	Citing standard operation procedure (SOP) for adenosine tri-phosphate (ATP) verification by QA.		
4	List steps for Pre-Op inspection.		
5	Correction for any visual findings and ATP fails.		
6	Repeated regular ATP failings sites must be placed into an area of concern. Incorporate the corrective action into the SSOP as an area of concern with training to the sanitors		
7	Take any picture(s) that may be helpful to describe the inspection procedures.		

Table 7.11 Generic Checklist for Step-7 Sanitize

	Description of Items	Yes	No
1	Methodology of applying the sanitizer(s).		
2	Time, location and record keeping of sanitizer(s) titrations.		
3	Drying of the production and warehouse floor for daily startup.		
4	Storage of sanitation tools such as brooms, brushes, squeegees.		
5	List steps for the sanitizer application process.		
6	Take any picture(s) that may be helpful to describe the washing procedures.		

7.4.8 Generic Checklist for Step-7 Sanitize

This is the final step of the sanitation process and the concentration and type of sanitizer must be listed on the SSOP. In addition, steps required for transitioning from sanitation to operation is also encouraged to be added to the SSOP for on-time smooth transition to production (Table 7.11).

7.5 SSOP Forms

The other components of SSOPs are forms that help to record and monitor the actual implementation of the procedure. Below are the examples of some common forms that are being used in documenting the SSOP.

7.5.1 Daily Sanitation Log

This is a form that record the daily cleaning of each element (equipment and environment) of a processing area in the facility. The type of chemicals used, the employee responsible for carrying out the task, and the review of the supervisor are common features in the form. An example is shown in Fig. 7.3.

7.5.2 Master Sanitation Schedule

Master Sanitation Schedule indicates items that are not cleaned and serviced daily. There are Master Sanitation Schedule software developed by companies to aid in the planning of the annual cleaning program. Fig. 7.4 is an example of a Master Sanitation Schedule form.

Company Logo	Sanitation Daily Cleaning Log		Version # :	X
			Date Issued :	XX/XX/XX
	Document ID #:	XXX-XXX-XXX	Facility Address	
	Approved By:	Name of Director of Sanitation		

Week Of:
Line Number:
Employee: All Sanitation

Items	SSOP Number	Chemical(s) Used	Initial By Shift All Areas ALL SANITATION PERSONNEL MUST INITIAL. NO EXCEPTIONS Sun	Mon	Tue	Wed	Thu	Fri	Sat
Production Tables									
Hand Slicer									
Tran-slicer									
Cutters									
Sorting Conveyor									
Blades									
Incline Conveyor									
Wash tank									
Dryers									
Dryer Baskets									
Walls									
Floors									
Drains									
Supervisor Review: Verify that items on sanitation checklist are completed. Initial and date daily.									

Revision History

Version	Revision	By	Date
1	New	Name	XX/XX/XX

Fig. 7.3 Sanitation Daily Cleaning Log Form

Company LOGO	Name of Form		Version # :	XXX
			Date Issued :	XXX
	Document ID #:	XXXXXXX	Supersedes:	XXX
	Approved By:	Name, Title	Company Address	

JOB DESCRIPTION	SSOP #	Responsibility	Frequency	Wk-1	Wk-2	Wk-3	Wk-4	Wk-5	Wk-6	Wk-7	Wk-8	Wk-9	Wk-10	Wk-11	Wk-12
Condensate Drip Pan	SSOP-002	Sanitation	2 Weeks												
Overhead Pipes/Drop Cords	SSOP-003	Sanitation	4 Weeks												
Ceiling/Lights	SSOP-005	Sanitation	Bi-annually												
Refrigeration Units (2)	SSOP-010	Refrigeration	10 Weeks												
Motor Housing of Ref unit	SSOP-011	Refrigeration	10 Weeks												
Clean behind vending Machines	SSOP-018	Janitorial	Weekly												
Buff and Wax Floors	SSOP-019	Janitorial	4 Weeks												
Stairwells	SSOP-020	Janitorial	Weekly												
Wash area around compactor	SSOP-021	Building	Weekly												
Exterior of Building	SSOP-022	Sanitation	Weekly												
Parking lot	SSOP-023	Sanitation	Weekly												

Fig. 7.4 A selected portion of a Master Sanitation Schedule Form

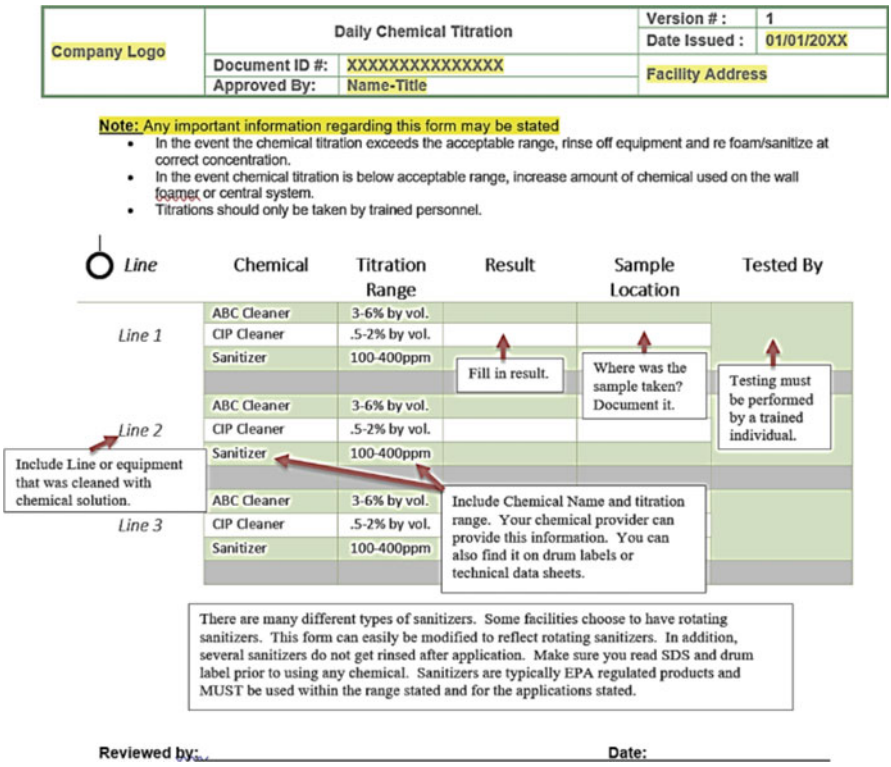

Fig. 7.5 Example of a Daily Chemical Titration Log Form

7.5.3 Daily Chemical Titration Log

Daily Chemical Titration Log (Fig. 7.5) is a form for recording the concentration of each batch of chemicals made daily. This is to ensure that the optimal concentration of the chemical is used for the sanitation procedures.

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7.5.4 Pre-operational Inspection Form

The Pre-operational Inspection Form (Fig. 7.6) is used to document daily observations obtained by the sanitation crew and the Quality Assurance team during the inspection process after the cleaning and rinsing steps. In the event of failing ATP verification swabs and/or visual inspection, corrective actions taken are also recorded.

Company LOGO	Name of Form		Version # :	XXX
			Date Issued :	XXX
	Document ID #:	XXXXXXX	Supersedes:	XXX
	Approved By:	Name, Title	Company Address	

Deficiency:
1- Debris/other
2- Small amount of Food Particles
3- Excessive Amount of Food Particles
4- Missing or damaged parts
5-Idle/Unused Equipment in Area 6-ATP fail

Corrective Action:
1-Swept clean area/picked up debris
2-Clean and sanitized
3- Area Specific Foam, Scrub, Spray
4-Parts/Equip replaced
5- Removed Idle/unused equip
6- Hot water rinse/sanitize
7- rinse/Water

Item #	Action P=Pass F=Fail	Santitation Checks P/F	CAR	QA Check P/F	CAR
Area #1					
1	Dumper 1 (inside, outside, and framework)				
2	Dumper 2 (F Lines) (inside, outside and framework)				
3	Conveyor/trim belt- Top and Bottom				
4	Ceiling Condensation				
5	Hopper (Inside, outside, and framework)				
6	Floor				
7	Drains				
8	Trim Table (A2, B, C, D, E,F) (Top and underneath)				
9	Coring machines and conveyor				
10	Platforms (floor, rails)				
11	Cutting board				
B. Shipping Area					
1	Packout tables and conveyors				
2	Floors and drains				

ATP and Allergen Results

Sample	Sampling sites	ATP Result	Circle Pass/Fail	Retest-Result	Retest-Result
#1			Pass/Fail		
#2			Pass/Fail		
#3			Pass/Fail		

Sanitation/ Production Supervisor Line Approved/Released BY:_____ Date_____ Time_____

QA Line Approved/Released By:_____ Date_____ Time_____

Verification Review Signature: Date Time

Fig. 7.6 Example of a Pre-operational Inspection Form

7.5.5 Sanitor Training Documentation Form

The Sanitor Training Documentation Form (Fig. 7.7) is used to document training conducted for sanitors, especially those newly hired. The training consists of three phases. The first phase focuses on training on the SSOP, the second phase involves hands on training and practices, and the third phase focuses on practical performance test to ensure that the sanitor understands and knows the technique described in the SSOP.

Company's Logo	SANITATION TRAINING FORM		Version # :	X
			Date Issued:	XX/XX/XX
	Document ID#:	XXX-XXX-XXX	Facility Address	
	Approved By:	Name of Director of Sanitation		

Name:_____ SSOP#:_____

In order to successfully complete training all sections listed below must be completed (SSOP Training, Hands on Training, and Performance Test). The date the sanitor has successfully completed the performance test will be the same date reflected on the skills matrix.

SSOP Training

Sanitor completed SSOP training. Sanitor was able to verbally recite the cleaning process to the SSOP being trained against without viewing SSOP. ☐ YES ☐ NO

Trained by: _____ Date Completed_____

Hands On Training

Sanitor worked with trainer and completed hands on training. ☐ YES ☐ NO

Trained by: _____ Date Completed_____

Performance Test

In order to successfully complete the performance test, the SSOP must be followed, no visual finding can be present, and all ATP results must pass on first attempt.

Did the sanitor follow the SSOP? ☐ YES ☐ NO

Did the area and/or equipment pass visual inspection? ☐ YES ☐ NO

Did all ATP swabs pass on the first attempt? ☐ YES ☐ NO

List ATP Locations	Pass/Fail
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Trained by:_____ Date Completed_____

Revision History

Version	Revision	By	Date
1	New	XXXX	XX/XX/XX

Fig. 7.7 Example of Sanitation Training Form

7.5.6 Concluding Remarks and Future Trends

In this chapter, we have shared sample checklists used for developing SSOPs and sample forms that can be used to document and record the implementation of the SSOPs. Considering that there are more than one processing line in a facility, a sanitation manager/supervisor might need to review and file a large number of forms

and logs daily. Data entry by electronic means (such as iPads) rather than manual entry by pens shall enhance the efficiency of the process. In addition, some of the commercially available software programs also allow on-the-spot instantaneous photo documentation and can be customized and programmed for data analysis and key performance indicators (KPIs). This improves the capability of identifying opportunities and areas of concern. The vast amount of information collected daily can be located within a few clicks on the iPad or computer for easy reference. All these changes would enable the collected data to support continuous improvement in lowering food safety risks efficiently.

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Chapter 8
Hazard Analysis and Critical Control Points (HACCP)

Kerri B. Gehring and Rebecca Kirkpatrick

8.1 Introduction

Although not new, the Hazard Analysis and Critical Control Point (HACCP) system is still one of the best tools available for preventing food safety hazards and producing safe food products. The HACCP concept was started around 1959, when the National Aeronautics and Space Administration (NASA), Natick Laboratories, and the Pillsbury Company, joined forces to ensure the safety of food products being developed for consumption by United States astronauts on missions in space (Bauman 1995). NASA was committed to developing food that could be consumed in zero gravity conditions without crumbling. Crumb-free food was necessary to prevent atmospheric contamination and potential damage to spacecraft equipment. Additionally, NASA wanted the food products to be nutritious and free of any contamination by pathogens, toxins, chemicals, or physical food safety hazards that could potentially cause illness or injury to the astronauts while on their space missions (Bauman 1995). The group charged with developing a process to ensure food products sent to space met these requirements determined that a system for controlling food safety hazards was needed to protect the astronauts (Sperber and Stier 2010).

Ensuring safety was not new to NASA, as it already required critical control points (CCPs) to be identified in various engineering and manufacturing processes to ensure safety and reliability of equipment during a mission (Sperber and Stier 2010;

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Table 8.1 Initial HACCP principles

Conduct hazard analysis
Determine critical control points
Establish monitoring procedures

Safe Food Alliance 2019). NASA had borrowed this approach from the Army Laboratories' Failure Modes and Effects Analysis that was used to evaluate the reliability of weapons and ammunition (Hulebak and Schlosser 2002). Therefore, rather than developing something new, the food scientists determined that a similar approach could be applied and that incorporating CCPs into food manufacturing would ensure the safety of the food. The process of identifying and implementing CCPs in the food manufacturing process meant finding and eliminating critical failure areas from the process (Sperber and Stier 2010). This allowed the scientists to identify potential food safety hazards and control the risk of those hazards at critical points throughout the entire food production process. Previous food safety systems relied heavily on evaluating the finished products for hazards. Therefore, preventing contamination throughout the production process was a novel approach for the food industry (Hulebak and Schlosser 2002). This proactive and preventive process control approach became known as the Hazard Analysis and Critical Control Points (HACCP) system (Lytton 2019). The initial concept was based on three principles (Table 8.1). HACCP was so successful at NASA that the Pillsbury Company decided to implement the concept within their food manufacturing process as well.

Due to the success of HACCP at the Pillsbury Company, which is a Minnesota based major food manufacturer, the systematic approach was presented at the 1971 U.S. National Conference of Food Protection (Hulebak and Schlosser 2002; Lytton 2019). Later that year, an outbreak in Westchester County, New York, prompted the recall of over 6,000 cans of vichyssoise and eventually led to a full recall of Bon Vivant canned products, totaling 1.4 million cans. Then, later that same year the Campbell Soup Company recalled 100,000 cans of soup, and Stokely – Van Camp recalled 15,000 cans of green beans after they tested positive for *Clostridium botulinum* (Lytton 2019). These foodborne illness outbreaks put pressure on the Food and Drug Administration (FDA) to reassess the safety of commercially produced low-acid canned products. FDA and the National Canners Association (NCA) developed stricter food safety guidelines for canned food processors. During this same time, Pillsbury established a training program for FDA inspectors to educate them on applying HACCP principles to food production. Although the principles were not specifically identified, the HACCP concept was incorporated into the FDA's low-acid canned foods regulations in 1974 (Lytton 2019; Safe Food Alliance 2019).

Over the years, the utilization of HACCP expanded from its beginnings at NASA for the production of food consumed by astronauts to being implemented in food

Table 8.2 Seven Principles of HACCP

Principle	Definition/Purpose
1. Conduct a Hazard analysis	Identify and list all reasonably likely to occur food safety hazards in the production process
2. Determine critical control points (CCP)	Each step in the process must be evaluated to identify a CCP. A CCP is a point, step, or procedure in the production process where a control can be applied to prevent, eliminate, or reduce a food safety hazard to an acceptable level.
3. Establish critical limits	At each step with an identified CCP, critical limits for the preventative measures associated with the CCP must be established. A critical limit is the maximum or minimum value that the process must maintain to control the identified hazards at a CCP.
4. Establish monitoring procedures	Monitoring procedures for each CCP must be established. Monitoring includes a planned sequence of observations and/or measurements that assess whether or not the CCP is under control. Three objectives: 1. Track control of the process 2. Determine when there is a deviation 3. Provide written documentation for verification procedures
5. Establish corrective actions	Corrective actions must be developed for each process that has an identified CCP. Corrective actions are actions that must be taken if the process deviates from the critical limit determined in principle 3. These actions identify the address the deviation and bring the process back into compliance.
6. Establish recordkeeping and documentation procedures	Recordkeeping procedures allow documentation to be kept for the entire HACCP process. Recordkeeping includes supporting documentation for hazards, CCPs, critical limits, monitoring procedures, corrective actions, and verification procedures. As well as, daily operational records.
7. Establish verification procedures	Verification procedures ensure that the HACCP system in place is working correctly and effectively.

Taken from Food Safety and Inspection Service (2016)

processing facilities across the world. Part of the expansion is credited to the U.S. National Academy of Sciences for its encouragement of using HACCP (National Academy of Science 1985), as well as the formation of the National Advisory Committee on Microbiological Criteria for Foods (NACMCF), which expanded the initial three principles of HACCP to seven (Table 8.2; National Advisory Committee on Microbiological Criteria for Foods 1998) and the adoption of HACCP guidelines by the Codex Alimentarius in 1993.

8.2 The Seven Principles of HACCP

8.2.1 Principle 1: Conduct a Hazard Analysis

Food safety hazards, items which cause illness or injury when consumed, have historically been categorized as physical, chemical, or biological hazards; however, recently other categories such as radiological hazards have been used. Conducting a hazard analysis includes two steps: (1) hazard identification and (2) hazard evaluation. To identify food safety hazards, each processing step is examined to determine if potential hazards are introduced, controlled, enhanced, or reduced to an acceptable level at that specific step of the process. This is frequently referred to as the "brainstorming" portion of the hazard analysis. After potential food safety hazards are identified, then each one is evaluated to determine if it should be addressed in the HACCP plan. When conducting the hazard evaluation, one should consider the "risk" or likely occurrence of the hazard and the "severity" or impact to the consumer, if present. The likelihood of occurrence is usually based on past history, production process, and plant specific data. Severity is often determined by considering the impact on the consumer, including items such as the consequences associated with consumption, duration of the illness or injury, physical impact, or reported illnesses and outbreaks.

Each establishment must conduct a hazard analysis for its specific production process and must be able to support/defend its decisions. While processes might be similar, no two are exactly the same due to the differences in suppliers, employees, equipment, and history, which is why decisions made in a hazard analysis may also be different for two companies making the exact same products. The remaining principles rely on the accuracy of the hazard analysis, so it is important that it is done correctly.

8.2.2 Principle 2: Determine the Critical Control Points (CCPs)

Hazards that are identified as reasonably likely to occur and cause illness or injury if not controlled should be addressed in the HACCP plan. The point or step at which control is applied to prevent, eliminate, or reduce the food safety hazard to an acceptable level is called a critical control point (CCP). A CCP decision tree can be used to help establishments identify which steps are critical control points. CCP decision trees are a series of questions to guide individuals through the process of identifying the CCPs (National Advisory Committee on Microbiological Criteria for Foods 1998). It is important to remember that every food safety hazard that is

identified as reasonably likely to occur must have a CCP somewhere in the process, but it could be at a subsequent step in the process rather than at the step where it is introduced. For example, in a cooked product, pathogens could be identified as reasonably likely to occur in incoming raw ingredients, but the CCP could be at the point of cooking.

8.2.3 Principle 3: Establish Critical Limits

A critical limit is "a maximum and/or minimum value to which a biological, chemical, of physical parameter must be controlled at a CCP to prevent, eliminate, or reduce to an acceptable level the occurrence of a food safety hazard" (National Advisory Committee on Microbiological Criteria for Foods 1998). Critical limits must be scientifically based and appropriate to control the identified hazard. Some critical limits may have one critical parameter, like temperature, while others may have more than one parameters, like time and temperature. Critical limits may be established based on regulatory standards, scientific results published in peer reviewed articles or from plant-specific experiments, or regulatory guidelines. It is important that critical limits set for the purpose of safety and not for quality or operational limits.

8.2.4 Principle 4: Establish Monitoring Procedures

Monitoring includes the measurements or observations used to determine if the critical limits are met. HACCP plans should include the procedures and frequencies for monitoring. When monitoring shows the critical limits are met, then the process is in control. When the critical limits are not met, there is a deviation and corrective actions must be taken. When done correctly, monitoring can also be used to identify trends towards loss of control before control is actually lost. For example, if monitoring temperature, one could notice gradual increase or decrease in temperature over time before the actual critical limit is exceeded. This allows establishments to take measures to keep the process in control before deviations occur and product safety is questioned. Establishments should be able to support the procedures and frequencies for monitoring to ensure that the monitoring takes into account items such as cold or hot spots, and to ensure that the frequency is sufficient to represent all products. It is important to remember that when there is a deviation, the establishment should address product safety back to the last acceptable monitoring activity. Monitoring should be performed by properly trained employees. These employees

must understand the importance of monitoring, the consequences of failing to monitor appropriately, and the procedures to take if monitoring shows a loss of control.

8.2.5 Principle 5: Establish Corrective Actions

A deviation is when the critical limits are not met, and deviations require corrective actions to be taken to prevent customers/consumers from receiving unsafe food. The HACCP plan should include actions that will be taken to determine the appropriate disposition of the product to prevent adulterated on otherwise injurious product from entering commerce. For some deviations, the product can be re-processed to make it safe and other times the product must be disposed of as inedible. Corrective actions should also address the cause of the deviation and establish procedures to prevent the cause of the deviation from happening again. HACCP is a process control tool for food safety, which is why identifying and eliminating the cause of a deviation and taking steps to prevent it from happening again are so important. As with monitoring, only appropriately trained individuals should be allowed to take corrective actions.

8.2.6 Principle 6: Establish Verification Procedures

Verification has been "defined as those activities, other than monitoring, that determine the validity of the HACCP plan and that the system is operating according to the plan" (National Advisory Committee on Microbiological Criteria for Foods 1998). The first important item to note in this definition is that verification must be different from monitoring. For example, if the monitoring procedure is to take the product temperature at cooking, then having a different individual take the temperature a second time should not be considered verification because it is just a repeat monitoring.

The second important item to note is that verification determines the validity of the HACCP plan. Validation is a component of the verification principle and many individuals confuse verification and validation. Validation is the aspect of designing a scientifically sound plan that will control the identified hazards and that it can be applied within the specific operation.

Lastly, verification demonstrates that the plan is operating as designed within the establishment and frequently include on-going activities like calibration of monitoring equipment, review of records, and observation of employees conducting monitoring. All of these on-going activities demonstrate process control of the food safety system.

8.2.7 *Principle 7: Establish Recordkeeping and Documentation Procedures*

The documentation and recordkeeping principle allows establishments to prove that they have developed and implemented a HACCP plan that will produce safe food products. Documentation starts with developing the flow chart, providing a description of the product, and including the intended use or targeted consumers. Forms can be used by establishments to document their hazard analysis, identify CCPs, critical limits, monitoring, corrective actions, and verification procedures. In addition to the written hazard analysis and HACCP plan, the establishment must also document the results from the monitoring and verification procedures. When deviations occur, the corrective actions should be clearly documented to demonstrate that the food is safe and that the process is back in control.

8.3 Mandatory HACCP

In the U.S., the primary impetus for shifting from encouraging the use of HACCP to mandating its use occurred in late 1992 and early 1993 when a major outbreak of *Escherichia coli* O157:H7 occurred from the consumption of undercooked hamburgers being served by multiple Jack-in-the-Box locations across the northwestern United States (Hulebak and Schlosser 2002). The outbreak led to over 700 illnesses across four states, 171 hospitalizations, and 4 deaths (Marler 2017). In response to the outbreak and public outcry, USDA's Food Safety and Inspection Service (FSIS) initiated the development of new regulatory requirements regarding food safety in meat and poultry production facilities (Hulebak and Schlosser 2002). Their goal was to develop regulations that would reduce the risk of foodborne illness associated with the consumption of meat and poultry products. However, there was an understanding that complete elimination of all food safety hazards would not always be possible (Hulebak and Schlosser 2002).

FSIS concluded that the best food safety tool available was the one previously developed by the Pillsbury Company and Natick Laboratories for NASA. Therefore, they proceeded with writing regulations to mandate HACCP for all meat and poultry establishments. FSIS released the proposed Pathogen Reduction/Hazard Analysis and Critical Control Point (PR/HACCP) regulation in 1994, and it was finalized in July 1996 (Food Safety and Inspection Service 2015). The HACCP final rule gave FSIS the authority to:

> maintain and enhance the food safety and other consumer protection benefits of the current carcass inspection system and to effectively and efficiently oversee, evaluate, and verify industry implementation of the PR/HACCP regulations (Food Safety and Inspection Service 2015).

Under the new PR/HACCP regulation, all meat and poultry establishments were required to develop and implement HACCP plans for their products. Due to the

complexities of developing a HACCP system and the need for allowing sufficient time for training, implementation of the regulation was done through a phase-in process. Large plants had from July 1996 to January of 1998 to develop and implement a HACCP program, followed by medium plants which had until January 1999, and the small plants had until January 2000. After 2000, all new establishments were required to have a written HACCP plan before receiving a grant of inspection from FSIS, and existing establishments were required to have a HACCP plan before producing products not covered by their existing HACCP plans. Failing to produce meat and poultry products under a HACCP plan that complies with the PR/HACCP regulation can lead to the production of adulterated products and recalls.

USDA's FSIS was not the only government agency moving forward with HACCP requirements. In 1992, the Canadian government implemented the Quality Management Program, which used the principles of HACCP and required all registered fish processing establishments to have quality control programs, thereby, becoming the first country-wide mandatory HACCP requirement (Canadian Food Inspection Agency 2015; Weinroth, Belk et al. 2018). In 1995, the European Union Directive 93/43 required HACCP principles to be used by all food companies in the EU trading bloc (GrijspaardtVink 1995), and the U.S. Food and Drug Administration (FDA) finalized the Procedures for the Safe and Sanitary Processing and Importing of Fish and Fishery Products HACCP regulation (Department of Health and Human Services 1995). The seafood HACCP program covered many products with an expansive range of hazards – often making it more complex than the meat and poultry HACCP programs (DeWaal 1997; Alberini et al. 2008). By 2001, the criticisms of HACCP in the seafood industry forced the FDA to reevaluate the program. This led to increasing the frequency of inspections, improving guidance and training for industry and inspectors, and increasing inspection efforts aimed at the most critical foodborne pathogens found in seafood (Alberini et al. 2008). In January 2001, the FDA finalized the HACCP Procedures for the Safe and Sanitary Processing and Import of Juice rule (Department of Health and Human Services 2001), which mandated HACCP for juice manufacturers and encouraged its use in retail and food service sectors. Compared to seafood, the overall implementation and enforcement of the juice regulation was fairly smooth.

8.4 Global HACCP

Although it took years from the time HACCP was initially developed in the United States as a mechanism for producing safe food products for astronauts, HACCP and food safety systems eventually evolved into an international trade standard. A major push for HACCP to be widely accepted and adopted around the world stemed from the World Trade Organization (WTO) and a desire to reduce trade barriers (Caswell and Hooker 1996). In an effort to enhance the safety of the food supply and to stem non-tariff trade barriers, the WTO designed the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS) (World Trade Organization 2010) and

the Agreement on Technical Barriers to Trade (TBT) (World Trade Organization 2014) during the Uruguay Round of talks. According to Sperber (2005), these agreements required countries to share information on food safety, notify each other of proposed regulations, and establish "well organized procedures and independent, objective, and transparent risk assessments." The agreements also specified that national level regulations may differ, but they must be based on sound science and be applied equally to both domestic and imported food products while harmonizing the food standards across countries (Caswell and Hooker 1996; Trienekens and Zuurbier 2007). The North American Free Trade Agreement (NAFTA) signed in 1992 was one of the first trade agreements to implement these standards and move towards a more consistent food safety system across the United States, Canada, and Mexico (Caswell and Hooker 1996).

Trade agreements that require countries to standardize their food safety regulations to be identical in all countries within the agreement is considered "harmonization." Harmonization is most often used to determine minimum inputs, processes, and product performance standards that all countries must meet to be able to trade food products, leading to HACCP being adopted by countries around the world. In 1972, the World Health Organization (WHO) began promoting the HACCP system for use across all countries. However, it was not be until 1993 when the joint Food and Agriculture Organization (FAO) and WHO's Codex Alimentarius Commission formally recommended that HACCP replace the traditional approaches to food safety in member countries (Motarjemi et al. 1996).

Codex Alimentarius (2019) is an international food standard that focuses on developing standards, guidelines, and codes of practices to ensure the safety, quality, and fairness of international food trade. Recommendations and guidelines produced by Codex are strictly voluntary for all member countries and are not a substitute for national legislation. Codex sought to standardize the application and terminology used in HACCP by producing guidelines for HACCP that food production companies around the world could base their own food safety systems on. As countries began adopting HACCP into their own food safety systems, some as mandatory regulations and others as voluntary, the implementation of HACCP systems varied from country to country (Motarjemi et al. 1996). While HACCP systems varied, sometimes widely, across both developed and developing countries, harmonization of HACCP systems seemed to work well in areas like the European Union (EU) (Caswell and Hooker 1996). Although there were differences in HACCP among establishments and/or across segments of an industry (Jouve 1994), the overall basis for HACCP and other food safety standards within each country in the EU were fairly similar (Caswell and Hooker 1996).

In terms of developing countries, the integration of HACCP into their food safety systems has been a slower and gradual. However, in countries with major food exports, implementing HACCP based systems into their processes has been more expedited to maintain trade opportunities (Motarjemi et al. 1996). The level of adoption of HACCP across the developing world also varies tremendously. For many food production facilities, the most common and largest barrier to overcome was the cost associated with implementing HACCP (Weinroth et al. 2018). On top of

the high cost of equipment and training, the desire to implement and learn about food safety programs was also seen as a deterrent to implementing HACCP in many of food production facilities. Negative attitudes and perceptions related to HACCP were barriers that impeded its implementation (Panisello and Quantick 2001). Over 60% of food businesses in Turkey reported one of the primary barriers to successfully implementing HACCP as a lack of understanding of HACCP, and over 20% stated it was too complicated to implement (Baş et al. 2007). A survey conducted in Spain found that negative guidelines for conducting a hazard analysis as well as a lack of understanding contributed to failure of developing and implementing HACCP (Ramírez Vela and Martín Fernández 2003). Other factors, such as size of company, have been identified as potential barriers to HACCP implementation (Taylor 2001; Taylor and Kane 2005). Over time, multiple resources have been developed to help overcome some of the concerns and barriers. As globalization of food continues to expand, HACCP will likely be implemented at a higher degree (Weinroth et al. 2018).

As the use of HACCP spread around the world, various other food safety standards, consortiums, and systems were developed to help with the development and implementation of HACCP systems. To promote a uniform HACCP program for meat and poultry products, both within the United States and internationally, the International HACCP Alliance (IHA) was formed in 1994 (Weinroth et al. 2018). The IHA is located at Texas A&M University, College Station, Texas, USA. To help standardize HACCP training, the founding members of the IHA developed an accreditation process to ensure that HACCP training programs address the basic learning objectives necessary for developing and implementing a HACCP system, and the IHA maintains a registry of individuals who completed the accredited training programs. The IHA continues to accredit training programs and serve as a resource for industry professionals, academia, and government officials.

In 2005, ISO 22000 was released to help ensure safety throughout the global food chain, and it was revised in 2018 (International Organization for Standardization 2019). ISO 22000 is compatible with other ISO management standards, including ISO 9000, which focuses on quality management. ISO 22000 provides another tool for companies to use to "improve its overall performance in food safety" (International Organization for Standardization 2019). ISO 22000 focuses only on food safety. Implementing the ISO 22000 may help companies improve risk management and ensure more consistency in effectively employing food safety programs.

8.5 HACCP Auditing

Consistency in food safety programs is a major concern. Due to the variation in understanding HACCP, the differences in regulatory requirements, the uniqueness of products and processes, and the expectations of customers, HACCP and food safety programs are rarely the same, even for similar products. Therefore, another area that has developed over time is auditing of HACCP and food safety systems.

Auditing techniques can be used by both customers and government agencies to verify that food products have been produced using desired food safety and/or quality standards (Powell et al. 2013). Audits can be carried out both internally by the food manufacturer or externally by a third party or customer auditors.

While there could be some overlap, food safety audits are not synonymous with governmental inspections. The terms "inspector" or "inspections" are frequently reserved for government officials and tasks performed to ensure that food processing establishments are complying with all regulatory requirements. As expected, not all government inspection systems are designed to be the same. The intensity and the frequency of inspections may vary based on the specific country requirements and/or based on the type of products being produced within the country. In some countries, HACCP is implemented on a voluntary basis and no inspections are conducted.

Outside of the governmental requirements and standards and government oversight, the globalization of food manufacturing also increased the number of audits food manufacturers were required to complete. In response to the number of audits being conducted, the redundancy of the audits, and the time and effort being devoted to audits, the Global Food Safety Initiative (GFSI) was formed in 2000. One of the goals of GFSI was to standardize audits, thereby, reducing the number of audits a company would be subjected to do during a single year and to minimize the time and expense associated with audits. GFSI has recognized several certification programs (Global Food Safety Initiative 2019). Although each one is slightly different, all GFSI schemes revolve around the development, implementation, and maintenance of a food safety management system, good manufacturing practices, good distribution practices, good agricultural practices, and HACCP. Individual companies can decide which GFSI certified program they want to follow to develop their food safety management system.

One of the major auditing programs is the Safe Quality Food (SQF) program which was developed to address food safety issues during the 1990's (Weinroth et al. 2018). This program provides certification to food processors to ensure their products are held to the highest possible global food safety standards across the total supply chain (SQF Institute-a, SQF Institute-b; Trienekens and Zuurbier 2007). SQF originated in Australia, but the program was purchased by the Food Marketing Institute (FMI), a U.S. based organization. SQF is based on the seven principles of HACCP, along with the principles from International Organization for Standardization (ISO) and Quality Management Systems (Trienekens and Zuurbier 2007; Mensah and Julian 2011). SQF has different codes for different sectors of the industry, including codes for primary production, retail, manufacturing, storage and distributions, manufacture of food packaging, and even one for quality (SQF Institute-b).

Another widely used auditing certification program is the British Retail Consortium (BRC) global standards, which began in 1996 (BRCGS 2019). Along with the Global Standards for Food Safety, standards for Packaging, Consumer Products, Storage and Distribution, Agents and Brokers, Retail, and Ethical Trade and Responsible Sourcing are available. The BRC Global Standards were designed to

reduce the number of audits, improve quality of products, enhance supplier and recipient control, and help ensure adequate records are maintained (Chudzik and Szymonik 2018).

Preventing food safety hazards and meeting consumer demands and expectations for safe food is a universal goal. Over time, HACCP was expanded from three principles into seven principles, and today it is a single component of a food safety system that aims to prevent, eliminate, or reduce risks associated with food safety hazards, including biological, physical, and chemical hazards, in both raw and cooked food products. Optimally designed, preventive, food safety programs combine strong prerequisite programs, HACCP, management systems, training, audits, and other programs in an effort to produce safe food.

8.6 Conclusions and Future Trends

HACCP is well recognized as a critical component of an effective food safety system. It has been incorporated into multiple regulations and global food safety standards and has become a major component of global auditing requirements. As the use of HACCP has grown and expanded across multiple sectors of the food industry, additional tools have been developed to support the implementation of HACCP. One area that continues to grow is the integration of automated monitoring and recordkeeping within the production process and throughout the supply chain. HACCP is a dynamic system and as science and technology improve, HACCP will also improve. The demand for safe food continues, and so does the demand for effective HACCP systems.

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Chapter 9
The Hazard Analysis Risk-Based Preventive Controls

Luke F. LaBorde

9.1 Introduction

Continuing efforts to prevent food from becoming contaminated during growing, processing, and distribution remains a high priority throughout the world. The World Health Organization (WHO) estimated that, in 2010, 600 million illnesses and 420,000 deaths occurred from consumption of contaminated food (Hoffmann et al. 2017). In the United States, the Centers for Disease Control and Prevention (CDC) estimates that approximately 48 million foodborne illnesses occur each year (CDC 2018). Many of these only cause minor symptoms that quickly pass. However, severe cases result in approximately 128,000 hospitalizations and 3000 deaths per year.

CDC surveillance data collected from U.S. State Departments of Health, hospital records, and death certificates show that less than half of reported illnesses are traced to known human microbial pathogens, such as norovirus, *Salmonella* spp., *Campylobacter* spp., *Clostridium perfringens*, pathogenic strains of *E. coli*, and *Listeria monocytogenes* (Hoffmann et al. 2017; Scallan et al. 2011a). More often, the microorganisms responsible for illness remains unknown (Scallan et al. 2011b). Most cases of foodborne illness are related to food handling and preparation practices in restaurants and home kitchens (Angelo et al. 2017; CDC 2018). However, highly publicized multistate outbreaks have been linked to contamination that occurred on farms, in packing houses, and in processing plants that distributed products over great distances.

Several factors have been identified to contribute to this trend (Lynch et al. 2009). Consumer preference has shifted away from thermally processed food products (e.g. canned or blanched/frozen) toward novel, minimally-processed, fresh tasting

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products such as cut fruits and vegetables, salad mixes, ready-to-eat deli products, and un-pasteurized beverages, which have sensory attributes similar to their fresh counterparts. Consumers may therefore be exposed to an elevated risk of illness from such products until new technologies for making them safe are developed. Manufacturers have expanded their global supply chain for foods and food ingredients to an extent where it can be difficult to assure that best practices for safe growing, packing, and processing of foods are followed. Advances in food microbiology and public health have led to discoveries of previously unknown threats to human health, such as highly virulent strains of bacteria and naturally occurring allergenic compounds that can cause severe illness or even death. At the same time, the number of individuals whose immune systems are impaired during recovery from medical treatments has increased.

Foodborne illness also has significant economic costs. In a survey of U.S. food companies that issued a food recall between 2006 and 2011, 77% reported financial losses of at least $30 million, with 23% stating that costs were even higher (GMA 2011). In 2015, it was estimated that medical costs, legal settlements, income, and productivity losses from plant closures accounted for an annual economic burden of over $15.5 billion (Hoffmann et al. 2015). It is, therefore, not unexpected that the increasing number of food related recalls, illnesses, and outbreaks have caused wholesale buyers to demand proof of compliance with new food safety standards and government regulators to issue food laws and regulations that raise the standards for safe food production and processing practices.

9.2 The Risk-Based Approach to Food Safety and the HACCP Concept

Great strides were taken in the twentieth century to assure the safety of the U.S. food system. A series of federal food safety laws culminated with the passage of the Federal Food, Drug, and Cosmetics Act (FD&C) in 1938 [P.L. 75-717]. Among the provisions of the Act, the U.S. Food and Drug Administration (FDA) was given the authority to investigate illnesses or outbreaks attributed to manufactured foods and to inspect food processing facilities and warehouses for compliance with federal food safety standards. Periodic visits by government sanitarians and end-point product testing were thereafter relied upon for assurances of the safety of food products. If someone became ill from eating a food product or if contamination was found during an inspection, the usual practice was for the government to request that the food company destroy or recall the product. However, over time it became clear to many that the existing resources available for on-site inspections were not adequate to oversee a growing and continually changing food industry. A new approach was needed that placed more responsibility on manufacturers to identify potential food safety hazards for their unique products and processes, develop ways

to prevent them from occurring before they become a problem, and to document that all practices and policies are consistently implemented.

The Hazard Analysis Critical Control Point (HACCP) food safety management system has emerged as a better alternative to the inspect and test approach and is now the globally accepted system for assuring buyers, the public, and regulators that they have taken all possible measures to reduce or eliminate potential food safety hazards in their operations. HACCP is a systematic and proactive way to consider risks at each step of a manufacturing process and then develop control measures to prevent or reduce food safety risks to acceptable levels.

Risk-based, preventive approaches to food safety began in the 1960s when the National Aeronautics and Space Administration (NASA) adapted "zero defect" engineering and quality assurance systems for assuring the safety of food taken into outer space. These included applications of "Modes of Failure" concepts that require a thorough understanding of the product and the process in order to predict when a food safety "hazard" can occur. In 1985, the National Academy of Sciences (NAS) recommended that HACCP be incorporated into U.S. food regulations. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) then developed uniform procedures for developing food safety plans known as the seven HACCP principles (NACMCF 1998).

Worldwide consensus on the utility of HACCP for maintaining the safety of the global food supply was achieved in 2003 when the World Health Organization's (WHO) Codex Alimentarius Committee on Food Hygiene issued Hazard Analysis Critical Control Point guidelines for international trade (Codex 2003). Soon after, the European Union (EU), Canada, Australia, and Japan issued regulations requiring food businesses within their jurisdiction to develop and implement food safety plans based on the NACMCF and Codex HACCP frameworks (Bernard and Scott 2007).

Over the last three decades, U.S. government agencies have issued a succession of regulations that required HACCP plan development for certain types of foods. In 1995, all U.S. seafood processing facilities were mandated by the Food and Drug Administration (FDA) to develop HACCP plans (Federal Register 1995). Soon after, the United States Department of Agriculture (USDA) required meat and poultry establishments to write HACCP plans (Federal Register 1996). In 2001, after a series of food borne illness outbreaks attributed to unpasteurized juice products, FDA directed wholesale juice and cider processors to implement HACCP plans in their operations (Federal Register 2001).

In the U.S., full adoption of the HACCP approach for assuring the safety of food came in 2011 when Congress passed, and the president signed into law, the Food Safety Modernization Act (FSMA) [P.L. 111-353]. The law is said to be the most sweeping reform of the U.S. food regulatory system since the 1938 FD&C Act was enacted. FSMA adopts the risk-based, preventive approach of HACCP and expands upon it to address potential hazards that have emerged with the development of modern food production and processing practices. The law grants new authority to the U.S. Food and Drug Administration (FDA) to establish and enforce food safety

Table 9.1 Food safety regulations issued under the U.S. Food Safety Modernization Act (FSMA)

Regulation	Scope
Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food (Federal Register 2015a)	Animal food facilities must have a food safety plan in place that includes an analysis of hazards that need to be controlled and risk-based preventive controls to minimize or prevent those hazards from occurring.
Current Good Manufacturing Practice and Hazard Analysis and Risk-Based Preventive Controls for Food for Animals (Federal Register 2015b)	Food facilities must have a food safety plan in place that includes an analysis of hazards and risk-based preventive controls to minimize or prevent the identified hazards from occurring.
Foreign Supplier Verification Programs for Importers of Food for Humans and Animals (Federal Register 2015c)	Importers of food into the U.S. must perform certain risk-based activities to verify that that food has been produced in a manner that meets applicable U.S. safety standards.
Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption (Federal Register 2015d)	Growers, harvesters, packers, and those who hold fruits and vegetables likely to be eaten raw must comply with science-based minimum farm food standards.
Accreditation of Third-Party Certification Bodies to Conduct Food Safety Audits and to Issue Certifications (Federal Register 2015e)	Establishes a voluntary program for the accreditation of third-party certification bodies, known as third-party auditors, to conduct food safety audits and issue certifications of foreign entities and the foods for humans and animals they produce.
Sanitary Transportation of Human and Animal Food (Federal Register 2016a)	Shippers, loaders, carriers by motor or rail vehicle, and receivers involved in transporting human and animal food must use sanitary practices that ensure the safety of that food.
Mitigation Strategies to Protect Food Against Intentional Adulteration (Federal Register 2016b)	Food facilities must develop risk reduction strategies to prevent intentional adulteration from acts intended to cause wide-scale harm to public health.

standards encompassing the entire U.S. food system, including farms that grow, harvest, pack, and hold fresh produce; facilities that process, manufacturer, pack, or hold human or animal food; and shippers and receivers involved in transporting human and animal food. The seven regulations issued under FSMA and a brief description of the scope of each are shown in Table 9.1. Complete information on each regulation can be accessed on the FDA's FSMA website at https://www.fda.gov/food/guidanceregulation/fsma/. The FSMA regulation that has the greatest impact on the food processing and manufacturing industry is "Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food" (Federal Register 2015a), often abbreviated to simply the "Preventive Controls Rule".

9.3 Writing a HACCP Food Safety Plan

Because the foundation of the Preventive Controls Rule is based on the risk-based HACCP approach for maintaining a safe food supply, it will be helpful to understand the NACMCF system for writing a HACCP plan (Barach and Hayman 2014). HACCP terms, their definitions, and a summary of the process of writing a HACCP plan are summarized in Table 9.2 and Fig. 9.1, respectively.

The task of writing a HACCP plan must first begin with preliminary steps that assure the plan accurately reflects the specific process, product, and environmental conditions within the processing facility. The five preliminary steps are described as follows.

9.3.1 Step 1: Assemble the HACCP Team

The HACCP team is responsible for writing the HACCP plan and is accountable for maintaining it. A HACCP coordinator is selected to help lead and support the team. The team should be drawn from individuals that have experience and expertise in the company's products and processes. The ideal team is composed of representative from quality assurance, sanitation, and plant operations with at least one member from upper management since investment in new facilities and equipment may be necessary. Individuals with expert knowledge and training in food microbiology and process engineering are obvious assets in the risk assessment process. If needed, outside resources such as consultants, trade or professional associations, or university extension specialists may be brought in to join the team. Each person on the team should have an appropriate level of training on the fundamentals of HACCP.

9.3.2 Step 2: Describe the Food and Its Distribution

Microbiological food safety risks are dependent on the inherent physical and chemical characteristics of the ingredients and the finished product. These are used later in the plan writing process for documenting handling, storage, and processing specifications. Names of ingredients in the product, any processing aids, potential food allergens, water sources, packaging materials, or potentially toxic chemicals used during manufacturing are recorded. The expected shelf life of the product and any temperature requirements during shipping (e.g. refrigerated, frozen, ambient) should also be recorded.

Table 9.2 Definitions of terms used in HACCP (NACMCF 1998) and HARPC in the FDA Preventive Controls Rule (Barach and Hayman 2014; FSPCA 2015)

Term	Definition	HACCP	HARPC
Control	(a) To manage the conditions of an operation to maintain compliance with (1) a critical limit in a HACCP plan or (2) a parameter or value in a HARPC plan or (b) The state in which correct procedures are being followed and criteria are being met.	✓	✓
Control measure	Any action or activity that can be used to prevent, eliminate, or reduce a hazard.	✓	
Control point (CP)	Any step at which biological, chemical, or physical factors can be controlled	✓	
Correction	An action taken to correct a minor and isolated deviation from an allergen, sanitation, or supply chain preventive control when the problem is not likely to result in distribution of non-compliant food entering the marketplace.		✓
Corrective action	Procedures followed when control is lost at a CCP and a process deviation occurs.	✓	✓
Critical control point (CCP)	A step at which process control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.	✓	✓
Critical limit	A maximum and/or minimum value, or combination of values, to which any biological, chemical, or physical parameter must be controlled to significantly minimize or prevent a hazard requiring a process control (the terms parameter or value are used more broadly in HARPC).	✓	✓
Reasonably foreseeable hazards	Those hazards that a person knowledgeable about the safe manufacturing, processing, packing, or holding of food would identify for a specific product and process (analogous to potential hazards in HACCP).		✓
Reasonably foreseeable hazards requiring a preventive control	Hazards, identified in the hazard analysis, that are of sufficient severity and likelihood of occurrence that one or more preventive controls are needed to significantly minimize or prevent the food from becoming contaminated or produced under conditions that could cause contamination. Analogous to significant hazards in a HACCP plan.		✓
Deviation	Failure to meet a (1) critical limit in a HACCP plan or a (2) parameter or value in a HARPC plan resulting in loss of control	✓	✓
Good manufacturing practices for human food (GMP)	The FDA regulation (21CFR Part 117 Subpart B) that describes conditions	✓	✓

(continued)

Table 9.2 (continued)

Term	Definition	HACCP	HARPC
	and practices that must be followed for processing safe food under sanitary conditions and which provides the foundation for a (1) HACCP and (2) HARPC food safety plans		
Food safety plan	A set of written documents based on risk-based food safety principles.		✓
Food safety system	The outcome of implementing the food safety plan and its supporting elements		✓
HACCP	Hazards Analysis Critical Control Point. A risk-based systematic approach to the identification, evaluation, and control of food safety hazards	✓	
HARPC	Hazards Analysis Risk-Based Preventive Controls. The risk-based systematic approach for writing a food safety plan that complies with the FSMA preventive controls rule (21CFR 117)		✓
HACCP plan	A written document based on the principles of HACCP and that contains the procedures necessary to control significant hazards.	✓	
HARPC plan	A written food safety plan based on the principles of HARPC that contains the procedures necessary for compliance with the Preventive Controls for Human Food rule (analogous to HACCP plan).		✓
Facility	A domestic or foreign food establishment that is required to register with FDA in accordance with the requirements of 21 CFR part 1, subpart H, "Registration of Food Facilities".		✓
Hazard	A biological, chemical, or physical agent that is reasonably likely to cause illness or injury in the absence of its control.	✓	✓
Hazard analysis	The process of collecting and evaluating information on hazards associated with the food under consideration to decide which are (1) significant and must be controlled in the HACCP plan or (2) known or reasonably foreseeable and for which a preventive control must be established in the HARPC plan.	✓	✓
Hazard requiring a preventive control	A known or reasonably foreseeable hazard for which a person knowledgeable about the safe manufacturing, processing, packing, or holding of food would establish one or more preventive controls to significantly minimize or prevent hazards from occurring.		✓

(continued)

Table 9.2 (continued)

Term	Definition	HACCP	HARPC
Monitoring	The act of conducting a planned sequence of observations or measurements to assess whether a CCP is under control and to produce an accurate record for future use in verification.	✓	✓
Parameters and values	A maximum and/or minimum value, or combination of values, to which any biological, chemical, or physical parameter must be controlled to significantly minimize or prevent a reasonably foreseeable hazard requiring a process, sanitation, allergen, or supply chain control (an expanded definition of critical limit).		✓
Prerequisite programs	Facility-wide policies and procedures that provide the basic environmental and operating conditions necessary to produce safe foods.	✓	✓
Preventive controls	Risk-based reasonably appropriate procedures, practices, and processes to minimize or prevent hazards identified in the hazard analysis as significant.		✓
Preventive controls qualified individual (PCQI)	An individual who has successfully been trained in the development and application of risk-based preventive controls or who is otherwise qualified through job experience	✓	✓
Risk	The threat of any particular hazard to cause harm to consumers based on its severity of outcome and probability of occurrence.	✓	✓
Significant hazard	A potential food safety hazard that, because it can cause severe illness or injury and is sufficiently likely to occur warrants control in the HACCP plan (analogous to a reasonably foreseeable hazard requiring a preventive control in HARPC).	✓	
Validation	Collection and evaluation of scientific and technical information to determine whether the food safety plan can effectively control significant hazards.	✓	✓
Verification	Activities other than monitoring that determine the validity of the food safety plan and that the system is operating according to the plan.	✓	✓

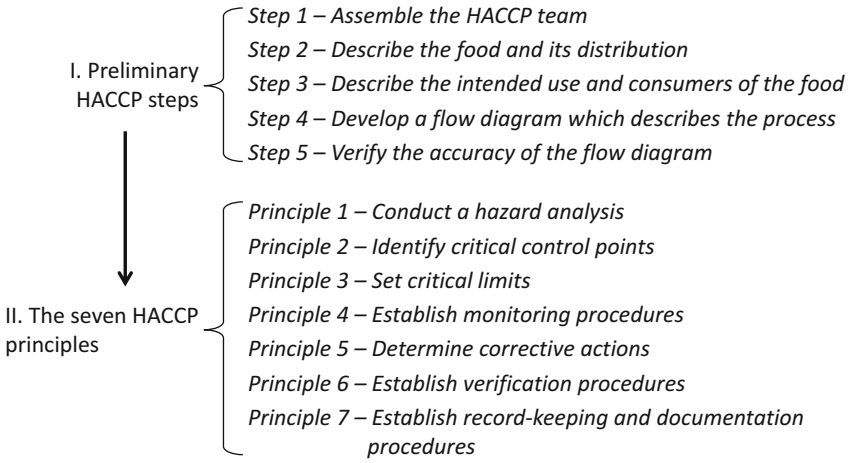

Fig. 9.1 Steps toward developing a Hazard Analysis Critical Control Point (HACCP) food safety (NACMCF 1998)

9.3.3 Step 3: Describe the Intended Use and Consumers of the Food

The expected use of the product by the consumer is important when assessing risks. For instance, is the product intended to be eaten without any further preparation or cooking (ready-to eat product)? Are there specific directions for preparation of the food? Will the intended consumers be the general public? Or, will the food be marketed to specific groups that are especially susceptible to foodborne illness such as infants, the elderly, those with weak immune system, or those taking immune suppressing medications?

9.3.4 Step 4: Develop a Flow Diagram that Describes the Process

Because a HACCP plan is process oriented, a clear description of each step under the control of the establishment is needed. Important process steps might include receiving and storage of ingredients, washing, mixing, grinding, chopping, heating, packaging, and shipping of the final product.

9.3.5 Step 5: Verify the Accuracy of the Flow Diagram

A process flow diagram written in a meeting room may not be accurate or up-to-date. The HACCP team should check the accuracy and comprehensiveness of the diagram by going into the plant and confirming that it accurately reflects the flow of food and ingredients as they are transformed into the finished product. On-site verification of each process step will help the team understand time and location relationships between steps that will be useful later in the hazard identification process. After the review is completed, any deficiencies should be corrected before proceeding to the seven HACCP principles.

Once the preliminary steps are completed, the HACCP team can begin to write the plan according to the seven HACCP principles (Fig. 9.1). The seven principles are a sequence of activities used to systematically identify and establish control measures and monitoring procedures for the most significant hazards, what to do if control measures fail, that the plan is based on the most up-to-date scientific knowledge, and that it is consistently followed as intended. The seven principles are described as follows.

9.3.5.1 Principle 1: Conduct a Hazard Analysis

A food safety hazard is any substance, object, or property that may cause a food to become unsafe for human consumption in the absence of its control. Potential hazards are typically categorized as:

1. **Biological hazards,** e.g. disease-causing bacteria, viruses, parasites, and molds,
2. **Chemical hazards,** e.g. naturally present food toxins above FDA tolerance levels or chemicals that can be poisonous if used improperly, such as cleaners, sanitizers, lubricants and fuels, or substances in food that can cause dangerous allergic responses in sensitive populations.
3. **Physical hazards,** e.g. bone fragments, metal pieces, glass shards, stones, and jewelry that could cause injury or choking if ingested.

A hazard analysis is the process of collecting and evaluating information on potential hazards that may be introduced, controlled, or enhanced at each step in the manufacturing process. Each step where a hazard must be controlled is termed a control point (CP). Because time, energy, and resources are always limited, the HACCP team must select a list of fewer hazards that pose the greatest risk to consumers and thus warrant control in the HACCP plan. These are classified as "significant hazards" because they can cause severe illness to consumers and their likelihood of occurrence is relatively high. Severity is a function of the potential magnitude and duration of illness or injury (e.g., how long an individual may be sick, and whether hospitalization, death or long-term complications are likely outcomes).

Table 9.3 Examples of food safety prerequisite programs

Allergen management
Buildings and grounds maintenance
Chemical control
Cleaning and sanitizing
Consumer complaint tracking
Employee hygiene
Employee training
Environmental testing
Equipment preventive maintenance
Food defense/security
Foreign material control
Glass control
Heating/ventilation/air conditioning (HVAC)
Labeling
Maintenance of hand-washing and toilet facilities
Pest control
Product tracing and recall
Raw materials and supplier specifications
Receiving, storage, and distribution
Transportation
Waste disposal
Water and ice safety

The likelihood of occurrence is estimated by considering past associations of the food product and processing method with outbreaks of foodborne illness or recalls, the method of preparation and processing, conditions during transportation, expected storage conditions, and whether the product requires further preparation or cooking steps on the part of the consumers before serving the food. This information should be gathered in the preliminary steps.

Lower risk (not "significant") hazards can then be managed outside of the HACCP plan through less stringently controlled facility-wide procedures and policies, known as prerequisite programs. These provide the basic environmental and operating conditions necessary to produce safe foods and are often not unique to any particular process or product. Prerequisite program standards are largely drawn from the FDA mandated Good Manufacturing Practices (GMP) (Federal Register 2015a) and any other food safety regulatory or customer specific requirements. Procedures for implementing prerequisite programs are generally documented as standard operating procedures. The types of prerequisite programs used by food manufactures are numerous and varied depending on the needs of the facility. Examples of typical prerequisite programs are shown in Table 9.3

9.3.5.2 Principle 2: Identify Critical Control Points (CCP)

For each significant hazard, a control measure must be implemented that will prevent, eliminate, or reduce the risk to an acceptable level. The control step takes place at one or more steps in the process known as Critical Control Points (CCP). In HACCP, CCPs are typically process control steps, which can include inspection test results upon receipt of raw materials, pasteurization or commercial sterilization, cooking, chilling, acidification, addition of chemical preservatives, metal detection, and labeling.

9.3.5.3 Principle 3: Set Critical Limits (CL)

For each control measure established as a CCP, critical limits (CL) must be set to distinguish between a safe and an unsafe process. These are ideally minimum or maximum numerical values that are easily monitored, such as heating temperature and time, cooler temperature, pH, water activity (aw), physical dimensions, product flow rate or residence time in a heating system, and ingredient weights. However, conformity or deviation from acceptable testing standards, presence or absence of metal, and correct labeling are also examples of critical limits.

9.3.5.4 Principle 4: Establish Monitoring Procedures

Monitoring is the planned sequence of observations or measurements to assess and accurately document whether or not a CCP is under control. Monitoring activities include a visual observation, an automatic readout from a temperature or flow rate recording instrument, or a check that test results fall within an allowable range. If monitoring shows that the requirements of the CL are met, the hazard is said to be "in control". If there is a deviation from the CL, the CCP is "out of control" and immediate action must be taken to correct the situation.

9.3.5.5 Principle 5: Determine Corrective Actions (CA)

When monitoring shows that a CCP is not under control, corrective actions (CA) must be in place to assure that non-compliant product does not enter the market place. By determining CAs well before a crisis happens, confusion on what to do when a deviation from a CL occurs can be avoided. Corrective actions include immediately isolating the non-compliant product for a subsequent determination of its safety and making an immediate process correction to assure no further products are affected. Once the CCP is back under control, a determination can be made on what to do with the affected product. Options include disposing of the product, re-processing it, or safely diverting it to animal feed. If there is a complete system

failure and the product left the facility, the company can issue a product recall. Later, an investigation must be conducted to determine the root cause of deviation and how to prevent it from happening again.

9.3.5.6 Principle 6: Establish Verification Procedures

Verification is defined as those activities, other than monitoring, that determine if the HACCP plan is operating as intended and the control measures are scientifically valid for producing a safe product. Verification activities may include regular reviews of monitoring activities and corrective actions to assure that the procedures established in the plan have been diligently followed. An annual review of the entire HACCP plan, most importantly the process flow chart and the hazard analysis, is essential to determine if there have been any changes to process steps, processing conditions, and product lines since the last review and that the scientific basis for the effectiveness of each control measure remains valid.

9.3.5.7 Principle 7: Establish Record-Keeping and Documentation Procedures

Records are written evidence that all aspects of the HACCP plan are continually followed. It is important to fully document how the HACCP team conducted its risk assessment in the hazard analysis, what basis it used to determine significant hazards and to keep an historical record of monitoring, corrective actions, and verification activities. For many food processors, intense record keeping is thought of as an onerous exercise. However, government inspectors and third-party auditors rely on records as verification that food products are consistently produced under the safest possible conditions. In the event of a recall for a product similar to yours, thorough documentation of all aspects of your food safety system could provide critical evidence that you are not at fault for introducing adulterated products into commerce.

9.4 FSMA and the Preventive Controls for Human Food Rule

The requirements within the Preventive Controls Rule apply to commercial food operations that manufacture, process, pack, or hold human food for consumption in the United States that are already required to register with FDA under section 415 of the FD&C Act. The rule is equally applied to businesses in other countries that export food to the U.S. Foods imported from other parts of the world must now be in compliance with the requirements of each of the FSMA regulations including

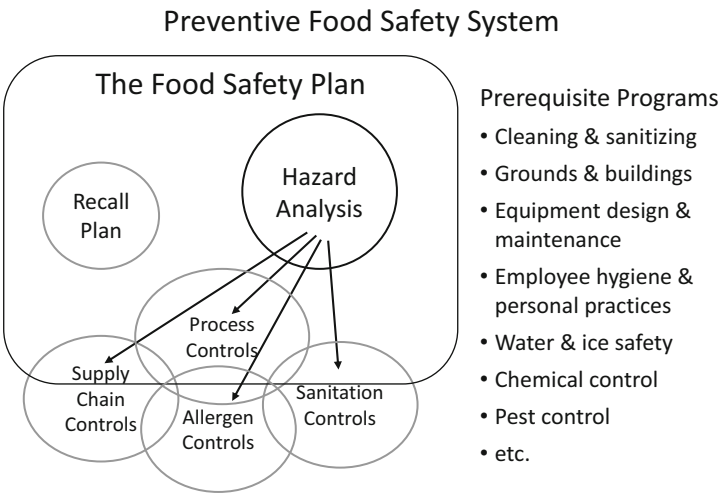

Fig. 9.2 The hazard analysis risk-based preventive controls food safety system. (Adapted from FSPCA 2015)

requirements for importers to perform certain risk-based activities to verify that food brought into commerce within the United Sates meets applicable U.S. food safety standards. Entities defined by FDA as retail food establishments, restaurants, and home-based businesses are not subjected to the Preventive Controls Rule requirements because they are not required to register. USDA inspected meat and poultry processors and home-based processors are specifically excluded from FSMA and therefore not subject to the Preventive Controls Rule. Seafood, low acid canned foods, and 100% juice products are not covered under FSMA because they are already covered under other federal food safety regulations. Although farms are not required to register, processing activities conducted on farms (mixed type facilities) are subject to the Preventive Controls Rule. Certain exemptions to parts of the rule are discussed later.

The framework of the Preventive Controls Rule has been established to provide the food industry with the tools they need to comply with the regulation, but most importantly, to reduce the incidence of outbreaks and recalls. The HARPC approach retains most of the elements within HACCP including developing and implementing a food safety plan that includes a hazard analysis, monitoring procedures, corrective actions, verification methods, and record keeping procedures. Compliance with Good Manufacturing Practices (GMP) managed through prerequisite programs also remains an important foundation for the food safety plan.

However, there are some differences in terminology (Table 9.2) and plan development stages (Fig. 9.2). FDA has introduced the term “food safety system” to mean all measures taken by the manufacturer to keep food safe. The food safety system is made up of two elements. The first is the “food safety plan”, known also as the HARPC plan. It is reserved for managing higher risk foreseeable hazards and is

analogous to managing "significant hazards" in a HACCP plan. The HARPC plan includes a hazard analysis, preventive controls and associated monitoring and corrective actions, and a recall plan. The second element of the food safety system consists of the already mentioned prerequisite programs that are used to control lower risk hazards and provide a supporting foundation for the HARPC plan (Table 9.3). Within the HARPC hazard analysis, the plan writers are provided with two options for how foreseeable food safety hazards should be addressed: (i) the hazards can be controlled within the HARPC plan through implementation of stringent preventive controls or (ii) the hazards can be controlled within prerequisite programs where the monitoring, corrections, and verification requirements are more flexible. The process begins with a set of preliminary steps as follows.

9.4.1 Preliminary Steps

Preliminary steps are the same as those developed for a HACCP plan. A food safety team is assembled, and the product, its distribution, and the intended use, and the end user of the product are identified. A process flow diagram must also be developed and verified. However, the Preventive Controls Rule requires that at least one member of the food safety team be a "preventive controls qualified individual" or PCQI. A PCQI is an individual who has successfully completed training in the development and application of risk-based preventive controls that is at least equivalent to that received under a standardized curriculum recognized by FDA. A individual can also attain PCQI status as proficient in developing and applying a food safety system by other means, such as through job experience. The PCQI is responsible for oversight of the food safety plan including determining that preventive controls are effective, conducting onsite audits of suppliers, reviewing records to assure that monitoring and corrective actions are complete, that corrective actions taken are appropriate, and that the plan is re-evaluated at least every year.

9.4.2 Hazard Analysis, Preventive Controls, Monitoring, and Corrective Actions

The hazard analysis remains at the core of the food safety plan. Under the Preventive Controls Rule, the term "reasonably foreseeable hazards", is introduced to mean all biological, chemical, and physical hazards occurring naturally or that are introduced unintentionally or for purposes of economic gain. These are analogous to the list of potential hazards identified at the beginning of the HACCP hazard analysis. FDA has characterized foreseeable hazards as those that a person knowledgeable about the safe manufacturing, processing, packing, or holding of food would identify. From

this definition, FDA makes it clear that education and training are important qualifications for members of the food safety team that will be conducting the hazard analysis.

The number of "reasonably foreseeable hazards" identified must then be pared down to those that "require a preventive control" to significantly minimize or prevent the food from becoming contaminated or produced under conditions that could cause contamination. This is analogous to the procedure for identifying higher risk "significant hazards" controlled in a HACCP plan. The remaining lower risk foreseeable hazards must still be controlled within the food safety system through one or more prerequisite programs.

FDA has established four types of preventive controls, each requiring monitoring, corrective action, verification, and record keeping procedures.

- **Process preventive controls** are procedures, practices, and processes to control reasonably foreseeable hazards occurring at specific process steps identified in the flow diagram. They are equivalent to process controls established as critical control points in a HACCP plan. Specific "parameters and values" must be set to indicate when the hazard is under control. Monitoring activities must be established to notify when a loss of control occurs and corrective action procedures must be carried out when a process preventive control has failed. Those who already have a HACCP plan, can easily merge already established CCPs, monitoring procedures, critical limits, and corrective actions into a HARPC food safety plan.
- **Sanitation preventive controls** are practices and policies that include cleaning and sanitizing food-contact surfaces, preventing microbial and chemical cross-contamination, and monitoring for environmental pathogens. In HACCP, risks related to inadequate sanitation practices were typically controlled in prerequisite programs and many can still be controlled that way in a Preventive Controls Rule food safety system. However, given an increasing number of outbreaks and recalls traced to post-processing contamination of ready-to-eat foods, it is no surprise that the Preventive Controls Rule now requires manufacturers to consider preventing sanitation deficiencies in the more stringent HARPC plan. When deficiencies related to cleanliness and cross contamination can easily be corrected in a timely manner, the full requirements of corrective actions proscribed for a HACCP plan (e.g. isolation of the affected product and evaluation of its safety) are not required. Instead, the FDA allows the manufacturer flexibility to make an immediate "correction" to a minor and isolated problem. For instance, re-cleaning a food preparation surface showing signs of residual food debris. However, more prescriptive corrective actions may be necessary if unsanitary conditions exist for an extended period of time or that pose an immediate and significant risk to consumers.
- **Allergen preventive controls** are procedures, practices, and processes to assure that the presence of food allergens in ingredients and final products are labeled

correctly and that cross-contamination during processing cannot occur. Compliance with the Food Allergen Labeling and Consumer Protection Act (FALCPA) (P.L. 108-282) is required to prevent unintentional illness on the part of sensitive individuals from exposure to allergens in milk, eggs, peanuts, tree nuts, fish, shellfish, soy, and wheat. Monitoring actions can include regular checks for mislabeled ingredients and finished products, or post-cleaning visual checks and allergen test kit results that could indicate a serious cross contamination risk. Corrective actions must be taken whenever monitoring indicates that measures taken to prevent exposure of the public to a food allergen are inadequate. Depending on the results of the hazard analysis, some allergen hazards can also be controlled through sanitation and supply chain preventive controls, or within prerequisite programs.

- **Supply chain preventive controls** are actions or procedures to minimize or reduce a hazard in raw materials or ingredients. These actions must be applied by the supplier and are monitored by the food manufacturer. Supply chain controls include inspecting for the presence of a certificate of analysis (COA) with each shipment, site visits by the manufacturer for assuring conformance with food safety standards, or results from third party audits. A supply chain prerequisite program can rise to preventive control status within the HARPC plan if no other preventive controls are adequate to control the foreseeable hazard.

9.4.3 Verification and Validation Procedures

The Preventive Controls Rule states that, for each preventive control, verification activities must be conducted to take into account the nature of the preventive control and its role in the facility's food safety system. Verification is required to assure that the food safety plan is consistently implemented including reviewing monitoring and corrective action records within seven working days after the they are created, that appropriate decisions about corrective actions are being made, and that process monitoring instruments are regularly calibrated.

A reanalysis of the entire food safety plan must take place at least every 3 years or whenever (1) significant changes in food products and processing methods within the facility could result in a new foreseeable hazards or significantly increase the risk level of a previously identified hazard, (2) the manufacturer becomes aware of new information on potential hazards, or (3) part or all of the HARPC plan is known to be ineffective. Process preventive controls must be validated through scientific studies or other means to assure they are adequate to control the foreseeable hazards identified in the hazard analysis. Validation of sanitation, allergen, and supply chain preventive controls do not require the HARPC plan although scientifically valid environmental and product testing procedures must be used for all verification activities.

9.4.4 Record Keeping Procedures

An integral part of the preventive control system is keeping good records. Written records benefit the manufacturer by providing evidence to buyers and regulators that that the HARPC plan is consistently followed as planned. The following records must be kept in order to comply with the Preventive Controls Rule:

- the hazard analysis,
- preventive controls for each identified hazard and verification that they effectively control the hazards,
- monitoring records to ensure preventive controls are consistently performed,
- a full account of any corrective actions taken,
- the supplier approval and verification program,
- the recall plan,
- all testing and auditing results, and
- the results of the food safety plan reanalysis

All the required records must be retained at the facility for at least 2 years after the date they were prepared.

9.4.5 Recall Plan

A recall is an action taken by a food establishment to remove a product from distribution. Despite all efforts to prevent food safety hazards from occurring, there is always the possibility that an unsafe product has left the control of the manufacturer and entered the marketplace. A recall plan is not intended to prevent food safety problems, but can limit exposure of the public to harm and limit liability to the manufacturer. Under the Preventive Controls Rule, a written recall plan is mandatory in a HARPC plan if a preventive control was established. If a company discovers a problem that has a reasonable probability of causing serious injury, illness, or death to consumers, an immediate recall is required. If FDA finds that a company is not responding quickly enough to a situation that requires a recall, it may issue a mandatory recall notification, and if necessary shut down the facility. FDA requires that recall plans to include all steps necessary to conduct the recall including assigning responsibility for taking those steps. Required procedures include:

- notifying customers about the food being recalled, including how to return or dispose of the affected product,
- notifying the public at large when appropriate to protect public health such as through a pre-prepared press release approved by FDA,
- conducting regular effectiveness checks to verify that the recall is being effectively carried out, and

- determining appropriate disposition of the returned or recovered recalled product such as reprocessing, reworking, or diverting to a use that does not present a safety concern, or destroying the food.

9.5 Exemptions to the Preventive Controls Rule and Modified Requirements

Although all FDA regulated food businesses that manufacture, process, pack, or hold human food for consumption are covered under the Preventive Controls Rule, certain exemptions are available where only some aspects of the rule apply.

A "qualified facility" exemption is available to facilities having (1) less than $1,000,000 in annual total food sales plus inventory (adjusted for inflation since 2011) or (2) less than $500,000 (inflation adjusted) in 3-year average annual sales provided that the average monetary value of all food sold directly to qualified end users is greater than that sold to other purchasers. A qualified end-user means (1) the consumer of the food, or (2) a restaurant or retail food establishment located in the same state or Indian reservation or no more than 275 miles from the qualified facility and is purchasing the food for direct sale to consumers. Qualified exempt facilities must comply with Current Good Manufacturing Practices (cGMP) (Federal Register 2015a) but are not required to write a complete a HARPC plan nor are they required to meet the full record keeping provisions of the rule. However, exemptions are not automatic. The manufacturer must submit a form to FDA every 2 years that discloses their 3-year average annual sales figures and/or sales distribution requirements determined from tax documents, invoices, or other accounting documents. They must also have conducted a hazard analysis that justifies their conclusion that they already have adequate preventive controls in place. If part of their claim involves compliance with state or local food safety regulations, they must also present evidence to that effect. It needs to be mentioned that FDA can withdraw a qualified exemption if they find that foodborne illnesses were directly linked to the facility or is otherwise necessary to protect the public health.

FDA also exempts certain low-risk products and processing activities conducted on farms (mixed type facilities) that have fewer than 500 full time employees or 3-year average annual food sales plus inventory of less than $1,000,000. Lower-risk products and processes that take place on a mixed type facility include baked goods, candy, jams, jellies, maple syrup, vinegar, and other processed foods that do not require time/temperature controls for safety. The complete list can be found in the body of the preventive controls for human food rule (Federal Register 2015a).

Food manufacturers who are eligible for exemptions to the rule need to keep in mind that wholesale distributors and buyers are under no obligation to accept FSMA exempt food and may require full compliance with the regulation as a condition of purchase.

9.6 Conclusions and Future Directions

The risk-based, proactive control approach used for HACCP food safety plan development is globally accepted as the most effective way for food manufacturers to prevent recalls, outbreaks, and associated financial losses. The Preventive Controls Rule has adopted many of the established HACCP principles while adding new terminologies and procedures for food safety plan development. Compliance dates for the Preventive Controls Rule have passed and food manufacturers in the U.S., and those importing food to the U.S., can expect increased scrutiny from FDA for assurances that all aspects of the regulation are followed.

Writing a HARPC food safety plan can be challenging, especially for those with no prior experience with HACCP. The materials presented in this chapter are only a cursory review of HACCP and HARPC. Readers are encouraged to seek out courses offered by university extension, commodity groups, or consulting businesses on risk-based food safety plan development. A high-quality course will generally take 2–3 days and will include active discussions and breakout work groups that provide the hands-on experience necessary to write a food safety plan. Risk-based preventive controls food safety plans are living documents that must be regularly updated over time to keep up with rapid changes in demand for new products, advances in food technology, and our understanding of potential biological, chemical, and physical hazards in the food supply chain.

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Chapter 10
Food Traceability

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10.1 Introduction

The complexities in understanding the origins of a food ingredient or product at the end of the food supply chain multiplied as advances in agricultural production capacity, cold chain storage, and rapid agricultural freight systems proliferated over the late nineteenth century and early twentieth century. Additionally, there was reduced incentive to trace and track product in the late nineteenth century to the end of the twentieth century due to the commodification of many agricultural products, such as wheat, corn, hogs, and beef (Friedberg 2017). However, the Anthrax attacks of 2001 as well as high profile food safety incidents highlighted the need to have documentation in the case of bioterror or other food protection scenarios, resulting in the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (CSPAN 2002). This codified 1-up, 1-down as the basis of traceability. For traceback investigations, this paradigm provides a minimum level of documentation to perform effective recalls. However, because recordkeeping requirements only mandated information on suppliers and customers, investigators may run into barriers, such as incomplete or inaccessible records, delaying traceback during food emergencies. This means that in the process of a traceback investigation, government officials must manually connect the dots from a variety of nonstandard records to find the convergence point of the outbreak.

Therefore, momentum has built within the industry to extend traceability information accessibility throughout the food supply chain system. Whole chain, or end-to-end, traceability, entails a system of linked internal traceability systems to enable the ability to quickly traceback products and their ingredients to their origin. The movement toward whole-chain traceability requires data standardization and

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system interoperability. Technical guidance enables supply chain participants to share traceability information with each other and investigators. The North American Produce Traceability Initiative (PTI) is an example of an industry-wide initiative developed for that purpose, which comprehensively developed best practices, technology solutions, adoption metrics, and IT protocols for successful implementation of traceability (PTI 2011). Traceability initiatives, like PTI, speed the creation and industry uptake of data schemas for food traceability, which consist of Key Data Elements (KDEs) and Critical Tracking Events (CTEs). KDEs are the essential pieces of information carried with the product to accomplish traceability goals (McEntire and Bhatt 2012). CTEs are the junctures at which KDEs are collected (McEntire and Bhatt 2012). Not every event needs to be captured to achieve robust traceability; in general, CTEs are where sufficient change ownership, transformation, or otherwise utilize the product to necessitate documentation (McEntire and Bhatt 2012).

Consumer demand, new regulations including the Food and Drug Administration's Food Safety Modernization Act (FSMA) or National Oceanic and Atmospheric Administration's Seafood Import Monitoring Program (SIMP), and technology advances such as Blockchain technology and Internet of Things (IoT) sensors are spurring investment in food traceability (Lin 2018). These factors are also extending the paradigm of traceability beyond chain of custody and effective recalls to underpinning other supply chain improvements, especially sustainability and consumer transparency. Many of these are linked to the growing adoption of whole chain traceability. With this transition to greater visibility of supply chain data, it has the capability to transform food safety outbreak investigations by enabling much quicker tracebacks, easier linkage of microbiological analysis to product, and additional metrics to make epidemiologic conclusions.

A key to traceability success is having a common vernacular for describing the system. Depending on the stage of the supply chain or the commodity, common terms may have different connotations or specifications. In Table 10.1, we describe traceability terms as they are used by traceability engineers and subject matter experts.

10.2 Consumer Behavior and the Return on Investment for Food Traceability

Most U.S. consumers expect food companies to fully address a recall or foodborne illness within one or two days (Jones 2017). Some consumers will either wait several months or never return to the implicated brands (Jones 2017). Food traceability systems that deliver transparency information build consumer trust and reduce fears, leading to increased sales and potentially higher profits for traceable foods (Choe et al. 2009).

Table 10.1 Glossary of traceability terms

Term	Definition
Batch/Lot	The batch or lot number associates an item with information the manufacturer considers relevant for traceability of the trade item. The data may refer to the trade item itself or to items contained in it.
Critical Tracking Event (CTE)	Point at which product is moved between premises or is transformed, or at which is determined to be a point where data capture is necessary to maintain traceability. A Critical Tracking Event has four dimensions: What: what physical objects were involved? When: when the Event took place? Where: where the Event took place? Why: what business step was being carried out?
External Traceability	External Traceability takes place when instances of a Traceable Item are physically handed over from one supply chain partner (source) to another supply chain partner (recipient).
Internal Traceability	Internal Traceability takes place when a supply chain partner transforms one or more input traceable items into one or more output traceable items.
Interoperability	Interoperability is the ability of information technology or software systems to exchange information with different traceability systems. Interoperable traceability systems enable members of supply chains to share and use information more readily. To be fully interoperable, systems must be able to utilize a common data format (syntactic interoperability) and they must interpret information based on shared definitions (semantic interoperability) (Bhatt et al. 2016).
Key Data Element (KDE)	Data input required to successfully trace a product and/or its ingredients through all relevant CTEs.
Link	Recording the information necessary to establish the relationship to other relevant information.
Location	A place where a traceable item is or could be located [ISO/CD 22519]. A place of production, handling, storage, and/or sale.
Record	Act of creating a permanent piece of information constituting an account of something that has occurred.
Share	Act of exchanging information about an entity or traceable item with another Trading Partner.
Traceability	[ISO 9001: 2000] Traceability is the ability to trace the history, application, or location of that which is under consideration.
Traceability Data	Any information about the history, application, or location of a traceable item, either Master Data or Transactional Data.
Traceable Item	A physical object where there may be a need to retrieve information about its history, application, or location.
Trace Request	A formal inquiry about the history, application, or location of a traceable item. A request can trigger subsequent trace requests up or down the supply chain in order to fulfill the original request
Tracing (Tracing Back, Traceback)	The ability to identify the origin, attributes, or history of a particular traceable item located within the supply chain by

(continued)

Table 10.1 (continued)

Term	Definition
	reference to records held. "Tracking back" and "tracking forward" are the preferred terms used in this document.
Tracking (Tracking Forward, Traceforward)	The ability to follow the path of a traceable item through the supply chain as it moves between parties.
Transformation	An irreversible change to the nature of a traceable item that changes the identity and/or the characteristics of the traceable item.

Food industry executives typically allocate technology and process investments based on quantified return on investment (ROI). However, contemplating the possible impact of a major foodborne illness event is challenging and frightening, while the upside associated with increased consumer trust is equally difficult to measure. Therefore, companies in the food sector typically focus their traceability investments on meeting regulatory requirements, industry initiatives, or components of third-party food safety, quality, and sustainability certification schemes.

Traceability is an area driven by consumer demands for safe and accountable food, regulatory demands to expedite recalls and outbreak investigations, and industry needs to mitigate risk from food safety and supply chain transparency issues. Despite initially being a regulatory requirement, enhanced traceability is developing into an essential business process for the food industry (GS1 2018).

10.3 Food Traceability System Design: Scope and Goals Through Use Cases

Food traceability schemas and systems are best framed by their use cases. In systems engineering, use cases conceptualize how goals are achieved through external actors utilizing the system. Without defining the use cases prior to the devising of a food traceability system, the resulting solution may be ineffective, inefficient, or contain gaps. Understanding the design and context of a given traceability solution or framework is essential to maximize the effectiveness of food safety scenarios.

Traditionally, food traceability has been associated with regulatory demands to address food safety and defense concerns. The United States devised traceability requirements as part of counterterrorism efforts (2002 Bioterrorism Act) and then food safety reform (2011 Food Safety Modernization Act) (FDA 2014; US Congress 2002). As part of a food safety strategy, a traceability system primarily focuses on making existing information more readily accessible in the case of a food contamination event and subsequent recall. In traceback investigations, regulatory and

public health agencies must find, and often manually trawl, through records in each link of the supply chain. Depending on the availability of records, cooperation of contacts, and complexity of the outbreak or contamination event, a traceback investigation can outlast the duration of the illness, such as in the case of the recent *E. coli* O157:H7 outbreak of spring 2018 in Romaine lettuce (Gottlieb and Ostroff 2018). Effectiveness and timeliness for cooperating with public health agencies and supply chain partners is the key metric for this use case.

Beyond traceback investigations, there exist other common use cases for food traceability. Animal health, legality and trade requirements, transparency of agricultural and food production practices, and consumer information/competitive advantages are among other reasons for creating food traceability systems. These do not have to be mutually exclusive to food safety capabilities of a traceability system but each KDE adds complexity to a given system and can impact implementation and cooperation of supply chain partners.

Animal health and food safety often overlap and use traceability to mitigate risk. Livestock traceability programs in Canada and the United Kingdom came about over concerns for Bovine Spongiform Encephalopathy (BSE) entering the food supply chain (Stanford et al. 2001). BSE requires robust traceability systems, because one infected animal may expose thousands of people through modern meat production processes. Additionally, monitoring of international shipments of livestock through traceability programs ensures that BSE and other livestock diseases are not introduced to susceptible herds. Variant Creutzfeldt-Jakob's disease (vCJD), a devastating and horrifying illness, is the epidemiologically linked disease associated with ingestion of BSE-contaminated beef. vCJD has a long incubation period (up to 10 years), incurable, only diagnosable at autopsy, and fatal (CDC 2018). Consequently, there are major trade implications to the discovery of BSE in a given country. Animal health concerns dictate tracing the attributes of the individual animal until slaughter, including lineage, location data, and feed (Stanford et al. 2001). However, these programs do not traditionally extend beyond slaughter.

Traceability frameworks designed for other use cases generally provide robust chain of custody to aid in tracebacks. For instance, traceability systems for legality often require mass balancing capabilities to ensure that undocumented product is not mixed in with legal, documented product, thereby making these systems adept for food safety and traceback as well. Seafood, often overfished or otherwise illegally caught, is looking to food traceability to address opaque harvesting, procurement, and processing in the industry, which can obscure practices that contribute to IUU fishing (GDST 2018). Brands have even started using origin attributes as market differentiation tools; companies like Chicken of the Sea® and Just Bare®, provide codes on their products wherein users may input and receive information on their product including origin (Chicken of the Sea 2019; Just Bare Chicken 2019). Consumer facing traceability have a variety of uses, including transparency and even food safety alerts.

10.4 Traceability Core Concepts

Progress towards better traceability systems necessitates interoperable semantics and data architectures capable of communicating essential digital information throughout the supply chain alongside the product. Information technology systems in food operations are complicated by lower than average investment, proprietary food technologies that may rely on legacy software, and limited personnel resources for customizing and augmenting existing systems. To accommodate these complications, food traceability efforts focus on fundamental aspects of the supply chain and how they relate to IT systems. Three core concepts to successful traceability frameworks are interoperability, KDEs, and Critical Tracking Events (CTEs).

KDEs are the core pieces of metadata necessary to accomplish the use cases of the given traceability system. In other words, KDEs are the pertinent attributes recorded to address traceability domains, such as food safety, authenticity, and/or legality. For traceback capabilities, the information requirements are minimal. KDEs needed are dates, weight, lot number, sender/receiver, and identifiers (e.g. UUID, GTIN) (Stehr-Green et al. 2004). Pre-competitive standards setting processes have been successful in creating best practices for data collection, KDE definitions, and incubating technology to address traceability operational gaps. Food companies have different and unique processes that go into their ways of doing business including capturing data. If this data is categorized and organized in an ad hoc way, the ability for investigators or supply chain partners to interpret and utilize the data is markedly reduced.

Correspondingly, CTEs are the points along the food supply chain where KDEs must be transferred, transformed, and/or verified (McEntire and Bhatt 2012). CTEs generally fall into three categories: transportation, transformation, and depletion (McEntire and Bhatt 2012). Additional business steps documented in product data standards, such as Electronic Product Code Information Services (EPCIS), include catch/harvest, inventory counts, inspections, and sensor monitoring. Transportation is the transfer of product between two locations and may be internal or external. A transportation CTE could be a meat processing facility shipping a product to customers (external) or a retailer transferring a product from its distribution center to a store location (internal). Transformation is any process where a substantial, irreversible product change occurs and necessitates documentation to maintain traceability. Transformation CTEs take inputs from ingredient KDEs and logs an output, which captures the finished product's suppliers, lot/batch, and product ID so that it may be available for subsequent CTEs. Depletion is the consumption or discarding of product. Depending on the commodity, there may be unique considerations to best practices at particular CTEs, which is part of the reason for industry-specific standards. For example, produce may be repacked and/or commingled, which requires best practices for combining lot/batches and/or identifiers into a final lot. Though catch/harvest could be represented as a transformation event, its location at the beginning of supply chains and its importance to capture important product information warrants its own category of CTE. Sensor, or Internet of Things

(IoT), monitoring is an emerging CTE being standardized in the industry, since these devices have the capability of constant documentation rather than traditional event-based logging.

Interoperability between IT systems is a key to robust traceability frameworks. Interoperability may be defined as "... a measure of the degree to which diverse systems, organizations, and/or individuals are able to work together to achieve a common goal" (Ide and Pustejovsky 2010). The principle of interoperability is familiar to our daily lives. These may be communication protocols such as Bluetooth® or compatibility requirements among software developers. In food traceability, agreed upon definitions of KDEs and CTEs are critically important and illustrate the necessity of pre-competitive industry initiatives (Tables 10.5 and 10.6). For logistical food traceability, Global Standards 1 (GS1) has done the most extensive, globalized work on standardizing how information is captured, identified, and shared among supply chain partners. GS1 is a global standards organization which centers its mission on product identity, and increasingly, traceability (GS1 2019). They are most well-known for the promulgation of barcoding to identify products (GS1 2019). Other products include the Global Data Synchronization Network and standards for the use of RFID and NFC tags in logistics systems (GS1). However, GS1 tends to be used downstream, from processors to retailers, while upstream producers may or may not be using GS1 identifiers, especially in non-Western markets. In the past, margins and perceived lack of need precluded upstream supply chain actors from adopting unified methodologies for capturing digital information and sharing that data with processors, distributors, and retailers. Pre-competitive standards setting processes have formed in response for these needs of upstream food traceability, such as the Global Dialogue on Seafood Traceability (GDST) or the Produce Traceability Initiative (PTI).

10.5 Food Traceability Frameworks: 1-Up, 1-Down Versus Whole Chain Traceability

Food traceability, especially in the United States, began with regulatory demands and national security concerns. The 2002 Bioterrorism Act mandated 1-up, 1-down record-keeping requirements for all FDA and USDA regulated food facilities, which was then was extended by FSMA (US Congress 2002; FDA 2014). The 1-up, 1-down paradigm for food traceability has many advantages from a regulatory point of view: it is easy to mandate as much of the information is readily accessible or already collected, it encompasses a wide range of technologies including paper records, and there are few considerations as to data governance, because information is kept within each supply chain partner's information system. However, in the event of a foodborne disease or contamination event, speedy responses and recalls save lives and reduce reputational risk. The information required to complete a traceback investigation may take weeks to months depending on the outbreak's epidemiology,

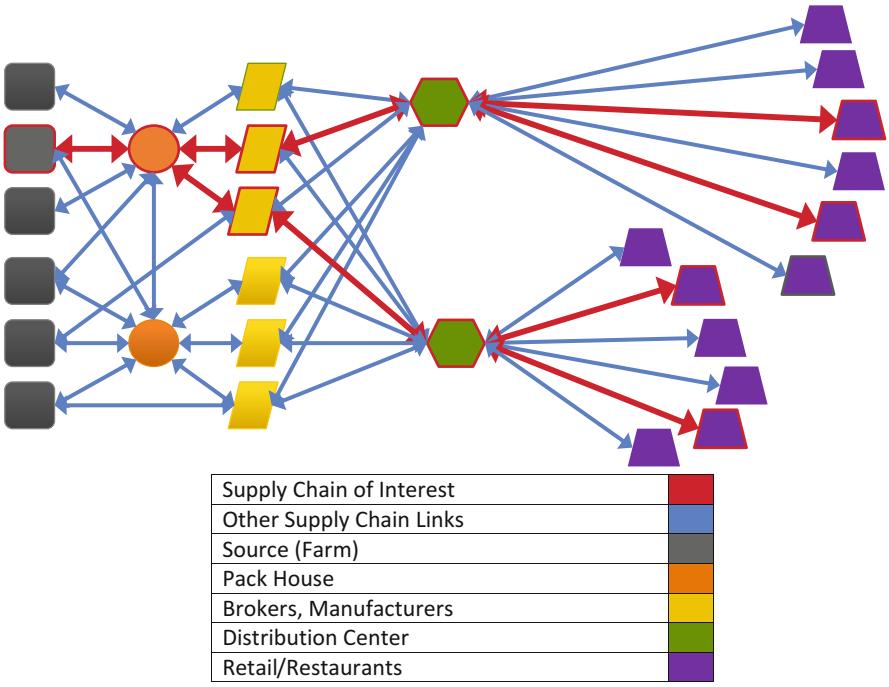

Fig. 10.1 Whole chain traceability

shelf-life of the product, and supply chain complexity. Records have to be individually requested, interpreted, and concatenated before traceback analysis. The 1-up, 1-down traceability siloes these records until the public health agency needs them.

Whole chain traceability, depicted by Fig. 10.1, is the ability to trace and track a product's pedigree throughout its journey in the supply chain. There are some barriers to this vision of traceability including concerns on sensitive business information and data governance. Figure 10.1 shows the hypothetical supply chains of a commodity using produce as an example. Companies on the left are producers who send their product to pack houses (orange circles). Product here then goes through brokers/manufacturers (yellow), distribution centers (green), and retailers and restaurants (purple). All of these stages of the supply chain have multilateral relationships, making 1-up, 1-down traceability impractical in the event of a food safety emergency. Whole-chain traceability enables the linkage of a product's pedigree associated with a unique identifier. Mainly, the promulgation of standardized KDEs and CTE best practice documentation enables whole chain traceability. Even if systems are not optimally interoperable, datasets extracted in the event of a food safety incident can be easily cross-referenced and analyzed to find the traceback convergence point.

10.6 Technologies in Traceability

Information technology is rapidly driving changes to most industry operations, responding to pressures of globalization and supply chain efficiency. Because food traceability includes aspects of logistics, digitization, and even sustainability, investors and technology startups have significant interest in developing and implementing new products and innovations. Consequently, the future of food traceability will dramatically transform, pending decisions by industry, government authorities, and consumers. However, the broad trends will not change, namely: embedded and networked devices such as Internet of Things (IoT), increases in network speeds and capabilities, and flexible and inexpensive database solutions. These capabilities shape the broad categories of food traceability technology: identity, capture, and share.

Identity is a fundamental aspect of logistics systems. Identifiers, such as barcodes, use simple contrasting patterns to convey information rapidly to a given reader. While not easily readable to a human, optical scanners may be able to read and identify object attributes from a barcode rapidly. Similarly, Radio Frequency Identification (RFID) tags, are designed to be queried at an industrial pace, but have added advantages in being rewritable and conveying more information.

Advancements in wireless networks are increasing connectivity in rural regions and the ability to network many devices simultaneously. Especially pertinent to data collection and capture are IoT devices, internet-enabled smart devices which can provide monitoring and data collection in industrial settings. The usage of internet-capable devices will depend on their cost per unit, compatibility, interoperability with other systems, and usefulness of data produced. Constant temperature monitoring, for example, may be useful for ascertaining food safety qualities in a traceability system, but if the data is not stored or successfully connected to a product's identity, the technology may be less than useful. Early adoption success is key to most industrial implementation of novel technologies, but it is especially true to the food industry, given lower profit margins. The food industry has fewer resources for large information technology investments compared to higher margin industries, such as the pharmaceutical industry. Therefore, food companies tend to look for early successes before making these investments themselves.

The Canadian Cattle Identification Agency is a prominent early example starting the last 20 years of efforts to digitize food traceability systems. Until recently, data sharing protocols were principally point to point arrangements using Electronic Data Interchanges (EDIs), File Transfer Protocol (FTP), and HTTP Get/Post. Currently, the potential for whole-chain sharing of traceability information has captured technologists' imaginations interest beginning in the mid-2010s. The advent of blockchain as a revolutionary database technology has invigorated considerable interest in food traceability, especially as a market driven force rather than simply regulatory compliance. The properties of immutability, decentralization, and transparency are attractive to technologists and food safety professionals. However, as of this writing, it is still an unproven technology with limited implementation and some

Table 10.2 Traceability functions

Traceability Function	Technologies
Identity	Human Readable Text, Barcodes, RFID/NFC Tags, QR Codes Internet of Things (IoT) devices
Capture	Optical Scanners, Radio Scanners, Cameras, Vessel Monitoring Systems, Global Positioning Systems, Scales, Thermometers
Sharing	Email, Fax, File Transfer Protocol, Electronic Data Interchange, Blockchain, EPCIS, APIs to Cloud Applications
Other (Use)	Artificial Intelligence Data Analytics, Facial/Image Recognition, Remote Sensing

fundamental questions still being answered. To append transactions onto a blockchain, the network nodes use a resolving algorithm to authenticate it. These resolving algorithms are intrinsic to the decentralized architecture of blockchain but are computationally intensive and makes data storage cost prohibitive on the blockchain. Though this technology is still immature, there is value in understanding blockchain architecture as there is a high probability that essential elements of blockchain, namely decentralization and immutability, will be attributes of future database technologies.

Table 10.2 is an outline of the range of technologies used in traceability, some established and others new and emerging. Robust traceability does not necessarily require utilizing new technologies, but they can alleviate potential disruptions to existing business processes through automation or enhance security or flexibility of recordkeeping. The table describes technologies used in traceability based on their function. Identification technologies denote uniqueness of shipments, pallets, and products for use in logistics and inventory management systems. Data capture technologies read identifiers as well as record KDEs, such as weight, location, and temperature. Sharing protocols vary in complexity and scalability; data may be able to be transferred over email or may be more automated and customized through an Electronic Data Interchange or Blockchain. Other technologies being used in traceability systems may apply to data processing and analytics. With the accumulation and aggregation of large datasets of logistics information, it is becoming possible to utilize the power of Machine Learning to augment decision making, reducing waste and inefficiencies.

Traceability technologies will change over the next decade depending on advancements in artificial intelligence, blockchain, and networking speeds. They will also depend on successful implementations with the approval of pertinent stakeholders. A solution may work in a proof of concept, but if scaled up and communicated to stakeholders poorly, a perfectly capable technology sometimes does not materialize into common use.

10.7 Laws and Regulations

In the United States, traceability was regulatorily introduced through the 2002 Public Health Security and Bioterrorism Preparedness and Response Act (US Congress 2002) and enhanced through the 2011 FSMA, specifically section 204 (FDA 2014). The European Union Commission has similar 1-up, 1-down requirements, but is exploring more robust traceability requirements (Zhang and Bhatt 2014). Detailed in Tables 10.3 and 10.4 are an assessment of food traceability regulations of major economies. Table 10.3 shows the metrics for Table 10.4's ratings. Overall, the countries analyzed have robust traceability regulations, with China having poor traceability and the US and Canada having average ratings.

10.8 Industry Initiatives

Because regulations generally do not dictate how traceability systems work, other than prescribing a 1-up, 1-down capability, industry has significant responsibility in creating frameworks which support interoperability and move towards streamlined traceback and recall management. Within each sector, there are processes and events that may require special documentation and guidance in the framework. Each food commodity group (e.g. produce, grain, animal proteins) may be stored, transported, and processed in such a way that needs unique consideration when drafting the framework. For example, grains are often stored in bulk grain elevators, making identification post-storage difficult to impossible. Produce is frequently repacked or comingled with other product. Table 10.5 shows a non-exhaustive list of

Table 10.3 Traceability assessment questions

1	Are there specific regulations/policies on national level for domestic products? When did these policies come into effect?
2	Are there specific regulations/policies for imported products? What documents required for import products to address traceability?
3	What is the clarity of the system of authority responsible for traceability regulations?
4	If no specific regulations, are there voluntary practices by industry?
5	What products or commodities are being regulated for traceability?
6	What kinds of identifiers are being used for tracking/registering of imports (e.g., ear tags, barcodes, RFID)?
7	Are Global Food Safety Initiative (GFSI) benchmark standards recognized?
8	Are GS1 services (i.e., traceability tools and coding standards) available?
9	Is there an electronic database system used for monitoring imports/export and their traceability? Are these systems accessible by importing countries?
10	What information on packaging labels is available for the consumer to understand traceability?

Adapted from Charlebois et al. (2014)

Table 10.4 Comprehensiveness of traceability regulations

	1	2	3	4	5	6	7	8	9	10	Aggregate Score
Australia	M	M	P	P	M	P	P	P	P	P	Average
Austria	P	P	P	P	P	P	P	P	P	P	Superior
Belgium	P	P	P	P	P	P	P	P	P	P	Superior
Brazil	M	M	P	P	M	P	P	P	P	P	Average
Canada	M	M	M	P	M	P	P	P	R	P	Average
China	R	R	M	M	R	P	P	M	R	M	Poor
Denmark	P	P	P	P	P	P	P	P	P	P	Superior
Finland	P	P	P	P	P	P	P	P	P	P	Superior
France	P	P	P	P	P	P	P	P	P	P	Superior
Germany	P	P	P	P	P	P	P	P	P	P	Superior
Ireland	P	P	P	P	P	P	P	P	P	P	Superior
Italy	P	P	P	P	P	P	P	P	P	P	Superior
Japan	M	P	P	P	P	P	M	P	P	P	Average
Netherlands	P	P	P	P	P	P	P	P	P	P	Superior
New Zealand	M	M	P	P	M	P	P	P	P	P	Average
Norway	P	P	P	P	P	P	P	P	P	P	Superior
Russian Federation	N	N	N	N	N	N	N	R	N	R	Insufficient Data
Sweden	P	P	P	P	P	P	P	P	P	P	Superior
Switzerland	P	P	P	P	P	P	P	P	P	P	Superior
United Kingdom	P	P	P	P	P	P	P	P	P	P	Superior
United States	M	M	M	P	R	M	P	P	R	P	Average

P = Progressive, M = Moderate, R = Regressive, N = No Data

Adapted from Charlebois et al. (2014)

pre-competitive industry initiatives, their relevant region, and a brief description. Table 10.6 shows a non-comprehensive list of global food standards which include traceability in their audits.

10.9 Case Study in Whole Chain Traceability

Below is a case study in traceability from farm (in this case, farmed fish) to retailer. The simplified supply chain shows how traceability is kept from farm through processing and distribution. To trace and track product as it moves through the supply chain, a color-coded box corresponds to each CTE is used (green for origin, blue for transportation, red for transformation, and yellow for retail/depletion) (Fig. 10.2). To illustrate what information (KDEs) has been added or changed at each CTE, the text is italicized (Tables 10.7, 10.8, 10.9, 10.10, 10.11, and 10.12). In seafood traceability, the company wanted to demonstrate the sustainable practices of its aquaculture facilities as well as differentiate itself as a premium product to its

Table 10.5 Pre-competitive industry initiatives

Initiative	Region	Description
Global Dialogue on Seafood Traceability	Global	Business-to-business framework for determining KDEs, CTEs, and IT architecture for global seafood economy to address IUU fishing, social issues, and traceback capabilities.
GS1 Global Standards	Global	GS1-led initiatives to create best practices and standards for food traceability. Initiatives include: Global Traceability Standard for Fish, Seafood, and Aquaculture Global Meat and Poultry Traceability Wine Traceability Traceability for Fresh Fruits and Vegetables
GS1 US	US	US-specific food traceability standards US Retail Grocery Initiative US Foodservice US Fresh Foods, including Deli, Dairy and Bakery; Meat and Poultry; Produce (Produce Traceability Initiative); and Seafood
GS1 Canada	Canada	Can-Trace: initiative with guidance on multi-ingredient, beef, pork, produce, and aquaculture production.
Cattle Trace	US	US-based industry-driven pilot for US cattle traceability to address animal health.
International Standards Organization (ISO)	Global	ISO 12875: Traceability of finfish products — Specification on the information to be recorded in captured finfish distribution chains ISO 12875: Traceability of finfish products — Specification on the information to be recorded in captured finfish distribution chains ISO 22005: Traceability in the feed and food chain — General principles and basic requirements for system design and implementation
AgGateway	North America	Traceability initiative for commodity grains.

customers. Negative consumer and public perception of aquaculture salmon has spurred the hypothetical company, Pacific's Best, to adopt a robust, whole-chain traceability system. These data may be organized into a file format, such as Comma Separated Value (CSV), eXtensible Markup Language (XML), or JavaScript Object Notation (JSON).

When devising a traceability system, mapping the business process (Fig. 10.2) and determining which KDEs are collected at each CTE are critical to developing data collection best practices and adopting an interoperable framework for communicating traceability information with supply chain partners.

The commissioning event (Table 10.7) occurs at harvest with all KDEs being filled out at this step including the product ID, batch/lot, product name, weight, location, catch beginning and end dates, and country of origin. For traceability

Table 10.6 Third party schemes

Third Party Scheme	Description
Global Food Safety Initiative (GFSI)	GFSI coordinates alignment and benchmarking of global food safety certification schemes including traceability requirements.
Marine Stewardship Council (MSC)/Aquaculture Stewardship Council (ASC) (https://www.msc.org, https://www.asc-aqua.org)	MSC/ASC are certification bodies for wild caught and aquaculture seafood for sustainability and best practices.
BRC (https://www.brcgs.com)	BRC is a global food safety standards organization which emphasizes traceability as part of its certification.
SQF Institute (https://www.sqfi.com)	International food safety certification entity based on Hazard Analysis of Critical Control Points (HACCP). Facilitates assured safety between buyers and suppliers including traceability.
PrimusGFS Standard (http://www.primusgfs.com)	Produce-based food safety initiative based on Good Manufacturing Practices (GMPs), Good Agricultural Practices (GAPs), HACCP, and Food Safety Management Systems (FSMS).
Global Aquaculture Alliance (https://www.aquaculturealliance.org)	Broad certification of aquaculture products encompassing food safety, sustainability, social responsibility, animal welfare, and traceability.
Global G.A.P (https://www.globalgap.org/)	Global standard for GAPs covering crops, livestock, and aquaculture.
FSSC 22000 (http://www.fssc22000.com/)	Food safety certification based on International Standards Organization (ISO) standards.
CANADAGAP (https://www.canadagap.ca/)	Canadian standard for GAPs in fruits and vegetables from production to retail.
Global Red Meat Standard (https://grms.org/)	Food safety and hygiene standard specific to meat production of sheep/lamb, pork, beef, goat, and horse.
Japan Food Safety Management Association (https://www.jfsm.or.jp/eng/)	Japan-focused general food safety standard for small and medium sized enterprises compatible with other global food safety schemes.
IFS (International Featured Standards) (https://www.ifs-certification.com/)	Managing food standards for storage and loose foods.
Japan Gap Foundation (ASIAGAP)(https://jgap.asia/en/home-2/)	Japan-focused food safety standard focused on fruits and vegetables, tea, grains, and livestock.

systems, this step often has the least advanced digital technology adopted. The origin information is critically important to traceback investigations, as recent outbreaks among Romaine lettuce have shown (Gottlieb and Ostroff 2018). As the product moves from Tables 10.7, 10.8, 10.9, 10.10, and 10.11, new or changed information will appear italicized.

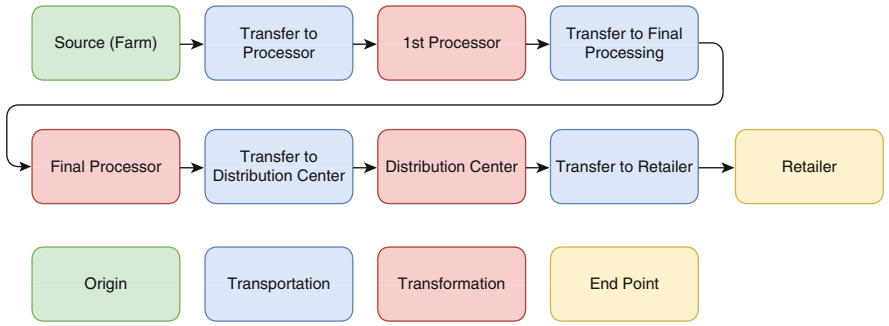

Fig. 10.2 Pacific's best supply chain

Table 10.7 Commissioning event

KDE Categories	Source (Farm)
When (DD.MM.YYYY)	*15.05.2018*
Who (Legal Identity)	*Legal Entity or ID*
Where	*Geocoordinates or Address* *Location Description (Farm)*
What	*Product ID* *Batch/Lot: BTA03* *Product name: Unprocessed Fish* *Unit of Measure: 3000 KG*
Catching End Date	*16.05.18*
Country of Origin	*Country*

Table 10.8 Sending event 1

KDE Categories	Transportation Example (Transfer to 1st processor)
When (DD.MM.YYYY)	*15.05.2018*
Who (Legal Identity)	Legal Entity or ID
Where	Geocoordinates or Address Location Description (Farm)
What	Product ID: 1234567890123456 Batch/Lot: BTA03 Product name: Unprocessed Fish Unit of Measure: 3000 KG
Sender	Location ID Location Description (Farm)
Receiver	*Location ID* *Location Description (1st Processor)*

Table 10.9 Transforming event

	Transformation Example (1st Processor)
When (DD.MM.YYYY)	*16.05.2018*
Who (Legal Entity)	*Legal Entity or ID*
Where	*Geocoordinates or Address* *Location Description (1st processor)*
What	(Input) Product ID Batch/Lot: BTA03 Product name: Unprocessed Fish Unit of Measure: 3000 KG
	(Output) *Product ID* Batch/Lot: BTA03 *Product name: Processed Fish, 1000 Cases* *Unit of Measure: 1000 Pieces*
Country of Origin	Country
Best Before Date	*26.05.2018*
Frozen Yes/No	*No*
Farms of Origin	*Geolocation(s)*

At sending events, the product ID and lot information stays the same, but dates and times are recorded and sender and receiver are documented to note the transfer of product. Transportation events ensure the chain of custody and are CTEs due to change in ownership.

The transformation event (Table 10.9) must account for the input and output at that CTE. During this step, the product ID changes to account for the processing while incorporating ingredient batch/lot numbers, such as in Table 10.10. Food safety related information, such as best before date and frozen status, may also be added here. To account for the geography of the product, geolocation IDs are given for the farms of origin.

Table 10.11 shows the final step of the supply chain, the receipt at the retailer from the distribution center. These final 3 transportation steps, from secondary manufacturing to distribution to retailer are critically important in foodborne disease outbreaks, because they are essential to ensuring that product is removed from the marketplace traced forward from the convergence point.

The above case study is a simplified supply chain but illustrates the process and core concepts to devising whole-chain traceability. For food safety, commissioning events are essential for when a point-source contamination occurs, and so recalls may be more precise than narrowing down to the growing region. Transformation events, such as Tables 10.9 and 10.10, are important to account for aggregation of multiple sources or combining ingredients for consumer packaged goods. Finally, distribution and retail CTEs are needed to find and discard products in the event of a recall.

Table 10.10 Transformation example (Final Processor)

	Transformation Example (Final Processor)
When (DD.MM.YYYY)	*16.05.2018*
Who (Legal Identity)	*Legal Entity or ID*
Where	*Geocoordinates or Address* *Location Description (Final Processor)*
What	(Inputs) *Product ID* *Batch/Lot: BTA03* *Product name: Unprocessed Fish* *Unit of Measure: 3000 KG* *Product ID* *Batch/Lot: XYZ33* *Product name: Spice Mix* *Unit of Measure: 100 KG* *Product ID* *Batch/Lot: ABC45* *Product name: Flour* *Unit of Measure: 10000 KG*
	(Output) *Product ID* Batch/Lot: ZZA99 *Product name: Breaded Fillets, 1000 Cases* *Unit of Measure: 1000 Pieces*
Country of Origin	Country
Best Before Date	*26.05.2018*
Frozen Yes/No	*No*
Farms of Origin	*Geolocation (0987654.00001.0)* *Geolocation (0987654.00002.0)*

Table 10.11 Endpoint example (retailer)

	Endpoint Example (Retailer)
When (DD.MM.YYYY)	*16.05.2018*
Who (Legal Identity)	*Legal Entity or ID*
Where	*Geocoordinates or Address* *Location Description (Retailer)*
What	*Product ID* *Batch/Lot: ZZA99* *Product name: Breaded Fillets, 1000 Cases* *Unit of Measure: 3000 KG*
Sender	*Location ID* *Location Description (Transportation Company)*
Receiver	*Location ID* *Location Description (Retailer)*

10.10 Conclusions and Future Trends

Food traceability is a rapidly evolving area of interest within the food industry due to advancements in technology and demands from customers, retailers, and governmental agencies for more detailed information on food products. By understanding the use cases for traceability, capabilities, and limitations of traceability technologies and the principles of interoperability, it is possible to create a traceability system that is cost effective, mitigates risk, and creates value for products. Employing a KDE/CTE approach to food traceability systems allows one to consider operational necessities while being technology agnostics. Utilizing global industry standards for traceability may increase market access and interoperability of data systems. Regulations primarily promulgate 1-up, 1-down traceability, but the industry and technology growth are moving toward whole chain traceability, or the ability to track and trace a product's and its ingredients' pedigree through the whole supply chain.

The future of traceability may be transformative to the entirety of the agricultural and food system. Digitization of ingredient and product information as well as the aggregation of data will enable predictive analytics to reduce food waste and loss while providing effective traceback and recall capabilities in the event of food emergencies. New embedded sensor devices and networking capabilities will enhance digital linkages of physical product supply chain systems, providing real-time feedback and data. Finally, food traceability standards setting processes will continue to intertwine with digital standards.

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Chapter 11
Product Recalls as Part of the Last Line of Food Safety Defense

Karina Martino, Warren Stone, and Ferhan Ozadali

11.1 Introduction

Even though the US food supply continues to be among the safest in the world, foodborne illness outbreaks or other food safety or mislabeling concerns can trigger the need to recall products. This may be due to an occurrence at a manufacturer's facility, or due to an ingredient obtained from an upstream supplier. Since global implications of recalls might be at different magnitude, the intent for coverage in this chapter will be on the U.S. and other countries that export to the US. US recalls information is also readily available to the consumer through a government website called FoodSafety.gov (www.foodsafety.gov). The latest information regarding recalls (what, where, and when) is available here, with free access, and email subscriptions if consumers want frequent updates.

In Europe and other parts of the world, the food industry heavily relies on food safety certification systems, such as FSSC 22000 for human foods (www.fssc22000.com), and FAMI-QS for animal feed (https://www.fami-qs.org), where both certifications have a strong requirement on recall plans.

The food industry and regulatory agencies that oversee it are committed to making the food supply as safe as possible, as well as to ensuring all food products are properly labeled. They share the common goal of eliminating foodborne illness and preventing the entry of unsafe or mislabeled products into the marketplace. While this is the standard that both industry and government should always be

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striving to meet, it is, however, not possible to completely eliminate risk from the food supply chain. Thus, it remains necessary for all food and consumer products manufacturers to be prepared, on short notice, to efficiently and effectively remove their products from the marketplace when necessary to protect public health.

The Food Safety Modernization Act's (FSMA) *Current Good Manufacturing Practice, Hazard Analysis and Risk Based Preventive Controls for Human Food* (PCHF, 21 CFR Part 117) regulation requires the development of a written recall plan when a hazard analysis identifies a hazard requiring a preventive control. Likewise, FSMA's Preventive Controls for Animal Food (PCAF, *Current Good Manufacturing Practice, Hazard Analysis and Risk Based Preventive Controls for Animal Food, 21 CFR Part 507)* requires a written recall plan as part of a facility's Food Safety Plan. In addition, certain products regulated by the United States Department of Agriculture's Food Safety Inspection Service (USDA-FSIS) are required to develop and maintain recall plans as well.

The Bioterrorism Act of 2002 (Public Health Security and Bioterrorism Preparedness and Response Act of 2002), passed by Congress after the tragic event of September 11, 2001 to enhance the security of the US, includes new traceability requirements regarding both the immediate source of incoming materials and the initial recipients of FDA-regulated products leaving a facility. It also provides FDA with new records access authorities under prescribed emergency conditions.

In 2007, because of several major recalls of FDA-regulated products, the US Congress mandated that FDA establishes a Reportable Food Registry for any food that their use or exposure will cause serious adverse health consequences or death to humans or animals. The Registry requires that FDA-regulated food firms promptly notify FDA when they become aware that such a food has left the manufacturer's control. FSIS has regulations requiring that establishments shipping or receiving an adulterated or misbranded product notify the Agency. More details can be found in Sect. 11.2 of this chapter. FSIS also now posts on its website (USDA FSIS summary of recalls 2018) a list of retail consignees that may have received recalled products. Even before this new FSIS requirement went into effect, the State of California passed a law and issued regulations requiring submission of extensive consignee information to the state by any entity handling recalled meat or poultry products within the state (FDA Commissioner Scott Gottlieb announced in 2018 that FDA will consider releasing consignee info in certain circumstances. https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm621692.htm.)

Before identifying the types of foods involved in recalls, roles and responsibilities of the parties involved in the recall process, some of the definitions for each regulatory agency may be helpful.

Recalls These are actions taken by an establishment to remove an adulterated, misbranded or violative product form the market. This section presents the different definitions used by both agencies, and how they classify recalls:

A. **FDA regulations related to recalls**:
(Enforcement policy: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=7)

- 21 CFR 7.3(g): ***Recall*** means a firm's removal or correction of a marketed product that the Food and Drug Administration considers to be in violation of the laws it administers and against which the agency would initiate legal action, e.g., seizure. *Recall* does not include a market withdrawal or a stock recovery.
- 21 CFR 7.3(j): ***Market withdrawal*** means a firm's removal or correction of a distributed product which involves a minor violation that would not be subject to legal action by the Food and Drug Administration or which involves no violation, e.g., normal stock rotation practices, routine equipment adjustments and repairs, etc.
- 21 CFR 7.3(m): ***Recall classification*** means the numerical designation, i.e., I, II, or III, assigned by the Food and Drug Administration to a particular product recall to indicate the relative degree of health hazard presented by the product being recalled.
  - **Class I** is a situation in which there is a reasonable probability that the use of, or exposure to, a violative product will cause serious adverse health consequences or death.
  - **Class II** is a situation in which use of, or exposure to, a violative product may cause temporary or medically reversible adverse health consequences or where the probability of serious adverse health consequences is remote.
  - **Class III** is a situation in which use of, or exposure to, a violative product is not likely to cause adverse health consequences.
- There are also requirements for a written **recall plan** in the Preventive Controls for Human Food (21 CFR Part 117.139) and Preventive Controls for Animal Food (21 CFR Part 507.38).
  - Preamble to Part 117: "*Each facility subject to the rule must have a recall plan for a food with a hazard requiring a preventive control*."

B. **USDA regulations:**

- FSIS Directive 8080.1, Revision 7, 9/9/13, (https://www.fsis.usda.gov/wps/wcm/connect/77a99dc3-9784-4a1f-b694-ecf4eea455a6/8080.1.pdf?MOD=AJPERES.)
  - **Recall:** A firm's removal of distributed meat or poultry products from commerce when there is reason to believe that such products are adulterated or misbranded under the provisions of the Federal Meat Inspection Act or the Poultry Products Inspection Act. "Recall" does not include a market withdrawal or a stock recovery.

- **Market Withdrawal:** A firm's removal or correction, on its own initiative, of a distributed product that involves a minor company quality program or regulatory program infraction that would not result in the product being adulterated or misbranded. For example, product does not meet company quality standards because of discoloration.
- **Stock Recovery:** A firm's removal or correction of product that has not been marketed or that has not left the direct control of the firm. For example, product is located on the premises owned by the producing firm or under its control.
- **Recall Classifications:** FSIS assesses the public health concern or hazard presented by a product being recalled, or considered for recall, whether firm-initiated or requested by FSIS, and classifies the concern as one of the following:
  - **Class I:** This is a health-hazard situation where there is a reasonable probability that the use of the product will cause serious, adverse health consequences or death. Examples of a Class I recall include the presence of pathogens in ready-to-eat meat or poultry products, or the presence of *Escherichia coli* O157:H7 or non-O157 Shiga toxin-producing *E. coli* (STECs) in raw ground beef.
  - **Class II:** This is a health-hazard situation where there is a remote probability of adverse health consequences from the use of the product. An example of a Class II recall is a recall because of the presence in a product of very small amounts of undeclared allergens typically associated with milder human reactions, e.g., wheat.
  - **Class III**: This is a situation where the use of the product will not cause adverse health consequences. An example of a Class III recall is the presence of undeclared, generally recognized as safe, non-allergenic substances, such as excess water in meat or poultry products.

11.2 Types of Food Covered by Each Government Agency

A. **Foods for which USDA has recall authority:** the FSIS inspects and regulates meat, poultry, catfish and processed egg products produced in federally inspected plants.

- Summary of recalls from USDA regulated products are shown in Table 11.1 (USDA-FSIS 2018):

B. **Foods for which FDA has recall authority:** all foods not regulated by the USDA.

Table 11.1 USDA FSIS summary of recalls 2018

Total		Number of Recalls	Number of Pounds Recalled
		125	20,552,911
Recalls by Class (N = 125)			
Class	I	97	19,328,046
	II	21	1,199,689
	III	7	25,176
Recalls by Reason (N = 125)			
Reason For Recall	STEC[a]	9	287,758
	Listeria monocytogenes	21	4,127,696
	Salmonella	6	12,963,341
	Undeclared Allergen	26	446,138
	Extraneous Material	23	1,587,250
	Processing Defect	8	88,285
	Undeclared Substance	3	4377
	Residue	1	69,016
	Unapproved Substance	1	15,363
	Other[b]	27	963,687
Recall by Species/Product (N = 125)			
Species	Beef	31	13,185,563
	Mixed	32	5,267,834
	Pork	25	670,369
	Poultry[c]	34	1,214,839
	Siluriformes fish (catfish)	3	214,306

[a]STEC includes recalls due to Shiga toxin-producing *E. coli* (STEC). STEC organisms include *E. coli* O157:H7, *E. coli* O26, *E. coli* O45, *E. coli* O103, *E. coli* O111, *E. coli* O121, and *E. coli* O145

[b]Other includes producing without inspection, failure to present for import inspection, and labeling issues, among others

[c]Poultry includes egg product

- Based on the FDA Recall Enterprise System (RES, Open FDA 2019), from 2004-present (date of last accessed to website), recall classifications are as follows:
  - Class I – 42%
  - Class II – 52%
  - Class III – 6%

C. In 2018, the leading cause for allergen recalls was milk, for recalls due to microbial contamination was led by Listeria, and about half of the recalls due to foreign material were caused by plastics (Food Safety Magazine 2019).

11.3 Industry Responsibility

Each government agency has their own procedures and requirements. This section presents different terminologies and definitions commonly used by the agencies and the food industry.

A. **FSIS jurisdiction:** Recalls are initiated by the manufacturer or distributor of the meat or poultry items, sometimes at the request of FSIS. The recalling firm is responsible for conducting the recall and for ensuring that its actions have been effective in removing the product from the marketplace. Recall activities by the firms are to start immediately upon deciding to conduct a recall or upon receiving notification of a recall. All recalls are voluntary. However, if a company refuses to recall its products, then FSIS has the legal authority to detain and seize those products in commerce.
B. **FDA jurisdiction:** Part 7.3 defines the specific responsibilities are delineated in 21 CFR Part 7 Subpart C - Recalls (Including Product Corrections) - Guidance on Policy, Procedures, and Industry Responsibilities.
C. **Terminating a recall:** A recall is considered terminated when FDA or FSIS determines that all reasonable efforts have been made to remove or correct the violative product in accordance with the original recall strategy, and when all possible products subject to the recall have been removed from distribution and proper disposition and/or correction has been made by the manufacturer. Recalls overseen by FDA are officially over when the agency sends a written notification to the recalling firm that the recall is terminated. The recalling firm may initiate the recall termination process by submitting a final recall status report to the District Recall Officer, making the case that the recall has been carried out effectively under criteria established in the recall strategy. The FDA needs to terminate the recall within 3 months after the firm completes the recall (FDA Recall Procedures, Chap. 7). FSIS will terminate a recall when it has completed its recall effectiveness checks and determined that the recalling firm has made all reasonable efforts to recall the product, and that proper disposition of the recovered product has been completed, or the product is under FSIS control (retention or detention) or documented control by the firm. To affect a timely termination of the recall, the firm should, upon its own determination that all possible product has been retrieved, provide all relevant information to the relevant FSIS District Office in the form of a "closeout memo" containing a list of customers, the amount of product retrieved, and the actions taken. Once the agency determines that the firm has made all reasonable efforts to recall the product, FSIS will notify the firm in writing.
   Upon termination of a recall, the recalling firm should notify all brokers, distributors, and retail customers. This communication should include an expression of thanks for their assistance and reassurance that the problem has been identified and corrected and that product currently in distribution is not involved in the action.

D. **Lessons Learned**
In the wake of any and every recall conducted by a firm, there should be a deliberation process for identifying lessons learned from the experience that can lead to continuous improvement and better, faster, more efficient recall efforts in the future, if that should become necessary. The greatest benefit from this review effort results from close examination, not just of the recalling firm's actions, but from collaboration exploration of the actions and interactions of all the key supply chain participants in the recall.

11.4 Recall Plan Recommendations

The public health of consumers in addition to a company's reputation are put on the line in a recall situation. Therefore, it's extremely important for a firm to have its recall actions planned and spelled out in advance. Waiting for the recall to occur and ultimately making up the plan as recall events unfold is a recipe for disaster. Resources spent in advance to obtain company recall preparedness by having a written recall team with designated members carrying out distinct functions is a wise investment.

A. **Company Recall Preparedness**
Every consumer products manufacturer must be prepared to deal with product emergencies. With focused effort in advance, firms will be able to respond without hesitation or unnecessary delay when the need to recall a consumer product arises. Prior planning will facilitate expeditious action to:

1. identify the problem and evaluate its significance,
2. notify all key stakeholders, including regulatory agencies, consumers and customers,
3. assure expeditious product removal and disposition,
4. replace product, and
5. analyze lessons learned

A key first step is to organize a recall team. The recall team should help prepare a recall plan that will detail the actions to be taken so that the company can rapidly and effectively address any type of situation that could require removal of product from distribution. The recall plan may or may not be a part of a broader company crisis management plan. Each company needs to decide how to organize its recall team. For example, oversight and coordination of a recall may be done at the corporate level, but actual production and shipping records may be coordinated at the factory or distribution center level. Regardless, recall teams should be organized in such a way as to connect with all levels of the supply chain.

B. **Recall Team**
A well-prepared company recall team can help assure that recalls and withdrawals are handled quickly, smoothly, and with the least possible disruption

to ongoing company operations. The actual composition of the recall team, and the roles and responsibilities of each member, need to be determined by each company. The recall team generally includes representatives with knowledge and authority regarding production, quality assurance, marketing, distribution, customer relations, finance, communications, and legal matters, and may include outside consultants as appropriate. In most cases involving corporations, the recall team reports directly to a senior line manager, although in some cases, the Chief Legal Counsel, Chief Compliance Officer, or the Senior Product Safety executive might be highest authority. Facility-specific recall teams generally report to the most responsible individual at the establishment (Hayman and Hontz 2014).

1. **Recall coordinator.** One person on the team should be identified as the recall coordinator (or recall team leader, in some companies), who will be responsible for initiating and coordinating safety or quality investigations, managing the decision-making process, and overseeing all activities related to recalls or withdrawals at that particular location. Many other duties that may fall to the recall coordinator are discussed below. An alternate recall coordinator should be identified to lead the team in the absence of the primary coordinator. In addition, for any recall or withdrawal operation, the recall coordinator or leader may designate a different individual to execute specific actions, oversee follow-up and effectiveness checks, and report to the regulatory agency with jurisdiction over the product being recalled.
   The recall coordinator should be knowledgeable about every aspect of the company's operations, including purchasing, production, quality assurance, and distribution.

   The recall coordinator should be empowered by company management to convene meetings of the recall team and other key company personnel whenever the need arises, regardless of other activities that may be underway.

   The recall coordinator is responsible for overseeing the development of an action plan for dealing with a recall situation and for presenting that plan to top management. The recall coordinator also must assure the systematic recording and maintenance of facts about each situation in a master file that will ultimately contain all details and decisions made about the recall and other relevant actions taken by the company, including all contacts and copies of all information transmitted and received.

   The recall coordinator will typically provide overall coordination of timing and contacts, assume responsibility for keeping the company media contact informed, and coordinate contacts with regulatory officials. When a recalling firm concludes that all reasonable efforts have been made to retrieve the product, the recall coordinator may also request termination of the recall by the overseeing regulatory agency. However, FDA or FSIS will make a final decision about termination or official closure of a recall.

   The recall coordinator also has responsibility for conducting "mock recalls" and coordinating recall team activities as necessary to maintain an

up-to-date, functioning recall plan. The recall coordinator should assure that all members of the recall team at every level of the company are familiar with the company's recall plans and policies, and have received up-to-date training in recall preparedness. Mock recalls are not a regulatory requirement though they are required by many third-party auditing standards.

The recall coordinator, in conjunction with company management, should identify additional personnel to participate on the recall team, when a special need arises.

2. **Other recall team members.** The following are key company functions that are commonly represented on the recall team, along with some typical responsibilities for the team members from those functional areas. Each individual member of the team should have a designated alternate with a working knowledge of the required discipline. A single alternate for the entire team might not have sufficient knowledge of critical information to be effective during a recall. For example, a well-trained technical service person might be a suitable alternate for plant quality assurance, corporate quality systems or regulatory compliance, but may be an inadequate substitute for the distribution manager.
3. **Key company functions:**

   (a) **Plant/operations management.** A team member from plant operations or plant management should identify all potentially affected lots, including quantities of finished goods, rework and work-in-progress, dates produced, and codes; provide all production and quality assurance records for the lot(s) in question; maintain distribution information; identify inventory under plant control; halt production, as appropriate, until it can be assured that the root cause(s) of the recall has been corrected; and help recover suspect product, if necessary.

   (b) **Scientific/technical/quality assurance.** A team member versed in scientific, technical or quality assurance issues for the firm may have several duties. They will obtain samples, if necessary, and oversee the technical investigation of the problem; investigate the root cause and review technical records to determine the extent of the problem. Additionally, they may determine required analyses for suspect lots and future production, if any; determine the need for third-party testing, work with suppliers in the event that problems may be related to incoming ingredients or supplies; collect technical information for presentation to regulatory agencies. If necessary, they also can determine disposition of affected product; handle all inquiries of a medical nature; put any implicated in-house product on "hold" status. It is highly recommended to physically segregate and conspicuously mark "hold" product, not simply "restrict" it from shipment in an electronic system. Finally this team member must assure that the company is prepared for an intensive investigation by regulatory officials, depending on the seriousness of the recall situation.

(c) **Distribution/inventory control/supply chain management.** Someone intimately knowledgeable about product distribution, inventory control or supply chain management will be needed on the team. This team member will halt all in-transit shipments of questionable product; prepare inventory and distribution status showing how much product was produced, what was shipped, to whom, where and when; arrange for return of affected product, if appropriate, to collection points; aid in the segregation and isolation of any suspect product; and be responsible for physical recovery of suspect lots from distribution centers and warehouses as appropriate.

(d) **Consumer affairs/relations.** This team member will arrange staffing to handle consumer inquiries and assure the preparation and maintenance of records about the specific nature and content of consumer calls (including any complaints of alleged illness), along with the names, addresses, phone numbers and emails of callers. It will be important for this person to be alert for any mention of consumer contact with a member of the legal profession, government agencies, or media outlets, as well as for any action by a store or retail chain to remove product from store shelves without being requested to do so.

(e) **Finance/accounting.** Someone from finance or accounting may be assigned to the recall team to set up account codes to properly assess the financial impact of the recall; including costs for recalled product, as well as costs for return, replacement, or destruction of product.

(f) **Legal counsel.** The company would be well-advised to assure that legal counsel is an integral member of the recall team and is engaged throughout the entire recall process. Such an arrangement will assure that all communications concerning a potential recall remain confidential until such time as authorized communications are released and authorized contacts with regulators are initiated. The company should be guided in its dealings with regulatory agencies by the legal counsel well versed in food law and experienced in dealings with FDA, USDA, and, if appropriate, the Consumer Product Safety Commission (CPSC). Counsel will advise regarding the need for a recall and the scope of products implicated based on regulatory requirements, as well as assist in recall planning and execution. The council should be engaged in the wording of any prepared statement or recall press release; the handling of consumer complaints and possible litigation; discussions with regulatory officials; confirmation of the status of product liability and product recall insurance coverage; and notification of the insurance carrier, if appropriate. If such counsel is not available in-house, provisions for retaining such counsel should be made in advance and spelled out in the plan.

(g) **Public relations/communications.** It is important that the company plans for immediate access to experienced communications experts, who are accustomed to handling media relations in a crisis-management context. Typical public relations functions include preparing scripted responses or

answers to anticipated questions for telephone conversations with consumers; setting up a toll-free consumer telephone hotline and/or a special website, if necessary; preparing all news releases and statements for the media, employees and shareholders and identifying sources of media training for the sole designated company spokesperson, preferably, or for any other personnel who may have occasion to deal with the media. If personnel with this expertise are not available within the company or corporate structure, then a relationship with such professionals should be established before, rather than after, such assistance is needed, and details of the relationship should be included in the plan.

(h) **Sales and marketing.** A team member from sales and marketing will communicate orally and in writing with customers to whom suspect product was shipped and request that the product be removed from sale and isolated for later disposition; advise customers that if they have further distributed the product, it is their responsibility to notify their downstream customers of the recall instructions; assist in pick-up of small case quantities and delivery of suspect product to collection points; hire temporary help, if necessary, to assist in recovering product; arrange financial credit and stock replacement for customers; make up and distribute to retailers any point-of-purchase materials or posters necessary for recall effectiveness; conduct in-store effectiveness checks to verify product has been removed from the shelves; and help restore brand image after a recall.

C. **Team Responsibilities.**

Each member of the recall team should have a current list of office, home and mobile phone numbers, fax numbers, and e-mail addresses (as appropriate) for every member of the team. The list should include the appropriate regulatory contacts. For example, the FDA district office emergency number and the number for the FDA district recall coordinator; and/or the FSIS inspection personnel, the FSIS District Manager for the district in which an FSIS inspected establishment or the company headquarters is located, and the FSIS Recall Management Division in Washington, D.C.

The recall team has a number of key responsibilities:

1. Review existing operating procedures (e.g., production, quality assurance, distribution, etc.) and recommend any changes that will lessen the probability of having to recall or withdraw defective products from distribution and/or that will make product retrieval or disposition easier when necessary.
2. Review and revise existing product recall procedures, as appropriate, or, if no recall plan exists, expeditiously develop a written, thorough, and comprehensive plan.
3. Submit the revised or new recall plan to the appropriate company official for approval.
4. In the event of any problem that could involve the need to remove product from commerce, promptly assess the situation by identifying and collecting

the information and data required to determine if the potential problem is real and, if so, by conducting a health hazard evaluation.
5. Determine the scope of the problem and develop a solid and defensible basis for distinguishing between implicated product and unaffected product.
6. Recommend to management whether a recall is warranted and, if so, the steps to be taken to recover, recondition, re-label, or destroy affected product.
7. Manage any stock recovery, market withdrawal, or recall, including communications with suppliers and/or consignees, government agencies, trade associations, legal counsel, news media (see details regarding a company spokesperson below), and consumers.
8. Assure that any required notifications to federal or state regulatory authorities are fulfilled in accord with regulatory expectations.
9. As required by regulations or as prudent for maintenance of good working relationships, keep the responsible regulatory agency informed of company plans as the recall unfolds.
10. Make sure that once a problem that could lead to a product recall is identified, the recall coordinator or someone else specifically identified for that task is the sole contact person for all discussions with the regulatory agencies and that detailed records documenting these contacts are maintained.
11. Keep appropriate employees and customers informed of actions being taken by the company. It is better that customers and company workers hear about a recall directly from the company than from newspaper, radio, internet, social media or television accounts.
12. Develop a plan for timely replacement of recalled product on store shelves, while avoiding the potential for confusion over distinguishing replacement product from recalled product.
13. After each recall situation, review the actions taken, assess the effectiveness of the plan and the performance of team members, and recommend improvements or enhancements, as appropriate.
14. Attempt to identify controls or procedures that can prevent similar incidents in the future.

D. **Company Recall Plan**

Each food company should have an overall recall plan that fully considers unique features of the company and the affected facility related to production, inventory, distribution procedures, existing personnel, etc. Should the need for a product retrieval action arise, this plan establishes a framework for proceeding. It should be a "living" document that will be updated as needed. The plan should be reviewed at least annually and any time there are significant changes in the company organizational structure, personnel, product line, or areas of distribution, or when a "mock" or genuine recall indicates a need.

A readily accessible and current list of supplier contacts should be maintained. The recall plan should include names, responsibilities and contact information for all essential personnel within the company and for external attorneys,

laboratories used for product analyses, scientific experts (e.g., toxicologists, microbiologists, chemists, physicians, food allergy specialists), trade associations, public relations firms, and other outside support elements whose prompt assistance may prove essential in a crisis situation.

1. **Laboratory services.** The recall plan should specify the conditions under which the company will utilize internal or outside laboratories for product testing and other analytical work that must be performed quickly to help define the nature and scope of the problem. At least one competent laboratory should be identified in advance for likely tests that may be required. Since time may be at a premium during a recall situation, it is a good practice to have detailed contact information, special sample handling requirements, delivery requirements, and account numbers for overnight couriers readily available. Recall plan details regarding sample testing should specify the method of analysis, if appropriate [e.g., The Association of Official Analytical Chemists (AOAC), US FDA's Bacteriological Analytical Manual (BAM)], the source of origin for samples (e.g., warehouse, Quality Assurance library, consumer's cupboard), and, where appropriate, provisions for protecting the chain of custody.

   Procedures for submitting recall-related samples for analysis should be reviewed by corporate counsel to assure they are consistent with legal protections for confidentiality of information.
2. **Third-party scientific experts.** The recall plan should identify third-party scientific experts who may be needed to provide authoritative statements and information about the particular types of product issues the company may face. For example, in the case of an undeclared allergen in a product, an expert on allergic reactions to food could be invaluable in presenting data to a regulatory agency or in providing accurate information for dissemination to consumers. Likewise, an expert in toxicology can assist in evaluating the effects of potential chemical contamination and a microbiologist or process safety expert can help assess the risks from microbial threats such as from inadequate processing. Finally, a physician may be needed to assist in determining the overall health hazard evaluation of the risk, if any, to human health.
3. **Trade association contacts.** The plan should include the names and phone numbers of trade association staff contacts if the company is a member of one or more associations whose staff have years of experience in assisting member companies in dealing with recalls.
4. **Other expert assistance.** Other experts that may be identified in the plan include people experienced in security and threat analysis, who may be able to help a company determine how to react to a tampering threat, and experts in undercover surveillance of plant and warehouse operations, who may be needed to help identify a perpetrator of in-house sabotage.
5. **Consumer phone call assistance.** Depending on the scope of a recall, a company's switchboard could receive an unusually high number of calls

from consumers seeking information or expressing concerns. Outside services are available that can handle hundreds or even thousands of consumer calls each hour, typically using a toll-free "hotline." Where feasible, these services should be contracted before the need arises, and the recall plan should include contact information and details of the arrangements. At a minimum, the company should identify sources of such services and the recall team should contact them in advance to learn how they can be of assistance when a need arises.

6. **Product retrieval services.** Expert assistance is also available from companies that routinely retrieve recalled product from the marketplace and conduct effectiveness checks on behalf of recalling firms. If there is a chance that the company might need to employ such a service, appropriate contacts and related details should be included in the recall plan.

11.5 Additional Key Considerations for the Recall Plan

The aforementioned fundamental building blocks are critically important. Once they are firmly established a firm needs to ensure the details below can be addressed to ensure rapid response thus maximizing consumer health and company integrity.

1. **Importance of accurate records.** The importance of accurate records detailing production and distribution of products cannot be overemphasized. Good records make it possible to pinpoint the problem and minimize the scope of a recall to as few lot codes and production dates or hours, and as limited a geographical distribution area as possible. In addition, it is recommended that all manufacturers have effective systems for maintaining records that will allow traceback of ingredients, as this might be needed to determine the root cause of and to resolve some problems.

   In recent years, elevated level of foodborne illnesses such as *E. coli* Romaine lettuce recalls highlighted the need for better tracking and traceability. Blockchain technologies are well-suited to help with the execution of the effective recalls. Recognizing the impact of recalls and their costs should encourage supply chains to increase visibility. Companies with large and complex supply chains, as well as those involving global trades, can turn to blockchain to gain a good end-to-end visibility. Also, the use of blockchain process provides the users with continuous validation of issues by all blockchain users through shared data. If or when a recall does occur, blockchain will minimize the impact on finances and brands by isolating all problems immediately and allowing for quick action of execution (ShipChain 2019).
2. **Maintenance of chain of custody**. The recall plan should make clear that all physical evidences and records related to product complaints must be handled carefully, to assure the integrity of the handling, storing, and testing of product samples or other evidence related to a recall. All evidence needs to be carefully

maintained. Likewise, care must be taken regarding the handling of verification of product disposition, including product destruction.

3. **Records of conversations and communications.** It is a good practice to make a record of communications with regulatory officials or contact with affected customers and consumers. Such records should include relevant facts as well as the date and time of the communication.
4. **Product labels.** For recalls of either FDA or FSIS-regulated products, the recall plan should include provisions for submitting product labels to the agencies for posting on their websites to aid consumers in identifying the specific product being recalled. Including copies of product labels in notifications to customers can also aid retailers in efficient removal of implicated product from their shelves.
5. **Timely and clear communications.** Clear, concise, and accurate communication during a product recall is critical. The recall plan should spell out who in the company is to be notified of the key developments at various stages of the recall or withdrawal process. The plan also should state which outside parties—regulatory agencies, associations, distributors, suppliers, company attorneys, customers, and news media—are to be notified and at what stages of the process.
6. **Company spokesperson**. The recall plan should identify the individual who will be the company's contact with the media in the event print or broadcast news media request interviews about the company's actions. The company spokesperson will be the company's face to the public and must be believable and credible and should have received training in dealing with the media. He or she must have the ability to state facts openly, honestly and concisely. Everyone in management should know who the spokesperson is, and all media inquiries should be directed to that person. No one else should talk to the media about the recall action. The company spokesperson needs to be given all reasonable support and training that he or she needs to serve as the company's voice during a crisis situation.
7. **Practicing the recall – mock recalls**. Once a recall plan is established, the team should periodically conduct practice or "mock" recalls to ensure that the plan really works. These trial runs should test the team's ability to use the recall plan to conduct an expeditious review of records related to receiving protocols, processing operations, raw products, ingredients and containers, and to determine the distribution of a given finished product. Such exercises can also determine a distributor's ability to locate product rapidly. There are companies that can assist in developing scenarios and carrying out activities such as these.

    (a) Mock recalls should test both trace-back and trace-forward scenarios. Management should hold follow-up meetings to assess the effectiveness of the practice recall exercise and to establish goals for enhancing the effectiveness of future recalls. Records of these meetings could be documented for future use. Companies may want to consider a mock recall performance standard to measure themselves against. For example, a 4-hour time limit could be the measure a success for participants in a mock recall exercise involving a potentially hazardous ingredient to account for 100% of the ingredient that might still be on hand, as well as any products in which it was used, whether

in current production or in finished products that have already been shipped (to the first consignee).

(b) Companies may also want to conduct mock recalls utilizing some or all of the backup personnel specified in the recall plan. This will truly test how effective a particular plan is likely to be under a variety of circumstances.

11.6 Cost Implications of Recalls

Recalls are expensive both financially and the impact on the company's good will. To fully grasp the economic impact of a recall, the firms should also be prepared to capture and address associated costs.

(a) Top nine factors to maximize financial recovery (GMA et al. 2011):

(i) Have a financial recovery plan: plan in advance how your company will recover from a recall, including procedures, and specific responsibilities and roles during recovery. Also get the business unit involved and educated about recall and any recovery program

(ii) Appoint a cost recovery leader: the ideal individual will have experience, knowledge, and overall know-how regarding the company itself.

(iii) Clarify recovery goals: the earlier the better, this could speed up and help maintain important relationships for the company.

(iv) Communicate with insurers: communicate with your insurer as soon as possible is highly recommended to secure a favorable outcome.

(v) Prepare an initial estimate: a detailed estimate of the losses within 30–45 days can help the company have a better sense of the magnitude of the losses.

(vi) Maintain detailed and timely documentation of losses: there are key financial and non-financial documentation that the company will need to provide and/or generate depending on the recall and already existing documentation at the company.

(vii) Engage outside service providers: dedicated resources have to be added to the company's cost to help the recovery team, such as a broker, forensic accountant, lawyers, and others.

(viii) Don't delay. Act as soon as possible and keep open communication with insurer.

(ix) Share lessons learned: considered to formalize the process of sharing within the company all lessons learned, this will help a fast recovery in the event of another recall.

11.7 Conclusion and Future Remarks

Brand protection and Public health are two important elements of a sound recall program that has the main purpose of preventing any food safety crises with devastating consequences. This chapter covers only the basics of this critical program that is considered as last line of defense. The intent in this chapter was not covering the details such as examples, templates, and model plans. These are readily available in the literature.

Hearing about recalls in the daily news may be concerning, however, it is also a great feeling to know that the recall mechanism works. Demanding consumer base encourages companies to come up with record level innovative ideas that may further challenge the food safety boundaries. Since the product safety is Industry's responsibility, a robust and sustainable tracking and recall systems have to be established and maintained to enhance the public confidence. Technological developments such as Blockchain applications can be used to improve real time tracking, tracing, and communications.

Traceability is the backbone of the recall programs. Establishing prerequisite programs to be able to track and trace ingredients, packages, and finished products are paramount to establishing a world-class recall program.

For an effective brand protections and utmost public health protection against food safety related issues, a validated recall plans with its supporting prerequisite programs are vital.

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Part III
Plant Layout, Equipment Design, Maintenance

Chapter 12
The Hygienic/Sanitary Design of Food and Beverage Processing Equipment

Ronald H. Schmidt and Helen M. Piotter

12.1 Introduction

In this chapter, the criteria for sanitary/hygienic design of food equipment will be described. The terms (sanitary and hygienic) may be considered interchangeable. Sanitary design is a term primarily used in the United States (US) to describe the key elements recommended for equipment and facilities to provide safe processing for human and animal foods. Hygienic design is the term used more broadly in Europe and many other non-US locations to describe the safe construction of food handling and processing equipment. For harmonization, the term "hygienic design" will be used in this chapter. Multiple interwoven concepts will be addressed here encompassing hygienic design, fabrication, installation, and usage of food equipment. Finally, recommendations will be described for facility design, and for location and operation of the equipment, as well as coverage of regulatory requirements and international industry guidelines and standards.

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12.2 Hygienic Design, Fabrication, and Construction of Food Equipment in the Food Processing Facility

12.2.1 Importance and Role of Hygienic Design in Food Safety Programs

The hygienic design of equipment (and facilities) provides a solid foundation for an effective food safety program and is necessary to fulfill the processor's responsibility to produce safe and high quality food. As shown in Fig. 12.1, hygienic design principles support other food safety related programs including Good Manufacturing Practices (GMPs), Sanitation Programs, and the Hazard Analysis Critical Control Point (HACCP) system.

12.2.2 The Scope of Hygienic Design Programs

As shown in Fig. 12.2, hygienic design programs should be holistic in nature, and include both prescriptive elements – establishing design criteria (see Sect. 12.7) with assurances that equipment conform to these criteria as described, and performance-based elements – expected performance by equipment manufacturers and users.

The performance-based elements of hygienic design include, but are not limited to, the following:

Fig. 12.1 Hygienic Design Provides the Foundation for Effective Food Safety Programs

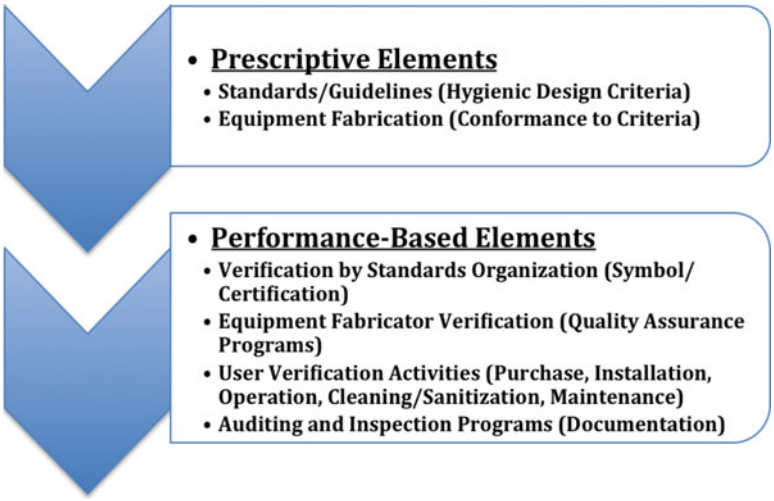

Fig. 12.2 Elements of a Holistic Hygienic Design Program for Food Equipment

12.2.2.1 Performance-Based Elements for Equipment Manufacturers

- Effective verification programs are implemented by standards developing organizations to assure that certified equipment meets specified criteria.
- Effective quality control programs are utilized by equipment manufacturers, with periodic verification to ensure that hygienically designed equipment is manufactured with consistency.

12.2.2.2 Performance-Based Elements for Equipment Users

- Appropriate programs are in place to assure that hygienically designed equipment is purchased, properly installed, and properly located to allow for sanitary operation, effective cleaning and sanitizing regimens, and easy access for observation of cleaning efficacy.
- Appropriate programs are in place to assure that equipment is properly situated to minimize risk of microbial contamination or allergen cross-contact. This would be accomplished by avoiding undesirable conditions that would create risks for environmental contamination of properly cleaned equipment through appropriate placement and/or shielding.
- Appropriate programs are in place to assure that all equipment brought into the facility is properly staged, inspected, and conditioned. This includes any purchased equipment (new or used) and any equipment moved in from warehouses, staging/storage areas, and/or other locations within the facility.

- Appropriate maintenance programs are in place to assure that all equipment is maintained in a hygienic state and that any equipment modifications (if needed) do not compromise hygienic design features.
- Appropriate programs are in place to assure that effective operation, cleaning, and sanitizing programs of equipment do not compromise hygienic design features.
- Appropriate verification and documentation programs are in place to support the items listed above.

12.2.3 The Role of Hygienic Design and Its Importance in Preventing Food Safety Hazards

Prevention of food contamination or cross-contact with known and reasonably foreseeable food hazards (including biological, chemical, or physical) should be the primary goal of a food manufacturing enterprise. Prevention of contamination and establishment of preventive control programs should encompass all levels of the food system from the originating source through distribution. It is imperative that a food processing and handling operation develop a food safety culture throughout and emphasize that prevention of food contamination is the requisite objective that every employee must embrace as their primary goal. Application of hygienic design criteria in equipment purchases, operation, installation, and maintenance provides an excellent basis for this food safety culture, and encourages the invaluable practice of superior hygiene.

12.2.3.1 Biological Hazards

The biological hazards often implicated in foodborne disease are pathogenic microorganisms including viruses, bacteria, and microscopic parasites. Of these, bacteria from food equipment or from the food facility environment, are most often a major concern of contamination . While often overshadowed by other factors in epidemiological reports, or overlooked in epidemiological investigations, poor hygienic design of facilities and equipment may, in fact, be the underlying root cause of foodborne illness outbreaks. Poor equipment design, construction, and/or maintenance may be either a direct, or an indirect, causative factor in foodborne illness outbreaks. Selected foodborne illness outbreaks directly and indirectly linked to hygienic design issues have been discussed previously (Schmidt 2012).

Bacteria may contaminate food products through a variety of sources and vectors in a food facility (e.g, aerosols of liquids and/or contaminants; air; personnel; food contact surfaces; environmental surfaces). Bacterial contamination in a facility may be classified as follows (FDA 2015a, b):

- *Non-persistent Contamination.* These more transient bacteria may directly contaminate food product, but do not persist in the food plant environment.

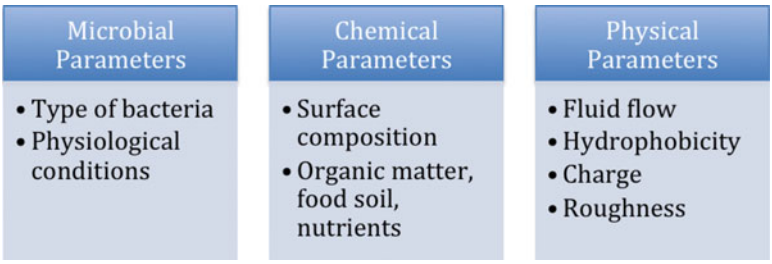

Fig. 12.3 Parameters associated with bacterial adhesion to (and detachment from) food equipment surfaces

- *Persistent Contamination.* The bacteria in this class are predominantly those that have the ability to persist in the food plant environment or as biofilms on food equipment and environmental surfaces. The primary pathogens in this category are the environmental pathogens (e.g., *Salmonella enterica* serotypes*; Listeria monocytogenes*).

Aside from poor personnel practices or direct physical contamination, microbial contamination of food contact areas is a direct result of the inadequacy of cleaning and sanitizing procedures and processes. This risk is higher with poorly fabricated and constructed equipment that is not easily cleanable. If bacteria are allowed to remain, adhere or form biofilms on food contact surfaces (possibly in surface cracks, crevices or micro-fissures), they will contaminate the food. Bacteria may simply adhere to a surface, or they may colonize as biofilms on the surface (Costerton et al. 1985; Faille et al. 2018; Wirtanen and Salo 2016). Once formed, biofilms require rigorous cleaning and sanitizing regimes for their removal. If allowed to persist, biofilms also may attack the surface and impact its hygienic design characteristics.

Adherence, as well as detachment in cleaning, is dependent upon complex microbial, chemical, and physical parameters (Fig. 12.3). These parameters are not necessarily independent and may be synergistic.

The types and characteristics of bacteria present are very important factors in the formation of biofilms. Certain types of bacteria (e.g., *Pseudomonas*) readily form biofilms by extruding extracellular polysaccharides (EPS), which aid in surface adherence of microorganisms. At times, other types of bacteria will assist in the formation of biofilm and/or become a part of a thriving community protected by the polysaccharide materials. Certain bacteria may require seeding points on the surface, or may passively inhabit the biofilm community. For example, *L. monocytogenes* has been shown to inhabit pre-established biofilms by *Pseudomonas* spp. (Puga et al. 2018; Sasahara and Zottola 1993) and *Escherichia coli* (deGrandi et al. 2018). While most research investigations have been with single-species biofilms, it is conceivable that, in food and beverage facilities, combined or multi-species biofilms may be predominant.

The characteristics of food equipment surfaces primarily fall under the physical parameter category as shown in Fig. 12.3. It is well documented that:

- a rough surface is more vulnerable to adherence and formation of biofilm;
- biofilms may form more readily on an inadequately cleaned surface due to the presence of nutrients and attachment sites for biofilm formation;
- the risk of biofilm formation is lower on stainless steel surfaces than on many plastic and rubber surfaces; and
- biofilms are easier to remove from stainless steel than from other surfaces.

A biofilm may form on a very smooth surface depending upon other surface compositional factors, which needs further investigation. There may be some variation between different stainless steels, as well as other materials, due to difference in material composition. For example, the nickel content in stainless steel has been related to the viability of bacteria in biofilms associated with waste treatment facilities (Lopes et al. 2005), and enhanced viability of adhered bacteria has been related to certain chemical components in nitrile rubber (NBR) (Storgårds et al. 1999).

12.2.3.2 Chemical Hazards

Metal or non-metal equipment surfaces meeting hygienic design criteria are less likely to have chemical contamination. However, if equipment is used in a corrosive environment, is not properly maintained, or is fabricated from an improper material, the likelihood of chemical contamination may increase. Improper or poorly maintained non-metal materials (such as plastics and rubber) that do not meet hygienic design criteria may also have the potential for leaching toxic chemicals into the food product, depending upon the composition and other factors. Further, imperfections in the material surfaces, as well as dead spaces, associated with poorly designed equipment may allow ingress of cleaning/sanitizing chemicals, as well as allergenic food residues.

Prevention of undeclared allergens in packaged food products should have a high priority, and regulatory agencies require the use of best practices to avoid improper labeling and allergen cross-contact during processing and handling (Gombis and Anderson 2001; Falci et al. 2001). Greater than a third of food recalls during 2009–2012 were associated with undeclared food allergens (Gendel et al. 2014). The importance of hygienic design in facilities and equipment is highlighted in the recommended best practices for preventing allergen cross-contact in a food manufacturing facility (FARRP 2018).

12.2.3.3 Physical Hazards

The primary sources of physical hazards associated with poor hygienic design of equipment are those hazards arising from the wear and tear that may result in food contamination with wood splinters, bolts, broken screens, wire fragments, and fragments (filings) from materials associated with food equipment. Such hazards

may enter the food directly from product contact surfaces or indirectly from non-product equipment surfaces, environmental surfaces (defined later in this chapter), and the facility environment.

According to FDA regulations (FDA 2005a), a hard or sharp foreign object that measures 7–25 mm in length is considered a choking hazard, especially for children. The presence of foreign particles in this size range in ready to eat foods is food adulteration under Sec. 402 of the *Food, Drug and Cosmetic Act*. However, the agency recommends that food safety plans in food facilities implement control measures to remove particles smaller than 7 mm, and most responsible food manufacturers set a much lower limit.

The likelihood of occurrence of physical hazards associated with food equipment is relatively low compared to physical hazards from other environmental sources such as glass chards and fragments from lights, packaging, and other sources. However, the likelihood may increase with poorly designed and poorly maintained equipment. In addition, certain types of equipment (e.g., slicers; peeling machines; mixers) with intricate design and moving parts can increase the likelihood. Meat and poultry regulations under USDA/Food Safety & Inspection Service (FSIS), emphasize concerns for physical hazards associated with farms, abattoirs, and retail outlets from a variety of sources (e.g., knives; mincers; meat slicers; vacuum bowl cutters; broken injection needles) (Wilm 2012).

It is recommended that the risks and likelihood of physical hazards for each facility be evaluated by doing a thoroughly targeted hazard analysis, and that control measures be developed, documented, monitored, and verified. Appropriate control measures for physical hazards in a food processing or handling facility must consider the following:

- Application of appropriate hygienic design principles in purchasing equipment;
- Proper installation of food equipment;
- A rigorous equipment maintenance program;
- Effective cleaning and sanitizing systems and procedures that minimize the risks; and
- Appropriate procedures for detection and removal.

A variety of detection and removal procedures are available (Anonymous 2015, 2016; Reimers 2012; Wilm 2012). These include in-line filters, magnets, metal detectors, X-ray detectors, and food radar system (FRS) detectors. It is important that the food facility be aware of the advantages, as well as the limitations, of the detection systems used in their facility. Food facilities must also assure that detection/removal devices are installed and operated according to the manufacturers recommendations and are properly maintained. Furthermore, the manufacturer of the detection system shall provide detailed instructions for operation, maintenance, and calibration, and all other appropriate information (e.g., sensitivity; limits of detection; variability) regarding the effectiveness of the detection system in removing potential hazards in the specific food system application. Finally, detection equipment with direct product contact shall meet the criteria of hygienic design.

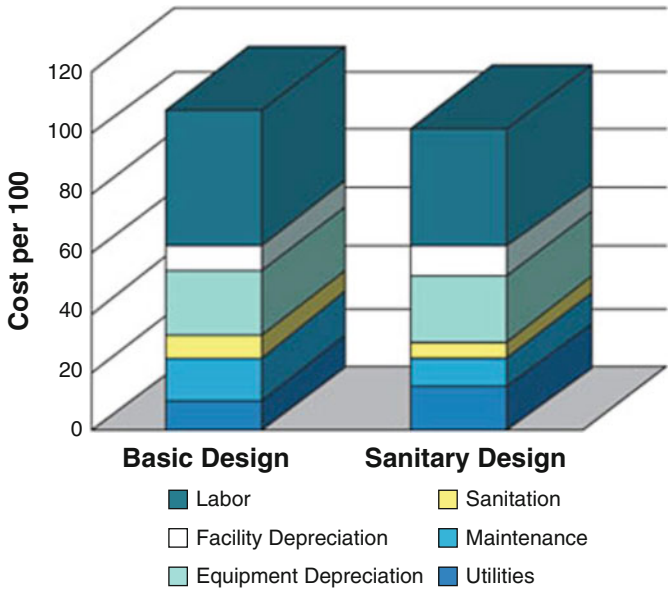

Fig. 12.4 Cost advantages of hygienic equipment. (Source: David C. Dixon, LLC)

12.2.4 The Economics of Hygienic Design Programs

Implementing an effective plan for hygienic equipment (and facilities) is also a sound business decision (Anonymous 2018b). While the initial capital costs may be higher compared to not meeting these criteria, basing the decision only on initial capital cost is short-sighted. A responsible business should consider that improved hygienic design reduces long term operating costs. The illustration in Fig. 12.4, shows that the reduction in annual operating costs associated with improved hygienic design outweighs the impact of amortized capital costs for improved hygienic design in facilities and equipment on a cost/case basis.

12.3 Definitions of Surfaces

There are several categories of surfaces in a food facility that, if not properly designed and maintained, may compromise sanitation programs. These include *product contact surfaces*, *solution contact surfaces*, *non-product contact surfaces* (including *equipment non-product contact surfaces* and *environmental surfaces*), which are generally described here:

12.3.1 Product Contact Surfaces

Traditionally, the primary emphasis of hygienic design principles and criteria has been focused upon *product contact surfaces* (e.g., *food contact surfaces*; *food product contact surfaces*). While there are several general definitions, the most comprehensive is found in 3-A Sanitary Standards (3-A SSI 2018a), in which *product contact surfaces* is defined as *"all surfaces which are exposed to the product and from which splashed product, liquids, or soil may drain, drop, diffuse or be drawn into the product or onto surfaces that come into contact with product contact surfaces of packaging materials"*. Under this broad-based definition, it is clear that the term *product contact surface* involves more than just the primary (or direct) contact surfaces of the equipment (sometimes referred to as "inside the pipe"). This terminology also encompasses certain surfaces that are not in primary contact but still have a high potential for contaminating food during normal processing operations (e.g., shields; covers; related surfaces; conveyor surfaces). Such ancillary surfaces must meet the same design criteria as those for primary contact with food product.

12.3.2 Solution Contact Surfaces

It is critical that solution contact surfaces are evaluated when designing and implementing automated or mechanical cleaning and sanitizing systems. A properly designed and implemented cleaning/sanitizing system will minimize the risk of potential contamination of food products from residual cleaning and sanitizing solutions. Proper design will also negate the possibility of indirect contamination due to leaching of adulterants from the exposed surfaces. *Solution Contact Surfaces* are defined as "*all interior surfaces of the equipment or system, including associated piping, that are used for supplying and recirculating cleaning and/or sanitizing solutions, except those lines only used to supply concentrated chemical cleaners/sanitizers from bulk storage to points of addition to the system"* (3-A SSI 2018a). In most cleaning systems, *solution contact surfaces* shall be considered as *product contact surfaces* with a few specific exceptions.

12.3.3 Non-product Contact Surfaces

Non-product contact surfaces are defined as *"All exposed surfaces from which splashed product, liquids, or other soil cannot drain, drop, diffuse or be drawn into or onto the product, product contact surfaces, open packages, or the product contact surfaces of package components*" (3-A SSI 2018a, b). While direct product contamination is minimized, microbiological contamination or allergen cross-

contact may occur from these surfaces through indirect routes (e.g., airborne or aerosol contamination; personnel; cross-contamination) during food processing and handling activities. The two general sub-categories include:

- *Equipment Non-product Contact Surfaces* – part of equipment (e.g., equipment and piping exterior; legs; supports; motors; housings); and
- *Environmental Surfaces* – includes all other surfaces in a food facility (e.g., walls; ceilings; lights; floor drains; electrical service components; other surfaces).

12.4 Overview of Regulatory Requirements Related to Hygienic Design

Equipment hygienic design is an important component of regulatory inspection programs throughout the world. Many regulatory programs today are based on the performance of the processor in producing safe food, and focus on a variety of performance-based programs and activities (e.g., HACCP, standard operating procedures, documentation, verification), with less prescriptive criteria. Thus, the recommendations for hygienic design are generically written, and the use of third party equipment standards (where available) is not mandated. However, conformance with certain standards is often recognized as meeting the regulatory specifications.

The regulation of the various sectors of the food industry in the U.S. falls under the jurisdiction of federal, state, and local agencies. These agencies provide routine inspection of food facilities, and to a varied degree, these regulatory programs address the general hygienic criteria for food equipment including fabrication, construction, design, and installation. For the sake of brevity and also variations from state to state, only federal requirements are discussed in this chapter. The programs of these agencies are compared with those in Canada and the European Union in the following sections.

12.4.1 U.S. Department of Health and Human Services (DHHS)/Food and Drug Administration (FDA)

The FDA enforces regulations under the *Food, Drug and Cosmetic Act (FDCA)* for the prevention of adulteration and misbranding of all foods in interstate commerce, except for meats, poultry, and egg products. These commodities are regulated by the US Department of Agriculture (USDA)/Food Safety & Inspection Service (FSIS).

12.4.1.1 Approval of Food Contact Materials

Materials used in food contact require FDA approval with regard to toxicity, and may also include restrictions for certain uses and application. FDA approval of these materials, published in the Code of Federal Regulations (CFR) or on the agency website, is as follows:

- *Prior Sanctioned Substance* – documented evidence of a sanction prior to passage of the 1958 Food Additives Amendment of FDCA (21 CFR §181);
- *Generally Recognized as Safe (GRAS) Substance* – 21 CFR§182-186;
- *Indirect Food Additives* – approved through a petition process, in which FDA evaluates data submitted by the manufacture (21 CFR§174-179); and
- *Food Contact Substance (FCS)* – meets the specific definition in FDCA 409(h)(6): *any substance that is intended for use as a component of materials used in manufacturing, packaging, transporting, or holding of food if such use is not intended to have any technical effect in such food.* Acceptance is either under a *Threshold of Regulation Exemption* (prior to 1997), or a *Food Contact Substance Notification (FCN)* and is listed on the agency website (FDA 2018).

12.4.1.2 Inspection of Facilities and Equipment Under Current Good Manufacturing Practices (cGMPs)

Criteria for FDA inspection of food processing and handling facilities have historically been delineated under Current Good Manufacturing Practices (cGMPs) regulations (21 CFR §110). Since these historical regulations were becoming outdated, the FDA set a plan to modernize the cGMPs in the late 1990s, and commissioned an expert panel to evaluate cGMPs (FDA 2005b). Poor plant and equipment sanitation and poor plant design and construction were listed among the top four food safety problems by this expert panel.

Several FDA regulations were promulgated under *FDA Food Safety Modernization Act (FSMA)* of 2011, including *Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food* (FDA 2015a), and *Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Food for Animals* (FDA 2015b). These regulations require that facilities manufacturing human and/or animal food conduct a hazard analysis, and develop and implement a risk-based food safety plan. In addition, the cGMP regulations were updated and moved to 21 CFR §117. Selected general criteria for equipment and utensils (adapted and paraphrased from 21 CFR §117.40) are listed in Table 12.1. Also, the topic of cGMPs is discussed in Chap. 6. Please refer to that chapter for more details.

Table 12.1 Criteria for equipment and utensils under cGMP regulations

Equipment and utensils must be:
Designed and of such material and workmanship as to be adequately cleanable, and adequately maintained to protect against allergen cross-contact and contamination.
Designed, constructed, and used appropriately to avoid the adulteration of food with lubricants, fuel, metal fragments, contaminated water, or any other contaminants.
Installed so as to facilitate the cleaning and maintenance of the equipment and of adjacent spaces.
Food-contact surfaces must be:
Corrosion-resistant when in contact with food.
Made of nontoxic materials and designed to withstand the environment of intended use and the action of food, and, if applicable, cleaning compounds, sanitizing agents, and cleaning procedures.
Maintained to protect food from allergen cross-contact and from being contaminated by any source, including unlawful indirect food additives.
Seams on food-contact surfaces must be:
Smoothly bonded or maintained so as to minimize accumulation of food particles, dirt, and organic matter and thus minimize the opportunity for growth of microorganisms and allergen cross-contact.
Non-product contact surfaces must be:
So constructed that it can be kept in a clean and sanitary condition.
Holding, conveying, and manufacturing systems (including gravimetric, pneumatic, closed, and automated systems) must be:
Of a design and construction that enables them to be maintained in an appropriate clean and sanitary condition.
Compressed air or other gases mechanically introduced into food or used to clean food-contact surfaces or equipment must be:
Treated in such a way that food is not contaminated with unlawful indirect food additives.

Adapted from 21CFR117.40(a) through (g)

12.4.1.3 Hazard Analysis Critical Control Point (HACCP) Programs

FDA requires the HACCP system for seafood (FDA 1995) and fruit/vegetable juice products (FDA 2001). Facilities regulated under these HACCP requirements are exempt from some of the food safety plan portions of the FSMA regulations, but must comply with the revised cGMPs. Under FDA HACCP regulations, facilities are required to develop, implement, monitor, and document written sanitation standard operating procedures (SSOPs) that address eight general sanitation areas. The sanitation areas in this list that relate to food equipment include: conditions and cleanliness of food contact surfaces, prevention of cross-contamination from unsanitary objects to food, food packaging materials, and other food contact surfaces, and protection of food, food packaging material, and food contact surfaces from adulteration with potentially hazardous chemical, physical, and biological agents. The regulations provide flexibility to the processor regarding how they meet the SSOP requirements. The topics of SSOP and HACCP are discussed in Chaps. 7 and 8, respectively. Please refer to those chapters for more details.

12.4.1.4 Federal/State Cooperative Programs

The FDA participates in federal/state cooperative regulatory programs for three food industry sectors including Grade A fluid milk and fluid milk products, retail foods, and shellfish. In general, these programs are cooperative interstate programs in which a designated regulatory agency is identified as the regulatory authority for each cooperative state. Oversight for these time-honored U.S. Public Health Service (USPHS) programs was transferred to FDA in 1968, when the agency was placed under the USPHS. A primary goal of these federal/state programs is to foster uniformity in state and local regulations throughout the US. The federal government develops and maintains model documents that list recommended sanitation criteria for adoption into state regulations and/or local ordinances. In addition, the FDA provides interpretations, guidance, and oversight over these programs. These FDA model documents are described in the following:

12.4.1.4.1 Grade A Pasteurized Milk Ordinance (PMO)

The PMO (FDA 2017b) is the official regulatory document for the *National Conference on Interstate Milk Shipments (NCIMS)*, the cooperative interstate program for inspection and regulation of Grade A fluid milk and milk products. Thus, the PMO has been adopted into the regulatory codes of all cooperating states. The PMO is revised and upgraded every 2 years, with input from state regulatory agencies and dairy industry groups through the NCIMS process.

The PMO provisions related to equipment used for raw milk include:

- Item 9r: Utensils and Equipment – Construction
- Item 10r: Utensils and Equipment – Cleaning
- Item 11r: Utensils and Equipment – Sanitization
- Item 12r: Utensils and Equipment – Storage

The PMO provisions related to equipment used for pasteurized milk and milk product include:

- Item 10p: Sanitary Piping
- Item 11p: Construction and Repair of Containers and Equipment
- Item 12p: Cleaning and Sanitizing of Containers and Equipment
- Item 13p: Storage of Cleaned Containers and Equipment

According to the PMO, *"equipment manufactured in conformity with 3-A Sanitary Standards and Accepted Practices complies with the sanitary design and construction standards of this Ordinance."* In addition, the PMO allows that 3-A Sanitary Standards may be used as a guideline to evaluate equipment not bearing a 3-A Symbol. The FDA and participating state agencies have developed an ongoing equipment evaluation program for equipment used in Grade A facilities. The criteria

used are specified in *Milk and Milk Product Equipment—A Guideline for Evaluating Construction* (FDA 2000), hereafter referred to as *FDA Milk and Milk Products Equipment Guidelines.*

12.4.1.4.2 FDA Food Code

The *FDA Food Code* (FDA 2017a) is a model document for adoption by state and local agencies involved in the inspection of the retail food sector (e.g., commercial food service; institutional food service; grocery stores; delis and related enterprises; vending machines). This document is either adopted into state codes or local ordinances in total, or by adoption of selected provisions. The *FDA Food Code* is updated every 4 years with input from federal, state and local regulatory agencies, as well as the retail food industry through the *Conference for Food Protection (CFP)*. The provisions relating to hygienic design of equipment are described in *4-1 Materials for Construction and Repair* and *4-2 Design and Construction*.

12.4.1.4.3 National Shellfish Sanitation Program (NSSP) Guide for the Control of Molluscan Shellfish

The NSSP Guide for the Control of Molluscan Shellfish (FDA 2015b, 2017c) is the FDA-issued model document provided to states participating in the *Interstate Shellfish Sanitation Conference (ISSC).* The ISSC is a cooperative venture in which states voluntarily use the NSSP Guide (or adopt by reference) in formation of state shellfish food safety regulations. Criteria for equipment used under the ISSC program are found in the *Shellfish Industry Equipment Construction Guidelines* (ISSC 2009).

12.4.2 US Department of Agriculture (USDA)

12.4.2.1 Food Safety & Inspection Service (FSIS)

The FSIS provides regulatory oversight for meat, poultry, and egg product facilities under the *Federal Meat Inspection Act*, *Poultry Products Inspection Act*, and *Egg Products Inspection Act.* Traditionally, FSIS sanitation regulations have been prescriptive, with pre-approval requirements for facility and equipment design, fabrication, construction and installation. In addition, the agency traditionally maintained a list of pre-approved equipment for use in meat, poultry, and egg product facilities.

In the 1990s, the agency moved towards more performance-based regulations with promulgation of HACCP regulations for meats and poultry (FSIS 1996). The HACCP regulations modified existing sanitation regulations at 9 CFR §417, and require the development and implementation of SSOPs. These SSOPs shall describe

all sanitation procedures sufficient to prevent direct contamination and adulteration of products, and address, at a minimum, the cleaning of food contact surfaces of facilities, equipment and utensils. The FSIS has also proposed HACCP regulations for egg products (FSIS 2018).

In 1997, the agency moved further towards a more performance-based regulation and discontinued the pre-approval process (with the exception of labeling requirements) (FSIS 1997a), and published general guidelines used for inspection of facilities and equipment (FSIS 1997b). These guidelines provide criteria for both product contact and non-product contact surfaces of equipment and facilities. With regard to materials used in equipment manufacture and food contact surfaces, manufacturers are required to provide written documentation of compliance with FDA food contact material regulations.

12.4.2.2 Agricultural Marketing Service (AMS)

Several agencies within the AMS provide voluntary grading programs for certain agricultural commodities (e.g., fresh fruits and vegetables, processed fruits and vegetables, poultry, eggs, livestock and meat, and dairy products). A sanitation inspection program is also provided for some of these commodities. The FDA honors this inspection and requires certain reporting provisions in the case of adulterated products. While the sanitation inspection program is a voluntary program, it is required by certain state regulatory agencies.

12.4.2.2.1 AMS/Fruits & Vegetable Program

The provisions for sanitation inspection of fresh and processed fruit and vegetable facilities are published in the *AIM Inspection Series Sanitation Manual* (AMS 2013). General criteria used by the agency for evaluation of equipment are provided in this manual.

12.4.2.2.2 AMS/Dairy Program

The AMS/Dairy Program provides voluntary grading for manufactured dairy products (e.g., cheese, butter, dry milk). For products graded under this program, a sanitation inspection of manufacturing facilities is included. In addition, the agency conducts a very thorough equipment evaluation program under the *USDA Guidelines for the Sanitary Construction and Fabrication of Dairy Processing Equipment* (AMS 2001a), hereafter termed *USDA Dairy Equipment Guidelines*. If a 3-A Sanitary Standard has been written for a specific class of equipment, the standard is used as a guide for equipment evaluation, and conformance to the standard is required. Conformance with 3-A Sanitary Standards is also required under several state regulations for dairy products.

Fig. 12.5 Certificate to indicate that meat and poultry equipment has been evaluated and accepted under the USDA/AMS equipment evaluation program

The AMS/Dairy Program also provides a meat, and poultry equipment evaluation service. This service is voluntary and available upon request (AMS 2001b). The following logo (Fig. 12.5) indicates the successful evaluation of the equipment.

12.4.3 Canadian Regulatory Requirements for Hygienic Design of Equipment

The Canadian Food Inspection Agency (CFIA) was created under the Canadian Food Inspection Act of 1997, by combining and integrating three federal agencies: Agriculture and Agri-Food Canada, Fisheries and Oceans Canada, and Health Canada. The Safe Foods for Canada Act (SFCA) and Safe Food for Canadians Regulations (SFCR), effective January 15, 2019, is streamlining food inspection programs across all food commodities. General regulatory provisions related to hygienic design equipment under the new SFCR are as follows:

- SFCR 8: Use of Food Additives and Other Substances;
- SFCR 50: Clean and Sanitary Condition;
- SFCR 53(b): Design, construction, and maintenance of conveyances or equipment; and
- SFCR 53(c): Materials used in construction and maintenance of conveyances or equipment

12.4.4 European Union (EU) Regulatory Requirements for Equipment Hygienic Design

The EU Directive 2006/42/EC states that *"machinery intended for the preparation and processing of foodstuffs, cosmetics or pharmaceutical products must be designed and constructed so as to avoid health risks"* (Lelieveld et al. 2014). A European Standard EN 1672-2:2005+A1:2009, Food Processing Machinery –

Safety and Hygiene Requirements has also been adopted for further clarification of the regulations. In addition, specific standards for various food industry sectors have been developed.

All machinery sold within the EU shall be marked with the "CE" mark to show compliance with the basic criteria listed here:

- Materials in contact with food;
- Surface smoothness;
- Welding or continuous bonding rather than fastenings;
- Surface drainage;
- Prevention of dead spaces which cannot be cleaned; and
- Prevention of contamination from ancillary substances, e.g., lubricants.

12.5 Organizations Involved in Developing Hygienic Design Standards, Guidelines, and/or Recommendations

12.5.1 Organizations Developing Standards/Guidelines and Certifications

Food equipment standards and guidelines, developed by third party organizations, prescribe criteria to be used in food equipment manufacture. Most of these standards organizations provide a symbol (or logo) to be displayed on equipment (see Fig. 12.6), or certificates to equipment manufacturers, to indicate that the equipment conforms to the specific criteria of given standards.

12.5.1.1 3-A Sanitary Standard, Inc. (3-A SSI)

3-A SSI is a non-profit organization with representation from three founding (or stakeholder) groups representing fabricators of equipment, users (e.g., processors) of equipment, and regulatory sanitarians (including academic representation), which has a predominant history of acceptance and value to the food industry (3-A SSI 2018a; Schonrock 2012, 2016). The process for developing 3-A Standards and Accepted Practices is consensus-driven and meets the procedural guidelines of the American National Standards Institute (ANSI) with input from the three stakeholder groups.

Although 3-A Sanitary Standards and 3-A Accepted Practices are primarily vested in the dairy industry, they are applicable to equipment and systems used in a wide variety of food and beverage industries. 3-A SSI is open to building collaborative relationships with other food industry segments. For the past 20 years, harmonization efforts have been ongoing between 3-A SSI and the European Hygienic Design Group (EHEDG) (Labs 2015).

a. 3-A Sanitary Standards

b. European Hygienic Engineering & Design Group (EHEDG)

c. NSF International

d. American Society of Baking (ASB)

e. International Organization for Standardization (ISO)

f. HACCP International

Fig. 12.6 Organizations that develop hygienic design standards and guidelines and certify food equipment. (**a**) 3-A Sanitary Standards. (**b**) European Hygienic Engineering & Design Group (EHEDG). (**c**) NSF International. (**d**) American Society of Baking (ASB). (**e**) International Organization for Standardization (ISO). (**f**) HACCP International

12.5.1.1.1 3-A Sanitary Standards

More than seventy 3-A Sanitary Standards are available, including the *ANSI/3-A 00-01-2018 3-A Sanitary Standard for General Requirements*, and standards for specific types (or classes) of equipment used in farm milking systems, processing facilities, and/or transportation (e.g., tankers, farm pickup). In addition, 3-A SSI has collaboratively developed three standards for meat and poultry equipment with NSF International (see Sect. 12.5.1.2).

12.5.1.1.2 3-A Accepted Practices

3-A Accepted Practices provide hygienic criteria for location, installation, and operation of equipment in food systems bearing the 3-A Symbol. Currently, the following 3-A Accepted Practices are available:

- *Sanitary Construction, Installation, Testing, and Operation of High-Temperature Short-Time and Higher-Heat Shorter-Time Pasteurizer Systems;*

- *Supplying Air Under Pressure in Contact with Product and Product Contact Surfaces;*
- *Permanently Installed Product and Solution Pipelines and Cleaning Systems*
- *Design, Fabrication, and Installation of Milking and Milk Handling Equipment*
- *Spray Drying Systems;*
- *Instantizing Systems;*
- *A Method of Producing Culinary Steam;*
- *Sanitary Construction, Installation, and Cleaning of Membrane Processing Systems;*
- *Farm Milk Cooling and Storage Systems;* and
- *Plant Environmental Air Quality.*

12.5.1.1.3 3-A Third Party Verification (TPV) Program

3-A SSI offers the Third Party Verification (TPV) program to assure that equipment or processing systems conform to the criteria of the covered 3-A Sanitary Standard (s) (3-A SSI 2018b; Hoffman and Rugh 2014). TPVs are conducted by Certified Conformance Evaluators (CCEs) who are required to pass the CCE examination and have participated in training and achieved the qualifications needed to do standardized equipment evaluations. The training encourages unified appraisals of equipment to avoid conflicting issues between different evaluators.

The following are elements of the TPV program:

- Onsite inspection and evaluation of equipment at the manufacturing facility or processing system at the processing facility;
- Review of all certifications for components fabricated from plastic, rubber or rubber-like materials, adhesives, or metal alloys;
- Verification of the manufacturer's written quality control program; and
- Evaluation of the *Engineering Design and Technical Construction File (EDTCF)* that contains the additional recorded information necessary to demonstrate that a machine and/or equipment, or process is in conformance to an applicable 3-A Sanitary Standard or Accepted Practice.

The TPV program is required for the 3-A Symbol and 3-A Certificate authorization programs described here:

- *3-A Symbol.* The 3-A Symbol (Fig. 12.6a) is a registered and licensed mark of 3-A SSI, and may only be displayed on equipment in accordance with licensing requirements of 3-A SSI. A listing of 3-A symbol holders is available at 3-A SSI. In addition, 3-A SSI maintains a continuously updated "Buyer Beware" section of the website which lists improper display of the 3-A Symbol, and improper use of symbol in advertising. When displayed on a piece of equipment, the 3-A Symbol provides assurance that the equipment conforms to all of the criteria of the covered 3-A Sanitary Standard(s) and has been evaluated and verified under the TPV program.

- *3-A Certificates*. The *3-A Replacement Parts and System Component Qualification Certificate (RPSCQC)* is an indication that the parts and components are in conformance with 3-A Sanitary Standards and have been evaluated and verified under the TPV program. Likewise, the *3-A Process Certificate (PC)* indicates that a processing system using 3-A Symbol bearing equipment has been evaluated under the TPV program and that it conforms to the applicable 3-A Accepted Practice and provides assurances of hygienic design to regulatory authorities.

In addition to the TPV program, 3-A SSI has procedures in place in which any interested party may file a Report of Alleged Non-conformance (RAN). A RAN may be filed when non-conformance issues to 3-A criteria are noted in field observation of equipment bearing a 3-A Symbol or a process holding a 3-A Certificate.

12.5.1.1.4 3-A SSI Educational Programs and Resources

The 3-A Annual Meeting features two educational programs: Basics of Hygienic Design (presentations cater to those individuals with little to no basic knowledge of hygienic design principles) and Educational Program and Update (session provides more detailed information on newer design principles, more complex equipment design concepts, and information on recent developments in hygienic design). On the 3-A website, 3-A SSI provides interactive training modules that may be used as educational material and links to additional educational resources.

3-A SSI is developing on-going relationships with academic institutions. Travel scholarships for students (based upon committee approval) are available to attend and participate in the annual educational conference. These scholarships are available to students in food science, engineering, and other related disciplines.

12.5.1.2 European Hygienic Engineering and Design Group (EHEDG)

The European Hygienic Engineering Design Group (EHEDG) is a consortium of European equipment manufacturer, food processors, research institutions, and public health authorities (EHEDG 2018a; Schonrock 2016). While EHEDG does not develop food equipment standards, EHEDG working groups have developed and published greater than 40 highly specific guidelines to interpret European regulatory requirements involving equipment and building design, cleanability, building element installation, and testing methodology (EHEDG 2018b).

12.5.1.2.1 EHEDG Testing and Certification Program

A testing and certification program is offered through *EHEDG* affiliated *Authorised Evaluation Officers (AEOs)* at *Authorised Testing Laboratories (ATLs)* located in several European countries, Taiwan, and the US, at the University of Tennessee

(Hoffman and Rugh 2014). Timperley (2012) published a review of the EHEDG test methods,which includes their relative advantages and limitations in certain applications, and how they are integrated into the EHEDG testing and certification program. The EHEDG certificate is an assurance to both equipment manufacturers and food processors that the equipment conforms to the requirements found in appropriate EHEDG guidelines. Several types and levels of certification are offered. For example, EL Class 1 certification (see Fig. 12.6b) includes testing for clean-in-place (CIP) cleanability under *EHEDG Doc. 2 – A Method for Assessing the In-Place Cleanability of Food Processing Equipment* (EHEDG 2002).

12.5.1.2.2 EHEDG Education, Training, and Research Programs

EHEDG has a very active education, training, and research program (EHEDG 2018c). This program includes a variety of training materials, training courses, university graduate study courses, and research journal publications.

12.5.1.3 NSF International

NSF International is recognized for developing and publishing internationally recognized standards for food, water, and consumer products. This organization has developed greater than 50 NSF/ANSI standards on food safety, drinking water and public health (NSF International 2018a). In addition, NSF International provides a variety of services to food and agricultural industries (e.g., auditing, certification, testing, training, consulting, and regulatory compliance). Services and programs span the following areas: animal feed and welfare, beverages/bottled water, food equipment, packaging, processing, retail foods, and storage and distribution.

12.5.1.3.1 NSF Equipment Standards, Certification and Registration

The NSF equipment certification and registration program (NSF International 2018a) is primarily focused on commercial foodservice and retail foods with greater than 20 standards available on a variety of subject areas (e.g., food preparation; cooking; holding and transportation; food and beverage dispensing; food vending; ice making; mobile food carts; ware washing; non-product surfaces; flooring; refuse handling; processing). In addition, three standards (cooperatively developed with 3-A SSI) for meat and poultry processing equipment are available. An NSF Symbol (Fig. 12.6c) on a piece of equipment indicates that the equipment is certified to be in conformance with an applicable NSF standard.

12.5.1.3.2 NSF Training and Education Programs

The NSF is actively engaged in training (NSF International 2018b), offering public training and webinars, on-line training, and on-site training at food facilities on food safety, food quality, and HACCP. In addition, a food safety certificate program is available.

12.5.1.4 American Society of Baking (ASB)

The standards for hygienic design and construction of bakery equipment have been in existence for many years under the *Baking Industry Sanitary Standards Committee (BISSC)*. The standards were condensed into a single booklet in 1977 (Schonrock 2016). The American Society of Baking (ASB) (Fig. 12.6d) became the Secretariat for the BISSC standard in the early 2000s (Frankenberg 2012) and has converted the standard document to an ANSI consensus standard, *ANSI/ASB Z50.2 American National Standard for Bakery Equipment — Sanitation Standards*.

12.5.1.5 International Organization for Standardization (ISO)

More than 110 ISO standards (Fig. 12.6e) related to food processing and handling systems are available (ISO 2018; Schonrock 2016). The primary ISO standards that relate to food safety systems and hygienic design, construction, and fabrication of equipment are listed here:

- *ISO 14159:2002 Safety of Machinery – Hygiene Requirements for the Design of Machinery* – provides specific criteria on hygienic design of food equipment and is referenced in EHEDG guidelines and EN regulatory directives (ISO 2002).
- *ISO 22000 Food Safety Management System (FSMS) Standard* – auditable standard for the HACCP system (Surak 2005) and Global Food Safety Initiative (GFSI) (Newslow 2014).

12.5.1.6 HACCP International

HACCP International (HACCP International 2020) is a certification body based in Australia (Fig. 12.6f). This organization offers food equipment certification under the *Food-Safe Equipment, Materials and Services* program.

12.5.1.7 Underwriters Laboratories (UL)

Underwriters Laboratories (UL) has been globally recognized for providing safety and energy certifications for electrical and gas appliances. More recently, the UL

developed food safety standards encompassing certain classes of equipment used in the meat and poultry industry and retail food industries and issues a "*Sanitation Certification"* (UL 2018).

12.5.2 Food Industry Organizations Providing Recommendations and Best Practices

12.5.2.1 North American Meat Institute (NAMI)

The North American Meat Institute (NAMI), a non-profit trade association formed in 2015 by merger of the American Meat Institute (AMI) and North American Meat Association (NMA), has adopted the *AMI Sanitary Design Principles.* These guidelines for the meat and poultry industries were developed by the AMI Equipment Design Task Force (EDTF) (AMI 2014; Bilgili 2006), and can be applied to both product contact and non-product contact surfaces of equipment. The AMI provides an auditing checklist with recommended point values for each general principle (and sub-criteria for each principle) using the following rating scale: Satisfactory (full points), Marginal (half points), Unsatisfactory (zero points), and Not Applicable. The AMI principles are listed here (along with the recommended point value for each):

- *Principle 1: Cleanable to a Microbiological Level (100).*
- *Principle 2: Made of Compatible Materials (100).*
- *Principle 3: Accessible for Inspection, Maintenance, Cleaning and Sanitation (150).*
- *Principle 4: No Product or Liquid Collection (100).*
- *Principle 5: Equipment is Designed to Eliminate or Minimize Hollow Areas (150).*
- *Principle 7: Sanitary Operational Performance (100).*
- *Principle 8: Hygienic Design of Maintenance Enclosures (50).*
- *Principle 9: Hygienic Compatibility with Other Systems (50).*
- *Principle 10: Validated Cleaning and Sanitation Protocols (50).*

12.5.2.2 International Fresh-cut Produce Association (IFPA)

The International Fresh-cut Produce Association's (IFPA) *Sanitary Equipment Design Buying Guide and Checklist* (IFPA 2003) provides voluntary guidelines, illustrations, and definition for use by fresh-cut produce facilities. This checklist contains criteria for both product contact and non-product contact surfaces.

12.5.2.3 Innovation Center for US Dairy Industry (IC)

The IC document, *Control of Listeria monocytogenes – Guidance for the Dairy Industry* references 3-A Sanitary Standards and provides checklists for use in evaluating the hygienic design and construction of dairy facilities and non-product contact surfaces (IC 2015).

12.5.2.4 Global Food Safety Initiative (GFSI)

The *Global Food Safety Initiative (GFSI)* (GFSI 2018) was developed to promote standardization and improvement of food safety audits for facilities providing food products to retail foods establishments. Several auditing schemes are accepted (or benchmarked) under GFSI for various food and agriculture industries. These auditing programs include general requirements for hygienic design of equipment, with variations between the programs. Those that apply to food and beverage processing and their hygienic design criteria are listed as follows:

12.5.2.4.1 Safe Quality Food Institute (SQF) (SQF 2018)

The current document is SQF Code, Edition 8 (SQF 2018). Equipment provisions are found in *Module 11: Good Manufacturing Practices for Processing of Food Products, 11.2 Construction of Premises and Equipment*.

12.5.2.4.2 British Retail Consortium (BRC)

The current document is *BRC Global Standard for Food Safety – Issue 8* (BRC 2020). Equipment provisions are found in *Clauses 4.6 Equipment* and *4.7 Maintenance.*

12.5.2.4.3 International Featured Standards (IFS)

The current document is *IFS Food Version 6.1 Standard* (IFS 2018). Equipment provisions are found in *QM 4.16 Maintenance and Repair* and *QM 4.17 Equipment* (IFS 2018).

12.5.2.4.4 Food Safety System Certification (FSSC) 22000

Equipment provisions are found in *Part II Requirements and Regulations for Providing Certification, 8.2 Hygienic Design,* and *8.3 Product Contact Surfaces*

(FSSC 22000 2018). FSSC 22000 requires conformance to existing food equipment standards, where appropriate.

12.6 Hygienic Design, Fabrication, and Construction Criteria for Food Equipment

12.6.1 General Criteria for Hygienic Design of Product Contact Surfaces

To ensure safe food and effective sanitation programs, all product contact surfaces of equipment used for processing and handling food products, as well as for conveying cleaning and sanitizing solutions, must be designed, fabricated, and constructed according to sound hygienic design principles (Anonymous 2012; Costa et al. 2013; Hastings 2009; Lelieveld et al. 2005; Lelieveld et al. 2014; Meireles and Simões 2017; Murray 2014; Schmidt and Erickson 2005). The fundamental terminology describing the desirable properties (e.g., physical/chemical; mechanical; operational) of product contact surfaces of food equipment are listed in Fig. 12.7.

The fundamental hygienic requirements for product contact surfaces and food equipment are described here.

Desirable properties of product contact surfaces:

- Non-toxic (approved under appropriate regulatory programs), and non-contaminating (do not allow leaching of toxic materials into the product).
- Non-porous and nonabsorbent (preventing ingress of food components, microorganisms, and chemicals).

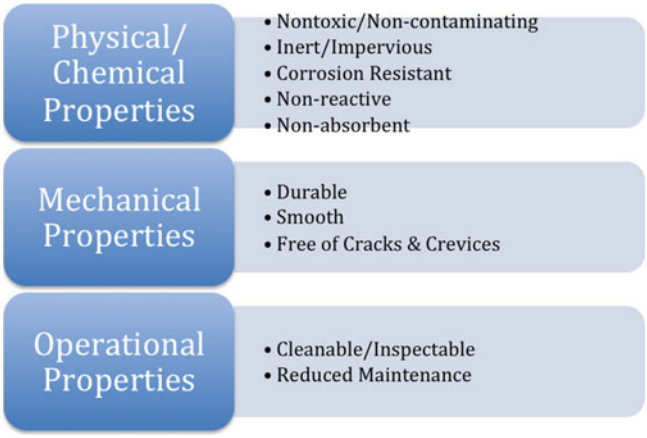

Fig. 12.7 Desirable properties of product contact surfaces. (Adapted from Schmidt and Erickson 2005; Hanson 2017)

- Non-reactive with food components, cleaning/sanitizing chemicals, sterilizing agents (e.g., ozone; hydrogen peroxide; steam) and/or water used in a food facility.
- Corrosion resistant under conditions of intended use.
- Smooth, impervious, and durable.
- Free of cracks and crevices (at the macroscopic and microscopic level).
- If modified by surface treatment or by coating with metal or non-metal materials, hygienic design properties are maintained.

Desirable properties of fabricated food equipment:

- No open areas and/or dead spaces that may allow accumulation of solutions or food residues resulting in potential allergen cross-contact or microbiological contamination.
- Accessible for inspection, providing for easy access to assure that food contact surfaces are adequately cleaned and that cleaning can be visually or test-verified.
- Allow for adequate draining to prevent accumulation of food, chemical solutions, or water.
- Cleanable (able to be both cleaned and sanitized, provided that proper techniques, chemical solutions and systems are employed).
- Any modification process used in fabrication (e.g., welding, bonding, soldering) is done using appropriate materials, and in a manner that ensures that the final surface meets the hygienic design criteria.

Photographic examples of improper hygienic design and poor maintenance are shown in Fig. 12.8.

12.6.2 Metal Materials Used for Product Contact Surfaces

A variety of metals and metal-alloyed materials are used when constructing and fabricating food equipment to be used in different applications. These materials range greatly in their properties with regard to workability, compatibility, and hygienic design features. Certain metals are not allowed to be used in food contact applications. These include (but are not limited to): antimony, copper-zinc alloys (brass), copper-tin alloys (bronze), cadmium, mercury, and lead.

12.6.2.1 Types of Metal Materials

12.6.2.1.1 Stainless Steel

Because of its desirable properties for food surface applications (e.g., corrosion resistance; strength; hardness; modulus; relative ease of machining and fabrication; relatively low cost), stainless steel is commonly used for product contact surfaces in

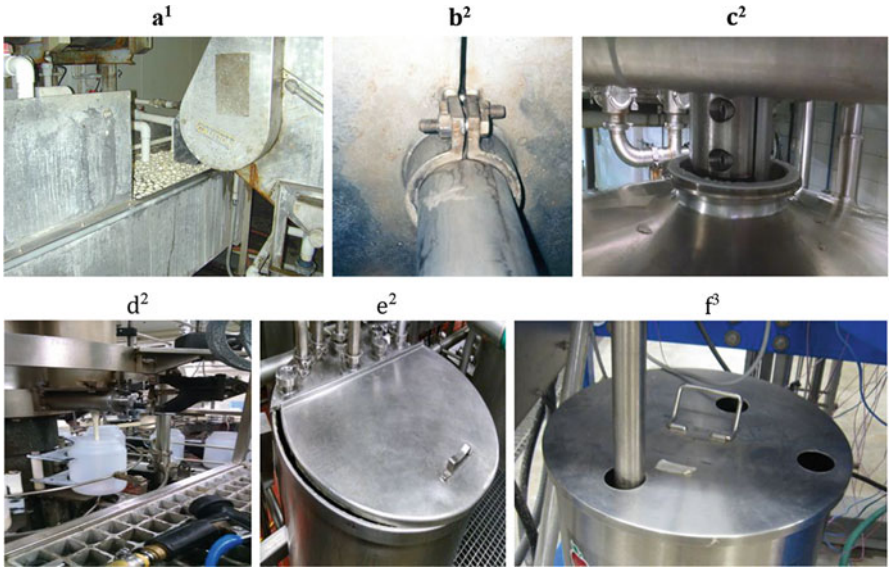

Fig. 12.8 Photo gallery of improper hygienic design (and maintenance) of food equipment. (**a**) Galvanized metal (should be avoided in food contact); (**b**) Threads and crevices in the interior of a processing vessel; (**c**) Lack of shielding on agitator shaft; (**d**) Lack of shielding during filling; (**e**) Misaligned tank lid; (**f**) Improper tank lid (lack of 'shoe box' openings; lack of diverter on pipe opening). ([1]Anonymous – photo from yester-year; [2]Photo courtesy of Gabe Miller. [3]Photo taken of pilot plant equipment in the Food Science and Human Nutrition Dept., University of Florida)

the food and beverage industries (Anonymous 2001; Euro Inox 2018; Partington 2012; Schmidt et al. 2012; Tuthill and Covert 2000). A wide variety of stainless steel materials and product forms are available with contrasting composition, surface finish, surface treatment, surface coating, and functional properties. It is essential that an awareness of the properties of the various stainless steels is recognized when purchasing stainless steel and food equipment. A comprehensive discussion of the composition and properties of stainless steel materials is available in *Design Guidelines for the Selection and Use of Stainless Steel, A Designers' Handbook Series No. 9014* (NDI 2018).

Steel (the base material in stainless steel) is, by definition, an iron (Fe)-carbon-(C) alloy with ferric carbide (Fe_3C) as the base component. The crystalline textures and associated properties (e.g., strength, hardness) of steel vary with the chemical combinations (and compositional distribution) of Fe and C used in the formulation, as well as the manufacturing method. Stainless steels are alloys of steel with chromium (Cr). This Fe-Cr alloy, or base alloy, is formed at a minimum Fe level of 50% and a minimum level of Cr of 10% by weight. The inclusion of Cr is what provides the name 'stainless steel', as it prevents this corrosion by forming a thin film of chromium oxide (Cr_2O_3) upon exposure to oxygen (Helmenstine 2008; Sourmail and Bhadeshia 2005). This insoluble, self-healing and nonporous chromium oxide layer (termed "passive layer") protects the "active layer" (e.g. iron),

which is susceptible to rust and corrosion. Generally, a minimum Cr level of 12% is considered necessary for adequate film formation, with 18% being recommended for corrosion resistance under most conditions (Gabric et al. 2016). Altering the relative amount of the base elements (C, Fe, Cr), as well as the level of other elements, in the alloy formulation will impact the functional properties of the material. Higher levels of Cr (ranging up to 30% in highly corrosion resistant stainless steel) will result in increased corrosion resistance, while increasing the Fe content will enhance strength and hardness. The carbon level in the alloy formulation may also be impact functional properties of stainless steel materials. These modified materials are designated with the letter "L" for low carbon or "H" for high carbon (e.g., 304L; 304H). A high carbon stainless steel has greater structural strength and hardness, as well as more resistance to oxidation and creep compared to a material manufactured from the base alloy. However, high carbon stainless steels may have an increased risk of corrosion. Conversely, low carbon materials with low strength might be used for their relative ease in welding.

The predominately used stainless steel in food industry applications is made from Fe-C-Cr-Ni alloys, whereby corrosion resistance varies directly with the Cr level and structural strength and hardness varies with the Ni level. It has been customary (especially in cookware) to identify stainless steels as a ratio of the general composition of these elements. A ratio of 18/8 indicates that the material is approximately 18% Cr and 8% Ni, while 18/10 indicates a higher relative level of Ni. However, these ratio designations do not necessarily relate to the more specific classification numbers (described later) and may not adequately classify materials according to their properties. For example, a variety of stainless steel materials with differing formulations, manufacturing conditions, and functional properties may be termed 18/8 under this system based solely on the ratio of Cr/Ni.

Stainless steel materials with enhanced properties can be attained using a variety of additional alloying elements (NDI 2018; Partington 2012) including:

- Copper (Cu), Nitrogen (N), and Molybdenum (Mo) – enhanced passive layer and increased corrosion resistance. Of these elements, Mo is considered to be most effective, especially in preventing 'pitting corrosion' associated with exposure to oxidizing chemicals;
- Manganese (Mn) – increased strength and hardness;
- Nickel (Ni) – increased strength and hardness (very high Ni stainless steel may be more susceptible to 'stress corrosion');
- Phosphorus (P), Sulfur (S), and/or Selenium (Se) – improved machinability; and
- Titanium (Ti) – improved strength, durability, heat resistance, and corrosion resistance (especially for acid materials).

From the possible variations in base alloy components, it is clear that all stainless steels are not created equal. A multitude of materials are manufactured under the name "stainless steel". There are greater than 50 stainless steel alloys and greater than 150 grades (or types) of stainless steel commercially available. These materials are further defined using the following general classification systems:

- American Iron and Steel Institute (AISI) numbering system (200, 300, and 400 Series);
- European Standard (EN 2005);
- Unified Numbering System (UNS) developed by the American Society of Testing and Materials (ASTM) and Society of Automotive Engineers (SAE).

The most common system used to classify stainless steel grades in the U.S. is the AISI numbering system, while the EN system is used in Europe (Cobb 2007; Gabric et al. 2016). The UNS (1-letter +5 digit) is currently being used on many newer grades. In addition, international specifications are used to identify and distinguish specialized stainless steel products (e.g. welding wire).

The stainless steels (classified by their metallurgical or crystalline structure) predominantly used in food applications (Gabric et al. 2016) include:

Austenitic Stainless Steel

This large category includes stainless steel materials with a wide range of properties. Generally, austenitic materials are formulated from Fe-Cr-Ni as the base alloy and are non-magnetic. The grades of *austenitic* used in food contact applications are as follows:

- AISI 300 Series – primarily composed of Fe-Cr-Ni alloys at varied combination and addition of additional elements. The 300 Series category is a very large with a diversity of formulations and properties. Those primarily used in food equipment applications are 304 (UNS S30400; EN 1.4301) and 316 (S31600; 1.4401). Of these, 304 stainless steels are the most commonly used. The 316 stainless steels are formulated with higher levels of Ni and Cr. In addition, Mo is added in the formulation. Thus, 316 is stronger and more corrosion resistant than 304, and is recommended for applications where these properties are desired. 'High grade' 316 materials, formulated with even higher Mo for additional strength properties, are also available. While 303 stainless steels do have high strength, they have relatively low corrosion resistance, and are not recommended for product contact surfaces in food and beverage applications. In fact, 3-A Sanitary Standards restrict the use of 303, and specify 304 or 316 depending upon the intended application.
- Super austenitic – formulated at very higher Cr and Ni levels. These materials have superior strength, hardness, abrasion resistance, and resistance to pitting and other corrosion. AISI 904L (EN 1.4539) is used in a wide variety of highly corrosive, high salt environments (e.g., marine environment, hot brine) or in lower salt, but slow moving (or stagnant) applications (e.g., hot water boilers) as well as steam heating applications.

Ferritic Stainless Steel

The ferritic stainless steels, in the 400 Series, are formulated from Fe-Cr alloys and they are magnetic. Generally, ferritic materials have high heat resistance, good

formability, strength, and machinability. When used in industrial applications, ferritic grades provide structural properties comparable to steel, but with enhanced corrosion resistance due to Cr. However, it is generally thought that most ferritic grades have undesirable corrosion resistance properties, for many food applications. The ferritic stainless steel of most common use in food industry applications is 430 (S43000; 1.4016) stainless steel, formulated with a higher Cr level and improved corrosion resistance compared to lower grades. This material is used in household and food service applications (e.g., dishwashers, refrigerators, sinks, cutlery), and in food industry applications that are moderately non-corrosive (e.g., vegetables; certain fruits; dry foods) (Gabric et al. 2016). Other ferritic stainless steels used in specialized food industry applications include: AIS 441 (S 44100; EN 1.4509) – used in heater or burner components; AIS 444 (S44400; EN 1.4521) – considered suitable for neutral, chloride containing medias (Gabric et al. 2016).

Martensitic Stainless Steel

Also in the 400 Series, martensitic stainless steels are generally formulated at higher carbon levels, which provides additional strength as well as wear resistance. Because of these properties, martensitic stainless steels are used for the manufacture of dental and surgical instruments. Food applications include bushings, buckets, ball bearings, molds and dies, utensils, and cutlery. For cutlery, the quality is related to the C and Mo content, as well as inclusion of additional alloying elements. For example, AISI 420 (S42000; EN 1.4021), formulated with moderate amount of Mo, is widely used for cutting and grinding applications and for medium-priced cutlery. Premium (or cooks' cutlery) is manufactured using AISI 440 (S44000; EN 1.4116), which has higher C, Mo, plus vanadium (V). These 400 Series materials are also used for cutlery and related utensils in the meat industry.

Duplex Stainless Steel

This magnetic stainless steel combines ferritic and austenitic structure in its manufacture, and has excellent strength and corrosion resistance. A numbering system often used is based upon the relative levels of Cu and Ni. Duplex 2205 (S31803; EN 1.4462), formulated with 22% Cr and 5% Ni, is used in higher salt and acidic food applications (e.g., mustard, vinegar, cheese, fish canning) (Gabric et al. 2016; Euro Inox 2018). Higher grade Duplex 2304 (EN 1.4462), formulated with 23% Cr and 4% Ni, is applicable for very corrosive environments (e.g., hot brine, stagnant and slow moving salty foods) (Gabric et al. 2016).

Precipitation Hardening (PH) Stainless Steel

Precipitation hardening, also called age hardening, is a heat treatment technique used to increase the strength of many structural materials (e.g., aluminum, magnesium, nickel, stainless steel), including most structural alloys of Al, Mg, Ni, Ti, and certain stainless steels. The PH stainless steels (e.g., Martensitic PH; Semi-austenitic PH)

are used for special applications (e.g., gear, valves, shafts) where high tensile strength, and moderate corrosion resistance are desirable. The most common grade in this category is "17-4 PH" (630, S17400) that is formulated with 17% Cr and 4% Ni, along with 4% Cu and 0.3% niobium (Anonymous 2001).

12.6.2.1.2 Aluminum

The aluminum materials in most common use (Davis 1999) are typically aluminum alloys containing one or more additional elements including Cu, Mn, Si, magnesium (Mg), and zinc (Zn). However, high purity aluminum (99.5%) may also be specified for certain food and pharmaceutical applications (Jellersen et al. 2006; Gabric et al. 2016). Depending upon the formulation, different mechanical, physical and chemical properties can be attained. The most commonly used in industrial applications are the wrought alloys. Cast alloys formed by pouring molten material into molds are also available. A common classifications system for wrought alloys is a numbering system involving a 5-digit number (Anonymous 2018a; Davis 1999; Kaufman 2000) depending upon their relative composition and alloying elements. The most commonly used aluminum alloys in food industry applications are the 3000 series alloys (formulated with Mn) used for kitchen utensils and cookware, and, to a lesser extent, in the manufacture of food equipment (e.g., storage tanks, citrus extractor heads) used in pilot plant or low volume applications. 3-A standards specify that aluminum of the 5000, 6000, and 8000 series (or other aluminum alloy that has adequate corrosion resistance when exposed to the conditions of the environment) may be used for dry product contact surfaces. Alloy materials in the 1000 Series are used for foil and packaging materials. Aluminum materials are used in a variety of non-product surfaces in the food industry (e.g., structural applications; air cooling evaporator fins).

Although aluminum does not have the overall strength and durability of stainless steel, its lighter weight allows it to have a higher strength to weight ratio. Thus, it is often the metal of choice for in certain parts and components where lighter weight and high strength to weight ratio is desired. While not as corrosion resistant as stainless steel, aluminum does have higher corrosion resistance than cast iron or galvanized steel. Further, aluminum is considered nontoxic, nonreactive, and recyclable (Davis 1999).

Aluminum alloys, when exposed to oxygen, form an aluminum oxide film, which improves corrosion resistance. However, many aluminum alloys are subject to pitting corrosion, as well as stress corrosion cracking (SCC), with continued exposure to corrosive environments. The corrosion resistance may be improved through the thickening of the natural oxide layer on the surface by a process known as anodizing, an electrolytic passivation procedure (Moerman and Partington 2014). Anodized aluminum has enhanced resistance to many inorganic chemicals. However, it is subject to pitting corrosion in the presence of chloride solutions.

The use of aluminum is restricted to specific applications by 3-A Sanitary Standards, the FDA Milk and Milk Products Equipment Guidelines, and the

USDA Dairy Equipment Guidelines. Because of a long historic use in the butter industry, the USDA Dairy Equipment Guidelines specify that aluminum is considered satisfactory for certain specified butter and dry product applications. However, when necessary for specific functional reasons, aluminum may be used as a product contact surface for certain high-moisture foods. This is only allowed when those aluminum parts are not subjected to caustic cleaning or sanitizing chemicals, and where dissimilar metals will not cause any corrosive reaction. In addition, the aluminum material chosen shall be demonstrated to be appropriate and acceptable for the intended use. A listing of specific aluminum designations considered acceptable has been included in 3-A Standards.

12.6.2.1.3 Titanium

A very durable, strong metal, that has excellent ductility and chemical corrosion resistance properties, titanium (Ti) is often used for in the manufacture of food equipment where heat transfer is involved and for processing and handling food products with high acid and/or salt (e.g., citrus juice; tomato products; pickles). Generally, titanium materials are considered to be a suitable alternative to stainless steel in many applications (Feliciani et al. 1998). As described above, titanium is used as an alloying element in the manufacture of stainless steel materials, and also used as a coating (electroplating) on stainless steel.

The primary limitation with the use of titanium is the relatively high cost due to high production costs. These costs are related to the special handling required due its reactivity at high temperatures and difficulty with tooling (Anonymous 2017). However, there has been some indication that the price of titanium has reduced (Jellersen et al. 2006).

12.6.2.1.4 Copper

This metal has the highest heat conductivity of metals used for product contact surfaces. In addition, Cu is relatively easy to fabricate, and has good malleability and ductility. However, it is a softer metal with less corrosion resistance than stainless steel.

Due to its heat conductivity, a Cu coating is often used in cookware. Copper is used as a food contact material in specialized industry applications (e.g., brewing vessels; cheese vats; chocolate; jam; candy), and is also used on non-product contact surfaces, such as tubes in evaporators used in refrigerators and freezers, electric wiring, and water piping. Copper use in the brewing and cheese industry is primarily due to tradition, where there are perceived advantages in these applications. Prior to the invention of stainless steel, copper was used for the manufacture of brewing kettles (or ‘coppers’), as well as vats used for Swiss cheese manufacture. While it is

still used to a degree in these applications, very often the vessels are just 'copper clad' (or coated) on the outside for appearance and to give a more traditional appearance.

Disadvantages of copper are that it is a relatively soft material that tarnishes readily, especially in non-product contact structural applications, and it is susceptible to fouling in food contact applications. An additional concern with copper in food contact is that there is the potential of copper to leach from the surface (depending upon conditions of use), resulting in migration of copper ions into the food (or water), especially with continued exposure to acidic materials. An additional problem with the leaching is that the surface roughness may increase, thus reducing the cleanability of the surface. There may be food safety concerns (depending upon the level) as well as quality concerns arise due to leaching of copper. Being strong oxidizing agents, copper ions may induce oxidation and off-flavor reactions in beer and/or other food products. Conversely, certain advantages to the leaching of copper ions have been suggested. Copper does have strong antimicrobial properties (Moerman 2014) and thus may prevent biofilm formation. However, it is not considered to be practical as an anti-microbial surface due to the high levels of copper required for adequate antimicrobial protection and the potential leaching problems (Robine et al. 2002; Gabric et al. 2016). In beer making, it is suggested that the presence of copper ions may prevent the formation of hydrogen sulfide off-flavors by forming insoluble copper sulfide (which is removed in filtering). Copper may also stimulate the growth of yeasts in beer making. A 'copper surface' in a Swiss cheese vat is thought to create a more desirable oxidation-reduction potential for the growth of the bacterial starter cultures and enhance flavor development reactions. Further, copper is required for cheese manufactured under the name Gruyere (McDonough 2017). The type of copper used and adequate maintenance are very important factors for avoiding potential migration issues. The use of "leachable copper" is specifically prohibited as a food contact material according to the *USDA Dairy Equipment Guidelines* (AMS 2001a, b).

12.6.2.1.5 Less Frequently Used Metals

Platinum and gold materials have excellent corrosion resistance, as well as strength, durability, and mechanical stability at high temperatures. It is because of these properties that platinum dishes have been traditionally used as weighing dishes in analytical laboratories. Platinum is used in alloys or electroplating for electrical contacts, rivets, and specialized welded components. The high cost of this rare metal, however, limits its use in primary food contact surface applications. Certain 3-A Sanitary Standards allow gold for special applications such as soldering optical sensors (e.g., fiber optics) into stainless steel fittings. Gold is desirable in these applications for its resistance to abrasion and compatibility with glass.

Carbon steel (aka 'mild steel') and cast iron are similar metals composed of C-Fe alloys. Carbon steel is composed of approximately 99 percent iron and 1 percent carbon, while cast iron normally contains 2–3 percent carbon and is 'cast' by pouring

molten liquid into a mold. Cast iron has a more irregular grain structure, is heavier, and has higher hardness and more brittleness compared to carbon steel, which is lighter and more pliable. Because of their heat retention properties, these materials are used for cooking surfaces and certain utensils used in food service. Carbonized steel is also used in very specific applications in the food processing industry (e.g., scraper knives in drum dryers). In addition, these metals are used for structural elements of equipment (non-product contact surfaces).

The primary disadvantage of carbon steel and cast iron is their poor corrosion resistance. Since they contain iron, they will rust unless properly treated to prevent it. In food contact applications, painting of the surface is not an option. Painting may be used in non-product applications, provided that it is appropriately applied so as not to chip or flake, and is properly maintained. Various conditioning techniques are recommended for cast iron cookware and cooking surfaces. The majority of these techniques involve spreading a thin layer of vegetable oil on a warm surface, followed by heating (curing) at medium-high temperatures. This creates a thin protective oil-based layer on the surface. Care needs to be taken with selection of the oil, as very unsaturated oils may be susceptible to oxidation reactions producing off-odors and off-flavors. This conditioning should be done at a defined frequency and following any rigorous cleaning. Porcelain-coated and ceramic-coated cast iron surfaces are also available. It is recommended that regulatory requirements and recommendations be followed when using cast iron and related materials in food industry applications.

Galvanized steel is a rust resistant material produced by forming a protective coating of zinc oxide on the surface of steel (or iron), either by immersion (hot-dipping) or electroplating. Galvanized steel has high strength and durability and good corrosion resistant properties (depending upon the application). Further, galvanized steel is deemed safe for food contact. However, the FDA recommends that galvanized metals be avoided for use in processing or handling acidic food products (FDA 2017a) due the potential of surface reaction and degradation causing pitting corrosion as well as the risk of leaching of zinc salts into the food product. While galvanized steel surfaces are considered to be a cleanable surface, if it is not properly maintained, cleanability may be dramatically compromised.

Galvanized steel is rarely used as a food contact surface in food facilities, with exceptions of certain segments of the food industry (e.g., ice plants; fresh produce), and especially in legacy equipment (see Fig. 12.7). Galvanized steel is commonly used in non-product surface applications such as shelving and structural elements (e.g., coolers; freezers; warehouses).

12.6.2.2 Corrosion Considerations with Metal Materials

As discussed briefly above, metal materials will corrode in moist environments. Corrosion is accelerated if used in an oxidizing, acidic, or ionic (salts) environment. The risk of corrosion may increase where metal surfaces are altered with continued use, especially under conditions where the outer protective layer is altered

(e.g. incompatible cleaners; abrasive cleaners; abrasive cleaning pads; chlorine and related sanitizers). The general types of corrosion (Cowan 1977; Tuthill and Covert 2000; Schmidt et al. 2012; Sourmail and Bhadeshia 2005) in food equipment applications are discussed here:

- *Uniform Corrosion* – associated with continued exposure to dilute acid or alkaline solutions, or by acute exposure to more concentrated acid or hot alkali.
- *Pitting Corrosion* – more localized destruction of the passive layer of stainless steel, with subsequent corrosion of the active steel layer. Pitting corrosion is also common with certain aluminum surfaces. Pitting corrosion is generally the result of exposure to chlorides, bromides, and other halides. Pitting corrosion is accelerated by high temperature and lower pH. Once formed, pitting corrosion has a tendency to continue to grow and is difficult to remove.
- *Crevice Corrosion* – may occur where there are crevices in a surface (e.g., under gaskets; incomplete or improper welds; overlapping surfaces), especially if acidic materials are allowed to remain stagnant.
- *Stress Corrosion Cracking (SCC)* – the result of stressing of materials, either during manufacture or during rigorous usage whereby pinholes or other stress areas become vulnerable to corrosion. SCC may also occur under continued exposure to high temperature solutions containing chlorides. In stainless steel materials, the risk of SCC increases with increased Ni, thus 316 stainless steel offers on advantage over 304 with regard to SCC. Because SCC is a common problem in the brewing industry, ferritic stainless steel grades (without Ni) are often used in this industry (Klang et. al. 1984). Resistance to SCC can be achieved by certain annealing processes and by using techniques that apply a compressive stress to the surface (e.g. shot peening).
- *Galvanic (e.g., Bimetallic; Electrolytic) Corrosion* – due to the flow of electric current, especially where two dissimilar metals are in contact. Prevention of galvanic corrosion can be achieved by avoiding mixed metal fabrications (e.g., non-stainless steel clamps; nuts and bolts; other)
- *Contact Corrosion* – occurs when small particles of foreign matter (especially carbon debris) are left on a stainless steel surface during manufacture. If allowed to continue, contact corrosion may lead to galvanic or pitting corrosion. Close attention to good manufacturing practices (GMPs) in addition to appropriately applied pickling and/or passivation steps (described later) are often recommended during material manufacture to reduce the risk of contact corrosion in the finished material.
- *Biologically and Microbiologically Influenced Corrosion (MIC)* – due to residual biological materials (e.g., microbial biofilms; food soil) remaining on the surface. Highly oxidizing bacteria, if allowed to persist on the surface, may accelerate pitting corrosion reactions.

Examples of corrosion are shown in Fig. 12.9. While this discussion is primarily focused on stainless steel, other metals exhibit similar corrosion reactions.

The most effective way to prevent corrosion is to “engineer it out” by selecting the appropriate material for the food application, especially for high acid, high salt,

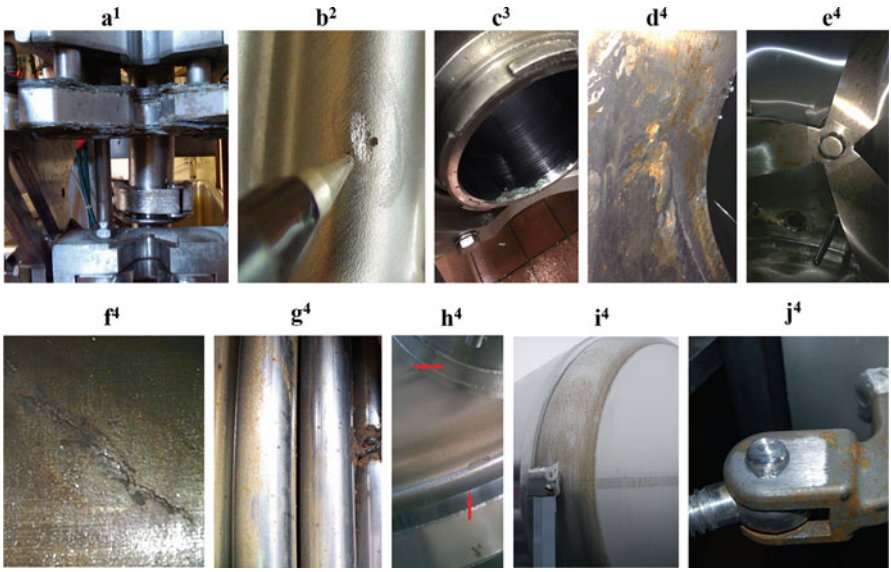

Fig. 12.9 Metal corrosion photo gallery. (**a**) Galvanic corrosion on a bin box filler; (**b**) Contact corrosion on a thin-walled heat exchanger; (**c**) Contact corrosion (due to chemical exposure) on a connection of a swept surface heat exchanger; (**d**) Pitting corrosion on tank surface (from chlorine); (**e**) Pitting corrosion on agitator (from chlorine); (**f**) Stress Corrosion Cracking (SSC); (**g**) Carbon steel contamination of stainless steel; (**h**) Corrosion caused by dust from tin roof of warehouse; (**i**) Corrosion caused by carbon steel contaminated knuckle; (**j**) Corrosion on man-way hinge. ([1]Photo courtesy of: [1]Robert Altobelli; [2]AGC Heat Transfer; [3]Dennis Glick; [4]Paul Mueller Company Repair Services)

or high temperature environments. Further, it is recommended that effective programs be developed and implemented. This program should delineate the risks of inappropriate chemical concentrations and temperatures, how to avoid corrosion, and procedures to remove corrosion once formed. Preventative maintenance programs should address the potential implications of corrosion on food contact surfaces through routine equipment inspection with follow-up procedures for handling corrosion, before it gets severe. When making modifications involving welding, it is critically important that the welding be performed by trained and certified stainless steel welders, and with care to avoid corrosion formation potential on and in the food equipment.

A common mistake made by food handling employees is using sanitizers at inappropriate concentrations, following the erroneous assumption that “if a little is good, more is better”. It is critical that supplier’s recommendation be adhered to when using sanitizers, as well as detergents, to minimize the risk of corrosion.

Because of additional risks of corrosion with aluminum, care should be exercised when cleaning and sanitizing aluminum components (in both product contact and non-product contact applications) as certain oxidizing cleaning chemicals and sanitizing chemicals (especially chorine) will accelerate the corrosion reactions.

Once formed, corrosion on metal materials can be difficult (or impossible) to remove without further damage to the surface. Mild corrosion can be removed by rigorous cleaning or, in some cases, re-working of surfaces. With stainless steel, more severe corrosion usually requires more rigorous treatment such as passivation (see Sect. 12.6.2.3). Severe pitting corrosion of stainless steel is not removable by passivation and can require a more rigorous treatment (e.g. pickling paste) for removal. These rigorous treatments would not be appropriate for softer metal surfaces (e.g., aluminum).

12.6.2.3 Passivation

The process known as passivation (Maller 1998a, b) is applied to the stainless steel surface to enhance and maintain the passive (non-reactive) Cr_2O_3 layer, and to protect the active (reactive) Fe layer from corrosion. Passivation of stainless steel surfaces should be done initially and at a defined frequency thereafter. It should also be done after any surface repair, polishing, or other modification. Prior to passivation of a stainless steel surface, it is recommended that an expert be contacted for assistance (Anonymous 2007). Detailed procedures for cleaning/passivation using nitric acid and other acids are provided under ASTM A380 *Standard Practice for Cleaning, Descaling, and Passivation of Stainless Steel Parts, Equipment, and Systems* (ASTM International 2018a). In general, a complete passivation process (immersion or spraying) must closely follow recommended procedures which involve the following steps: cleaning, degreasing, inspection, neutralization /rinsing, and complete drying. It is imperative that the surface to be passivated is clean, as passivation will not remove surface contaminants added during fabrication or during food processing operations. In fact, surface contaminants will impede the effectiveness of the passivation process. Since passivation is accomplished by exposing the surface to a solution of nitric acid (or other strong oxidizing acid), extreme care must be exercised with regard to worker safety, environmental discharge, appropriate concentrations, and appropriate exposure time. Further, if not neutralized correctly, these chemicals will corrode the surface being passivated, will corrode surrounding non-product contact surfaces (e.g., equipment non-product contact surfaces; sewer drains and piping), and will etch or damage concrete or tile floors. Any leak or spill must be immediately diluted with water or neutralized with a basic solution. Photographic examples of the consequences of improper passivation are shown in Fig. 12.10.

Fig. 12.10 Consequences of improper passivation (Miller 2012). (**a**) Corrosion from prolonged contact with passivating solution; (**b**) Material not compatible with passivating solution

12.6.3 Non-metals Used for Product Contact Surfaces

A variety of non-metal materials are used as food contact surfaces in specific applications (e.g., probes; gaskets; membranes; conveyor components; agitator paddles; pallets; filters; screening material). When used in primary product contact, these materials must meet the same hygienic design and cleanability requirements as metals. Since many non-metal surfaces lack the corrosion resistance, strength, and durability of metal surfaces, maintenance programs should include frequent examination for wear and deterioration under continued use and replacement when needed. Use of certain nonmetal materials are restricted including unapproved plastics and rubber materials, paper (except for single service gaskets and related items), and cloth (except where specified).

12.6.3.1 Plastic, Rubber, and Rubber-Like Materials

A wide variety of plastic and rubber materials are used in food applications ranging from solid to more elastic structures. The basic difference between plastic and rubber is that plastic is synthesized from petroleum, while rubber is manufactured from high molecular weight natural products containing isoprene (e.g., sap; latex). Structurally, these materials are hydrocarbon polymers, or large molecules composed of monomeric repeating units. The elastomeric polymers (e.g., rubber) have highly elastic or "memory" properties (the ability to return to their original shape when a compression load is removed) due to their cross-linked molecular structure. However, elastomers are not exclusively rubber, as many synthesized plastic polymers (e.g, rubber-like) have elastomeric properties and are used in similar applications.

These materials are also categorized as either thermoplastic or thermoset materials, defined as follows (Moerman 2014; Moerman and Partington 2014):

- *Thermoplastic Materials*. Structurally, thermoplastic materials are primarily linear and branched polymers, held together by inter-chain hydrophobic bonding and Van Der Waals Forces. These structures will soften upon heating and can be

reshaped upon cooling, a cycle that can be repeated several times. The properties of thermoplastic polymers may be altered by various manufacturing techniques and by the addition of other elements (e.g., hydrogen; fluorine; nitrogen; oxygen), covalently bonded to the carbon polymers. Due to their ability to melt, many thermoplastic materials can be recycled.

- *Thermoset Materials*. Thermoset materials (e.g., cured plastics; rubber-like plastics; rubber) have extensively cross-linked structures with stronger covalent interchain bonding. Thermoset plastics are "set" to a rigid consistency, which cannot be re-melted with heating. Most thermoset materials are stronger and more heat resistant than thermoplastic materials. Further, thermoset materials are not recyclable.

Comprehensive reviews of the properties and food contact applications of various plastic, rubber, and rubber-like materials are available (Gabric et al. 2016; Moerman 2014; Moerman and Fikiin 2016; Moerman and Partington 2014). The following general discussion has been adapted from these sources.

When compared to metals, the plastic, rubber, and rubber-like materials have lower electric/dielectric properties (less metal-metal contact issues), lower heat conductivity, and higher machinability. These materials are used in the food and beverage industry in both multi-use and single service food contact, as well as non-product contact applications. When used in food contact, the plastic, rubber, and rubber-like materials must, at the minimum, be nontoxic in accordance with regulatory requirements described earlier in this chapter. In addition, these materials must have the ability to withstand the stresses associated with a food processing system without cracking, breaking, or deteriorating during continued use. They must have the ability to withstand a broad temperature range that may extend from low temperature freezing [−58 °F (−50 °C)] to steam sterilization [250 °F (121 °C)], and resist various chemical conditions from food materials, as well as cleaning and sanitizing chemicals. It is recommended that the equipment manufacturer/supplier provide documentation that the material is inert, nontoxic, nonabsorbent, non-contaminating, fat-resistant, non-odorous or off-flavor producing, and resistant to the chemical solutions and gases used in food applications (e.g., acid; alkaline; reducing/oxidizing chemicals; cleaning and sanitizing chemicals) under conditions of intended use. 3-A Sanitary Standards further specify that these materials be relatively resistant to scratching, scoring, decomposition, crazing, and chipping under normal use conditions.

The mechanisms and progression of degradation for metals compared to non-metal materials are different. Degradation of metals usually occurs at the surface, and, thus, is usually visible upon surface examination. Similarly, certain types of degradation (e.g, exposure to harsh chemicals) may also be readily visible on non-metal surfaces. However, some reactions (e.g., absorption of water, fats, and oils) may initiate degradation at the interior of plastic and rubber materials, which migrates towards the surface. This interior process of degradation may not be initially visible upon surface examination (See Fig. 12.11).

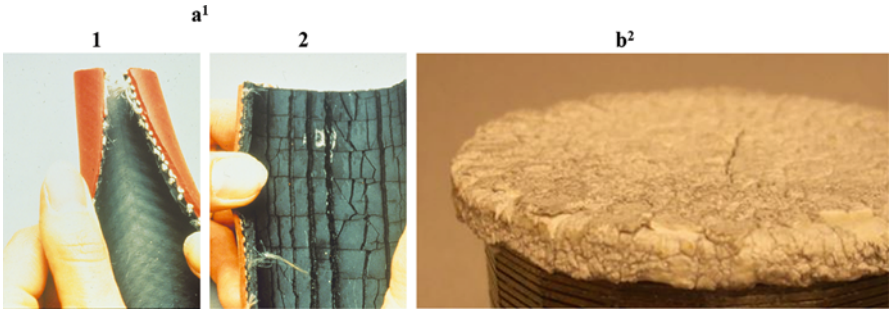

Fig. 12.11 Deterioration of plastic and rubber materials. (**a.1**) New rubber hose, (**a.2**) Same type of hose after prolonged use in harsh environment; (**b**) Plastic material, after use in an inappropriate application. (Photo courtesy of: [1]Tom Boufford; [2]Gabe Miller)

While the following was adapted from the summary of the causes and consequences of failure of plastic materials when used in food applications provided by Moerman and Partington (2014), similar failures occur with rubber materials.

- Water Absorption. Nearly all plastics and rubber materials absorb water to a certain extent, and water absorption is often listed as a cause of failure. The presence of absorbed water (as well as food components and chemicals) will impact chemical and intermolecular structural bonding causing loss of stiffness and softening of the material, increased porosity, loss of mechanical properties, and internal stress and failure. Certain thermoplastics, especially polyamide and acrylonitrile-butadiene-styrene (ABS) materials are more prone and may not be recommended in high moisture applications. Conversely, drying out may cause embrittlement of certain plastic or rubber materials.
- High Temperature and Steam. Under conditions of prolonged exposure to high temperature, certain plastic and rubber materials, especially polyethylene (PE), polycarbonate (PC), and polyvinylchloride (PVC), will exhibit cracking, spalling, embrittlement, and mechanical failure. More appropriate choices for high temperature or steam applications are polypropylene (PP), polytetrafluoroethylene (PTFE), or Teflon®, and poly ether-ether ketone (PEEK).
- Exposure to corrosive food and solution chemicals. Prolonged exposure to high levels of acid, alkali, and salt in food systems as well as cleaning chemicals will result in corrosion, loss of mechanical property, absorption, dissolution, material shedding, and embrittlement of many plastic and rubber materials. Absorption of food or cleaning chemicals could lead to food quality issues as well as food safety issues. Polyvinylidene fluoride (PVDF), PP, and PTFE are often recommended for their resistance to highly acidic environments, while PP, PTFE, and PEEK perform well in highly alkaline environments.
- Exposure to high fat and oil. Some plastic materials that are continuously exposed to fats and oils in food products will end up with damages such as physical degradation, material shedding (lamination), and cracking. PEEK, PTFE, PVDF,

and the polyamides are often recommended for use in high fat and oil applications. Synthetic rubber materials, especially ethylene propylene diene monomer (EPDM), will exhibit swelling with prolonged exposure to high fat applications.

- Exposure to specialized processes involving ozone, oxidizing chemicals, and ultraviolet (UV) light. When using specialized processes involving ozone and other oxidizing chemicals, and ultraviolet (UV) light, care must be exercised in selection of plastic and rubber materials, as many of these materials are not compatible. It is recommended that the manufacturer of equipment for these uses provide assurances that the materials used have adequate resistance properties.

It is important that worn plastic and rubber components (including gaskets) be replaced as soon as possible. A common error made by food processors is to use less expensive and often inferior components, which are not of the same material and may not meet hygienic design requirements. Though there may be a cost savings, using inferior replacement parts will lead to problems with cleanability and ultimately to failure of equipment.

12.6.3.2 Ceramics and Carbon Composites

Moerman and Partington (2016) provided an overview of the types of ceramics available, as well as their properties, and hygienic design criteria. Ceramics are very hard, non-metallic, nonorganic materials formed from the fusion of mineral substances at high temperatures. The broad category of ceramic materials ranges from pottery and china to carbides (e.g., silicon carbide; tungsten carbide), oxides (e.g., aluminum oxide) and nitrides (e.g., silicon nitride) used in industrial applications (Huebner 2018). In general, ceramic materials have resistance to high temperatures and chemicals, as well as the ability to withstand high pressure. Common disadvantages of ceramic materials, depending upon the type and formulation, include brittleness and porosity. This restricts the use of certain ceramics in food applications.

The allowed use of ceramics is dictated by regulatory status. Ceramics used in primary food contact applications should be documented as being free of leachable heavy metals (e.g, cadmium; lead) and other toxic metals (Moerman and Partington 2014). Further, the surfaces shall be smooth and free of crazing, cracks, and blemishes). The primary food application is the use of ceramic-coated materials for membranes in filtration systems and dynamic sealing surfaces in mechanical seals. Ceramics are also used in the manufacture of pipes and specialized equipment. They may also be used in other limited applications if wear resistance is necessary.

In recent years, carbon composites, formulated with plastics or graphite, have been used in many industrial applications. Carbon composites are generally inert and possess high lubricity, high strength, and rigidity, with low friction and low density. Several carbon formulations have been accepted as food contact substances by regulatory authorities for use in a variety of food applications (Anonymous

2018c). The primary food contact applications for carbon composites are in dynamic sealing surfaces in mechanical seals (Huebner 2018) and in packaging materials. Carbon materials are also used for molded parts and sensor components.

12.6.3.3 Glass

The potential for breakage limits widespread application of glass in food processing systems. Specially formulated glass materials such as Pyrex® have proven successful in certain applications. Whenever glass is used, it must be made of durable, break resistant or heat resistant glass. Some applications where glass is used are light and sight openings into vessels and in very limited glass piping applications. Shatterproof plastic materials are also available for glass replacement.

12.6.3.4 Wood

In general, the surface of wood material is highly porous and difficult to clean and sanitize effectively. Thus, wood should be avoided as a food contact surface, as well as in non-product surfaces. Wood is restricted in food processing and food service applications, with the exception of hardwood cutting boards and tight grain butcher blocks. Wood materials, however, are fairly common in certain segments of the food industry. Examples include: wooden boxes and lugs in fresh produce and citrus industry, aging and storage barrels for wine, beer, alcoholic beverages and steak sauces. In addition, wood pallets are still in widespread use, and wood is used for structural and environmental surfaces. The use of pressure treated or creosoted wood as a food contact material is prohibited.

12.6.3.5 Adhesives and Related Materials

Adhesives may be used in certain applications (e.g., holding gaskets in place; seals; bonding plastic components). A wide variety of adhesives are available including organic polymer adhesives (e.g., natural materials; synthetic materials) and inorganic adhesives (silicates, borates; metal oxides). When used, adhesives shall be non-toxic and are required to comply with 21 CFR Part 175 – "Indirect Food Additives: Adhesives and Components of Coatings." Further, their use must comply with supplier recommendations to minimize issues with localized corrosion in certain uses (Lewan and Partington 2014; Moerman and Partington 2014). All bonds shall be continuous, mechanically sound, and smooth (without crevices), shall remain firmly bonded and not separate from the base materials, and shall withstand the conditions of intended use, including temperature.

12.6.4 Surface Modification and Surface Finish

Surface modification is often necessary to improve the surface of materials to create a smooth surface that is durable, and free of cracks and crevices. Care should be used to assure that the surface treatment process doesn't create problems, such as crevices at the microscopic level or traces/flecks of material used in the treatment process. With stainless steel, a variety of surface modification methods are used to achieve desirable surface finish (Tuthill and Avery 1992).

12.6.4.1 Surface Treatment

A surface treatment is any process used to alter the chemical composition or mechanical properties of an existing surface with no appreciable build up (μm or less). In general, surface treatment procedures include grinding or polishing, cold rolling on polishing rollers, grinding with abrasives and cloth buffing, polishing with progressively finer abrasives, extensive buffing, tumbling, dry etching/sandblasting, wet etching using acidic solutions, and wire brushing. 3-A Standards and USDA Dairy Equipment Guidelines specify the following surface treatment procedures:

- Mechanical treatment including shot peening, polishing, and glass beading (with certain restrictions to limited applications);
- Thermal treatment including surface hardening laser, and electron beam;
- Diffusion treatment including carbonizing and nitriding;
- Chemical treatment including etching and oxidation;
- Ion implantation; and
- Electro-polishing.

12.6.4.2 Surface Coating

A surface coating process involves the depositing of a layer of different material (greater than μm) on the existing surface to create a new surface with altered functional properties (e.g., improve durability; protect from harsh environments; reduce friction between surfaces; reduce fouling; modify chemical, mechanical and thermal properties) (Moerman and Partington 2014). To be used, coating materials must be nontoxic and meet appropriate regulatory requirements, and must conform to hygienic design criteria. Coatings shall be free of surface delamination, pitting, flaking, blistering, and distortion when exposed to conditions of intended use.

According to 3-A Standards and USDA Dairy Equipment Guidelines, coating processes include, but are not limited to:

- Chemical (conversion, coatings);
- Engineering Plating (electro-deposition);

- Thermal Spraying [e.g., flame; plasma; arc spray; high velocity oxygen fuel (HVOF)];
- Physical Vapor Deposition
- Chemical Vapor Deposition
- Overlays and Encapsulation

PTFE (Teflon®) and similar plastic materials, are commonly used as coatings on aluminum and other metal surfaces in the manufacture of non-stick cookware. However, such products are of limited use in food industry applications because they have the potential for chipping, flaking or peeling. This creates two problems, 1) the coating material may contaminate the food product, and 2) the base metal layer may be exposed, thus increasing the risk of corrosion as well as loss of cleanability and/or potential metal migration into the product.

12.6.4.3 Surface Finish

Stainless steel finishes are given a number depending upon their characteristics and properties, with higher numbers indicating higher smoothness. The roughness average (R_a value) has been used to evaluate the smoothness of food contact surfaces (ISO 1984), and is accepted by 3-A Sanitary Standards. The R_a value is determined using a profilometer, an instrument that uses a diamond tipped stylus to measure peaks and valleys in a relatively smooth surface.

The No. 4 (150 grit) ground finish (32 μin. R_a, 0.8 μm R_a) on stainless steel is the most commonly used and specified stainless steel finish for food contact surfaces. 3-A Sanitary Standards, FDA Equipment Guidelines, USDA Dairy Equipment Guidelines, and EHEDG guidelines (EHEDG 2018b) specify that all surfaces (metal or non-metal), including fabricated, welded, and soldered joints, shall be at least as smooth as a 32 μin. R_a (0.8 μm R_a) finish, and shall be free of pits, folds, crevices, cracks, folds, and other imperfections in the final fabricated form.

Because of its superior release properties for higher fat products, the 2B milled finish, attained by cold rolling process, has been traditionally used in cheese, butter, and meat processing equipment. According to an investigation by Steiner et al. (2000), a 2B mill finish on stainless steel sheets may be as cleanable as a No. 4 (150 grit) finish. 3-A Sanitary Standards accept the 2B finish to be equivalent to a No. 4 finish, provided that the stainless steel sheets have been inspected and selected to be free of pits, folds, cracks, inclusions, and similar defects. Further, because mean R_a is not sensitive to individual extreme surface flaws, it is not accepted by 3-A Sanitary Standards as an assurance of the acceptability of a 2B finish on stainless steel sheets that have not been inspected and found free of these serious flaws. A comparison of a 2B finish and a No. 4 finish of stainless steel under magnification is shown in Fig. 12.12.

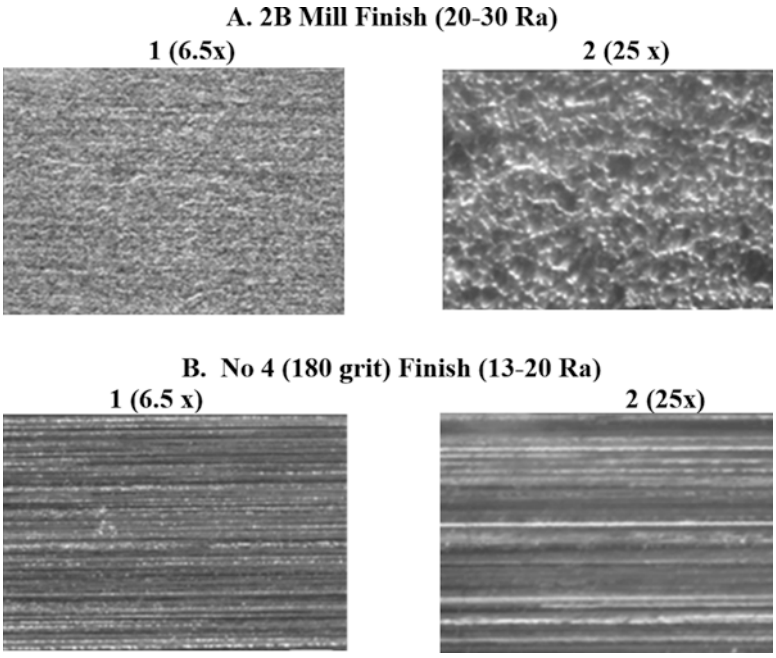

Fig. 12.12 Comparison of a No 4 and a 2B finish on stainless steel, under magnification. (From Schmidt et al. 2012). (**a**) 2B Mill Finish (20–30 Ra). (**b**) No 4 (180 grit) Finish (13–20 Ra)

12.6.5 Fabrication and Construction

The following discussion has been adapted from 3-A Sanitary Standards, EHEDG, and regulatory recommendations. Food equipment shall be designed, constructed, and fabricated to meet the following criteria:

12.6.5.1 Readily Accessible for Cleaning and Inspection

Food equipment must be designed, fabricated and constructed such that it is readily accessible for effective cleaning and sanitizing operations, as well as for inspection and verification that the surfaces are properly cleaned. 3-A Sanitary Standards define *Readily Accessible* as *"A location that can be safely reached by personnel from the floor, other permanent work area, or stable platform (permanent or moveable).*"

12.6.5.2 Self-Draining or Pitched to a Drainable Port

Food equipment shall be designed and constructed to be self-draining or pitched to a drainable port with no potential hold up of food materials or solutions. Illustrations

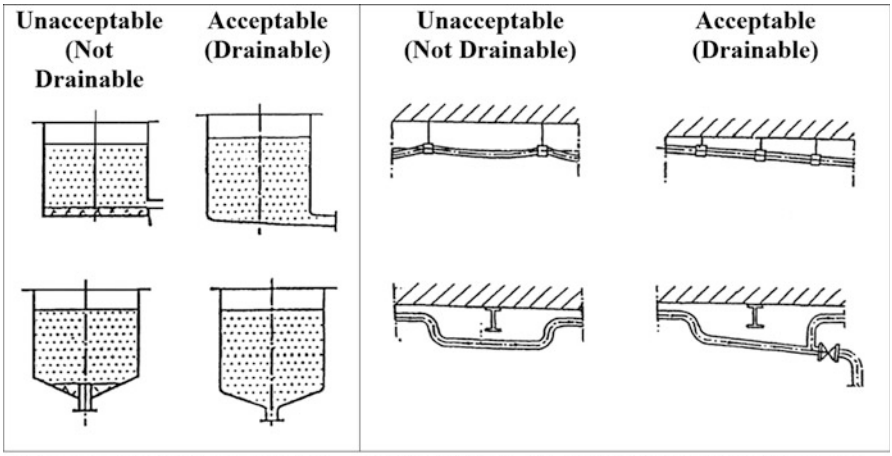

Fig. 12.13 Illustrations of acceptable vs. unacceptable hygienic design and construction for self-drainage. (Adapted from EHEDG 1995; FDA 2000; Schmidt and Erickson 2005). (**a**) Acceptable vs. unacceptable self-drainage design in tanks and vessels. (**b**) Acceptable vs. unacceptable self-drainage design for pipes

of acceptable and non-acceptable self-draining design are shown in Fig. 12.13. Piping systems installed in modern food processing systems designed for cleaning-in-place (CIP), require special consideration and close monitoring with regard to drainage. In equipment where self-draining is not feasible for functional reasons (e.g., heat exchangers; pumps), the surfaces must be pitched to a drainable port.

12.6.5.3 Free of Sharp Corners and Crevices

12.6.5.3.1 Internal Angles

For effective cleaning and sanitizing, all internal angles should be radiused (coved or rounded) (see Fig. 12.14). A square or right angle is not considered to be cleanable. Hygienic design standards and guidelines define and specify appropriate radii for specific equipment applications and components. 3A Sanitary Standards specify that *"all internal angles 135 degrees or less shall have a minimum radii of 1/4 inch (6.35 mm)."* These standards allow for smaller radii (e.g, gasket grooves; grooves in gaskets) where needed for functional reasons, and require that retaining grooves for removable O-rings and seals shall be no deeper than their width and have a minimum radii according to the nominal size of the O-ring.

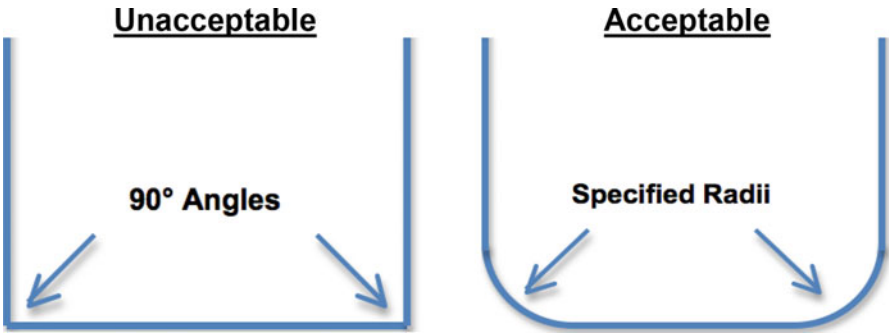

Fig. 12.14 Illustrations of acceptable vs. unacceptable internal angles

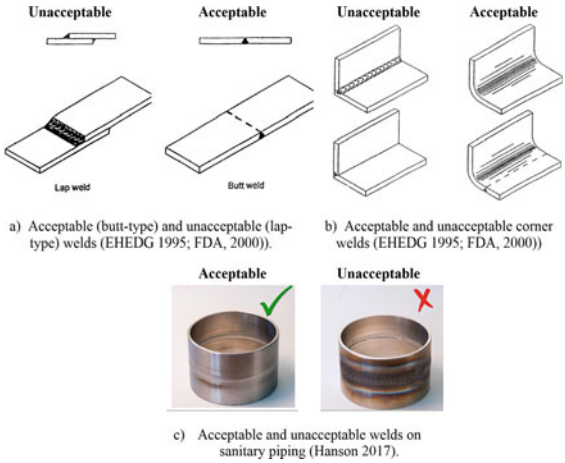

Fig. 12.15 Illustrations of acceptable and unacceptable permanent welded joints. (**a**) Acceptable (butt-type) and unacceptable (lap-type) welds (EHEDG 1995; FDA 2000)). (**b**) Acceptable and unacceptable corner welds (EHEDG 1995; FDA 2000)). (**c**) Acceptable and unacceptable welds on sanitary piping (Hanson 2017)

12.6.5.3.2 Mating Surfaces Substantially Flush

Mating surfaces (including gasketed connections) that are off-set from each other may create a possible ledge for accumulation of materials or niche for bacteria. 3-A standards specify that such mating surfaces be not more than 1/32 in (0.794) off-set from each other.

12.6.5.3.3 Permanent Joints

All permanent joints shall be continuously welded to be smooth, durable, with no crevices or pits. Welding of stainless steel and other metals used in food contact requires specialized expertise and must meet hygienic weld requirements (Eastwood et al. 1993). Welded joints on stainless steel surfaces should be continuous, butt-type joints (Fig. 12.15a) and ground to be at least as smooth as a 32 μin. (0.8 μm) R_a

finish. If the welded joint is at a corner, it must be coved to the appropriate radius and ground smooth (Fig. 12.15b). ***Extreme care shall be taken in welding sanitary piping, as often a weld that appears smooth on the outside may not be smooth in the interior*** (Fig. 12.15c).

Soldering and brazing should be restricted in food equipment fabrication, except for specific applications where welded joints are not possible. If done, soldering/brazing must use only non-toxic materials and the surface must meet the hygienic design criteria.

Metal-to metal, or interference fits, may be used in certain applications to assemble metal-to-metal parts. Such joints are permitted in certain 3-A standards, provided that the joints are free of external shoulders or relieved areas, and that the tightness of the fit is validated in accordance with EHEDG Guideline 2 testing method (EHEG 2018b) or a visible dye penetration test (ASTM International 2018b).

Bonded joints may be used for joints in non-metal applications (e.g, rubber; plastics; ceramics). Such bonds must be continuous and mechanically sound, meets hygienic design criteria, and does not separate from the base material when exposed to conditions of use.

12.6.5.3.4 Non-permanent Joints

Gaskets, O-rings, seals and similar components are necessary to obtain a tight seal or juncture. However, they may create hygienic issues if they are manufactured from improper materials that do not meet regulatory requirements, are not suitable for the intended use, and/or are improperly installed.

All joints involving removable gaskets shall be of a design that allows the groove to be inspectable and cleanable when the gasket is removed. Retaining grooves for removable O-rings and seals shall be no greater than ¼ inch (6.35 mm) and width of no less than ¼ inch (6.35 mm) and have a minimum radii in accordance with the nominal size of the O-ring, as described in 3-A Standards.

Bonded joints involving non-removable gaskets or similar components shall be designed such that any grooves in the gasket are no deeper than their width. When used between flat sealing surfaces, bonded gaskets shall be installed to be substantially flush. In applications intended for CIP cleaning, the gasket joints and O-ring installation shall be designed and installed such that the O-ring is adequately exposed to the cleaning solutions.

Metal-to-metal (e.g., mechanical force) seals may be used provided that the tightness of the seal is documented. As with any metal-to-metal contact, it is very important that liquid ingress is avoided. Such ingress creates a risk of contamination as well as contact corrosion and eventual failure of the seal. Use of this type of joint should be limited only to specific applications where welded joints are not possible (e.g. bushings), and the tightness of fit shall be documented.

12.6.5.3.5 Fasteners

Improperly installed fasteners may create issues with metal-to-metal contact corrosion, and, if they work loose, create a risk of physical contamination. Exposed threads, bolts, screws, or rivets on (or above) product contact surfaces should be avoided, unless where it is absolutely necessary for non-permanent joints in piping and for making various attachments (Lelieveld et al. 2005; Lelieveld et al. 2014; FDA 2001). If used, fasteners must be of appropriate hygienic design (e.g. acceptable sanitary threads; domed heads). Further, any equipment with exposed threads or bolts must be accessible for manual cleaning. Where enclosed threads are allowed (e.g., acorn nuts to attach pump impeller blades), the equipment must be designed and certified to be CIP cleaned. Fabrication that involves threads that are improperly enclosed such that it creates a pocket for accumulation of chemicals or food residues, is not acceptable.

12.6.5.4 No Dead Spaces

Care should be taken when connecting pipes or other equipment (e.g., gauges; thermometers; probes) to pipelines, tanks, vessels, or other food equipment. Improper connections could create a dead space (e.g., dead end; dead leg) or an area outside the product flow, where product, cleaning and sanitizing chemicals or other extraneous matter may accumulate. As shown in Fig. 12.16, the flow velocity of a liquid food product (or cleaning solution) is dramatically reduced as the depth of a dead space increases. This area would not be accessible and would not allow

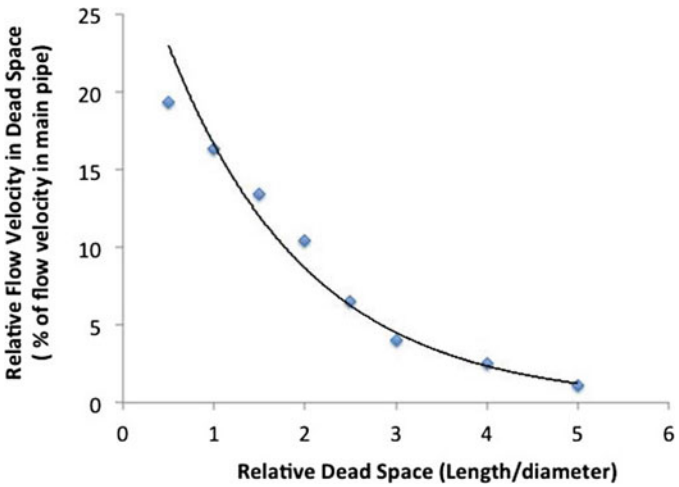

Fig. 12.16 Relationship of the relative liquid flow velocity in the dead space of a pipe T-section (averaged across pipe flow velocity ranging from 1.0 to 3.5 m/s), compared to the depth of the dead space. (Adapted from Lelieveld et al. 2014)

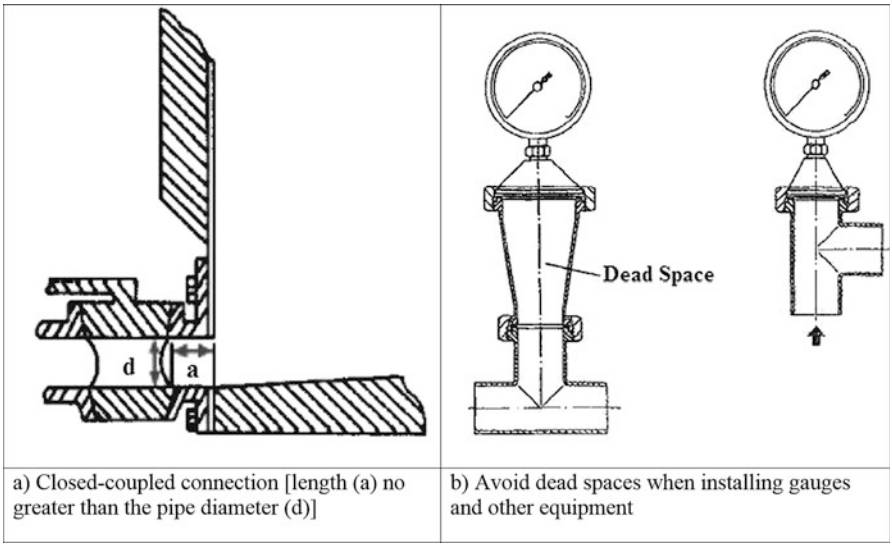

Fig. 12.17 Illustrations of acceptable and unacceptable connections (FDA 2000; Schmidt and Erickson 2005). (**a**) Closed-coupled connection [length (*a*) no greater than the pipe diameter (*d*)]. (**b**) Avoid dead spaces when installing gauges and other equipment

materials to be completely removed during Clean in Place (CIP) cleaning procedures due to insufficient flow velocity. If the equipment is used for thermal processing, the liquid food product in a dead space would not receive the appropriate heat treatment.

To avoid dead spaces, all connections should be close-coupled (e.g., pipe connection should not be of length greater than one pipe diameter) as shown in Fig 12.17a. Dead spaces created in the installation of gauges, probes, instruments, or other equipment are shown in Fig. 12.17b. All demountable connections that create a dead space must be disassembled and manually cleaned. Dead spaces may also be created through valving and pipeline installation design flaws. Thus, all new or modified piping plans need to be evaluated with this in mind.

12.6.5.5 Protection from Contamination from Environmental Sources

12.6.5.5.1 Ancillary Equipment and Their Connection

Any equipment installed into the product zone, must meet all hygienic design requirements. This includes probes, thermometers, magnets, shafts, bearings, agitators, and other equipment. Further, such ancillary components should be attached to food equipment in such a way that the food contact zone is sealed from contamination caused by leakage of lubricants or other contaminants into the product zone, and the components must be accessible and removable for cleaning.

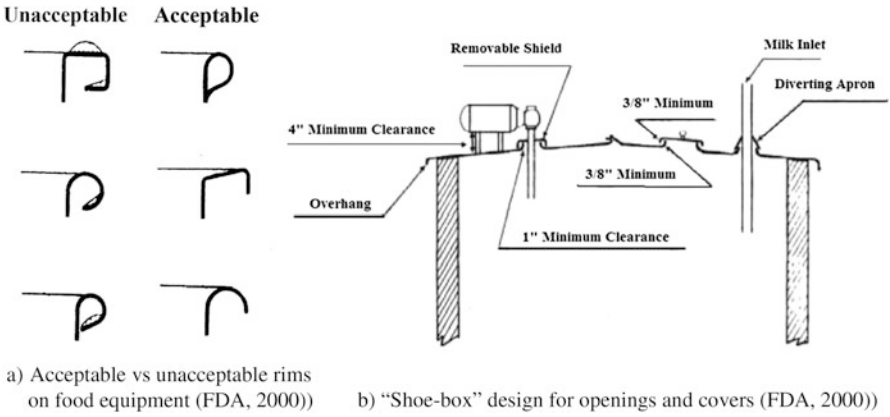

Fig. 12.18 Illustrations of acceptable and unacceptable designs for rims and lids on food equipment. (**a**) Acceptable vs unacceptable rims on food equipment (FDA 2000). (**b**) "Shoe-box" design for openings and covers (FDA 2000)

12.6.5.5.2 Top Rims, Openings, and Covers

Top rims of equipment should be constructed and fabricated to avoid the collection of water droplets or dust (Fig. 12.18a). Any opening or cover shall be designed, fabricated, and constructed in such a manner as to adequately protect food products from contamination and to divert potential contamination away from the food product zone. Openings should be lipped at a minimum height of 3/8 in, and protected with a shoe box type covering (Fig. 12.18b).

12.6.5.5.3 Conveyors

Conveyor and related surfaces (e.g., belts; rollers) have been a well-documented source of microbial contamination, as well as allergen cross-contact (Lelieveld et al. 2005; Lelieveld et al. 2014). Many hygienic design issues have been noted with conveyor belts in traditional use in the food industry (Sheffler 2013). Hygienic issues noted with flat belts are cracks and crevices on the surface due to aging and wear, as well as moisture ingress between layers, which create potential microbial niches. Modular plastic belts, with intricate design, are difficult to clean and sanitize. Hollow rollers also may have issues with moisture ingress and should be of solid hygienic construction or properly sealed (Fig. 12.19). Newer designs are available which are filled with dye to show when the seal has been compromised. The edges and ends of conveyor belts must also be adequately sealed.

Rigorous (and appropriate) cleaning/sanitizing and preventive maintenance programs are needed on conveyor systems. Material used must be easily cleanable and must withstand chemicals and temperatures used. Conveyor belts should be easily

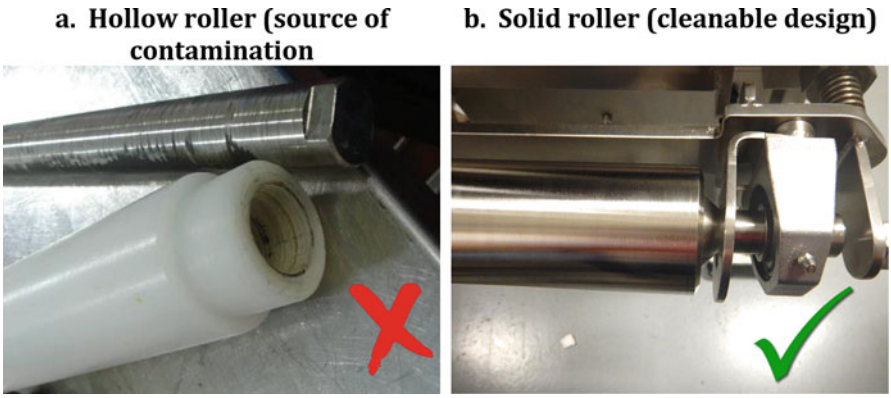

Fig. 12.19 Acceptable vs unacceptable conveyor rollers. (From Hanson 2017). (**a**). Hollow roller (source of contamination. (**b**) Solid roller (cleanable design)

removable and conveying systems should be designed for easy access to all areas for thorough cleaning.

Conveyor belts should be installed in a fashion that allows for easy removal and easy access to underlying areas of equipment, rails, and other appurtenances. If re-circulated cleaning is needed, belt length should be designed to allow for the belts to fit into Clean-out-of Place (COP) tanks.

Framework, chains, drive motors and casings, protective shielding, and other equipment shall meet, at minimum, the hygienic design criteria listed. Access to (and for ease of cleaning) these areas is a vital link in being able to control environmental sources of contamination.

12.6.5.5.4 Shields and Related Equipment

Appropriate shields over conveyors and other areas where open food products may pass are an absolute necessity. Such shields, however, if not properly designed may be a catch area for accumulation of moisture, dust, and other contamination. It is important that shields meet all hygienic design criteria and that they are sloped to prevent accumulation. Shields that are overlapped should be installed so that the overlapped area does not create drainage or ingress onto products, product contact surfaces, or product packaging.

12.6.6 Hygienic Design in Dry Cleaning Applications

Because of the unique ability of *Salmonella enterica* serotypes to survive in conditions of low water activity, *Salmonella* contamination has been a concern in facilities manufacturing dry food products. Non-hygienic design of equipment in use in these

facilities has been suggested as a possible root cause of food-borne illness outbreaks. Equipment design and air handling systems for drying equipment were implicated as causative factors in major recalls due to *Salmonella* contamination of dry milk and dry milk products in the 1960s (Anonymous 1966). 3-A Sanitary Standards developed standards for this industry, which are used as guide for equipment inspection under the USDA/AMS/Dairy Division (AMS 2001a, b). More recently, poor equipment and facility design have been identified among several potential environmental factors as a root cause of *Salmonella* contamination in dry foods (Podolak et al. 2010).

Avoidance of aqueous cleaning is often recommended in dry milk facilities, especially where dusty conditions are predominant. Introducing moisture may result in material clumping in crevices, creating niches for microbial growth and increasing the risk of microbial contamination (Chen et al. 2009; Du et al. 2007; GMA 2009). An overview of dry and non-aqueous cleaning methods, and their relative advantages and disadvantages, has been published (Moerman and Mager 2016). The Grocery Manufacturers Association (GMA) has developed a guidance document, *Control of Salmonella in Low Moisture Foods* (GMA 2009), for use in low-moisture product facilities. This document describes cleaning methods and includes hygienic design of equipment and facilities as important *Salmonella* control elements. It is sometimes recommended that a thorough wet cleaning be done in certain applications, provided that surfaces are completely dried after the wet cleaning. Additional concerns exist in food facilities involved in drying liquid food products (e.g., spray dryers; drum dryers; fluidized bed dryers; tunnel dryers). These facilities should completely separate the equipment involved in wet food handling and aqueous cleaning and sanitizing operations from the equipment to be dry-cleaned (e.g., cyclones; dust collection; powder discharge; powder handling equipment).

The hygienic design criteria, established for equipment to be cleaned by aqueous (wet) cleaning and sanitizing methods, are readily applicable to equipment to be cleaned by non-aqueous methods (Mager 2016). However, special emphasis should be placed on radii, avoiding pockets or areas where clumping of dry material may accumulate, static seals (gaskets) and flexible connections (where used), and on-air systems used in drying equipment. In addition, materials should be non-abrasive, especially in facilities handling more abrasive dry materials, and, since many dry cleaning methods are manual, accessibility for cleaning is also very important.

12.7 Hygienic Design, Fabrication, and Construction Criteria for Non-product Contact Surfaces

12.7.1 Hygienic Design Features/Concerns with Non-product Contact Surfaces

Traditionally, *non-product contact surfaces* have not been held to the rigid hygienic design, fabrication, and construction criteria associated with *product contact*

surfaces. However, non-product contact surfaces of food equipment are well-documented sources for contamination of a food facility with environmental pathogens as well as sources for allergen cross-contact. These areas can also be harborage areas for insects and rodents, which carry a variety of pathogenic microorganisms. The increased importance of environmental sanitation programs using corrosive chemicals and environmental monitoring programs, have elevated the importance of hygienic design of non-product contact surfaces. Therefore, a responsible sanitation program should include evaluating both *equipment non-product contact surfaces* and *environmental surfaces* in the food and beverage facility, and making the appropriate corrections and modifications needed.

12.7.1.1 Equipment Non-product Contact Surfaces

A photo gallery of non-product contact surfaces is presented in Fig. 12.20.

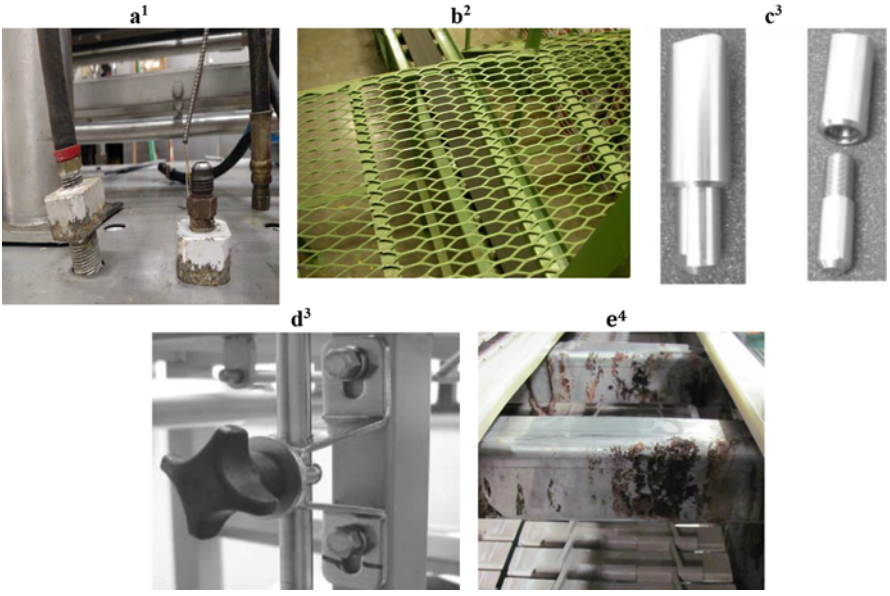

Fig. 12.20 Hygienic design issues with non-product contact surfaces. (**a**) Threads (if of improper type) and ledges with connections create potential contamination issues. In addition, corrosion from contact with dissimilar metals (aluminum thimble and stainless steel); (**b**) Catwalk installed over a product conveyor (source of overhead contamination); (**c**) Hygienic leveling components with enclosed (and sealed) threads (Marconnett 2004; Schmidt and Erickson 2005); (**d**) Hollow framework penetrated (with bolted attachments) – environmental contamination source (Marconnett 2004; Schmidt and Erickson 2005); (**e**) Uncleanable inner surfaces of a conveyor. (Photo courtesy of: [1]Paul Kennedy, Pragmatics Eng.; [2] Glades Crop Care, Inc.; [3]Kraft Heinz Co.; [4]Gabe Miller)

The following are recommended hygienic design criteria for *equipment non-product contact surfaces* to minimize microbiological contamination or allergen cross contact:

- Constructed and fabricated with appropriate materials that are compatible with the environment of use, and in such a manner as to be reasonably cleanable, corrosion resistant, and maintenance free. Contact between dissimilar metals, which may lead to corrosion, must be avoided in making connections (See Fig 12.20a).
- Ledges, or "catch areas", must be avoided. Tops of equipment, shields, covers, boxes and similar surfaces should be sloped at a 45 degree angle or more to avoid accumulation of dust, moisture, food residue, and other materials.
- Potential overhead contamination from catwalks and similar structures must be avoided (See Fig. 12.20b)
- Threads and threaded surfaces must be avoided. Socket and screw head bolts should be installed in a vertical position so that residues do not collect and to allow adequate drainage. The use of "all thread rod" should be avoided for hangers and similar applications, and where threads are necessary (e.g., leveling components; connections) they must be hygienic threads or are of the enclosed type (see Fig. 12.20a, c).
- Hollow areas of equipment, potential niches for microorganisms, are avoided, eliminated, or permanently sealed in a manner such that it does not create an improper hygienic condition. This includes, but is not limited to: legs, supports, frames and framework, rollers, junction boxes, nameplates and mounting plates, end caps, sleeves, bolts, studs, mounting bolts, studs, mounting plates, brackets, and other items. Sealed hollow areas should not be penetrated or compromised by drilling or other perforation of sealed areas (see Fig. 12.20d).
- Electric motors are of proper design and are maintained in hygienic condition. By nature, electric motors present a challenge with regard to hygienic design. Even stainless steel motors of improved design have areas where dust and contamination may accumulate (Higgins 2012).
- Non-product contact components of conveyor systems are of proper design and are maintained in hygienic conditions (see Fig. 12.20e). Conveyors have intricate construction and present many opportunities for contamination or allergen cross-contact.
- Piano-style hinges on cabinets, access areas to motors or chain drives on conveyors, or other restricted areas, should be avoided or eliminated. This type of hinge is not hygienic and will provide recessed areas that cannot be cleaned.
- Hygienic design features are maintained through rigorous preventive maintenance programs.

12.7.1.2 Environmental Surfaces

A variety of niches for microorganisms and for accumulation of allergens exist in food facilities. These environmental surfaces should be examined carefully to prevent, eliminate, or avoid contamination of food from environmental pathogens or cross-contact with allergens. This evaluation must include all the elements of facility hygienic design (Graham 1992a; Graham 1992b; Holah 2014; Moerman 2014) including facility sealing; walls, floors, ceiling construction and fabrication; coving of junctures, sloping of wall bumpers, and other building or facility elements. Since the "*Listeria Hysteria"* of the 1980s, there have been many advances in hygienic design concepts relative to materials used in sealing of walls, ceilings, and floors, and especially with regard to plumbing and floor drains; refrigeration equipment; refrigerated water (or 'sweet water') systems; heating, ventilation, air conditioning (HVAC) systems; electrical components and connections; lighting; and maintenance enclosures. Such newer, more hygienically advanced equipment is more compatible with aggressive environmental sanitation programs than that found older or legacy facilities. In the case of heating/cooling units, the materials used should be able to withstand the harsh chemicals used to clean and sanitize radiation fins, ductwork, filter housings and point of application vents. These units have the potential to affect large areas of production and packaging. A frequent maintenance and cleaning program is needed to alleviate issues with microbial contamination and/or allergen cross-contact in the facility.

Wood pallets are a documented source of microbial contamination and should be avoided in processing areas (Higgins 2012). However, plastic pallets, if not properly cleaned and maintained, may also have contamination concerns.

Refrigeration units and equipment (e.g, trays; drains; pans; fans; fan guards; fan motors), and drains present concerns as a harborage point for mold and mildew (see Fig. 12.20f), as well as *L. monocytogenes*, a psychrotroph that will grow in a cold, moist environment. Thus, they should meet appropriate hygienic design and construction criteria. Special attention should be given to the location of more portable refrigeration units. These should not be placed in an area where ready to eat food product is being packaged. Further, drains from these units should be of appropriate hygienic design and should not be plumbed into critical food handling areas. These refrigeration components should be cleaned and maintained at an appropriate frequency. Each facility needs to evaluate the conditions these units are subjected to and determine through environmental testing programs how often the units should be cleaned and treated for bacterial growth. Drain pans and drain lines need to be graded to drain, and there should be adequate clearance under the units to be able to clean drain pans easily. Drain lines should be designed of materials that will not sag over time and should be supported to allow for complete drainage. The exit of these drains should not allow condensate and drain water to flow over traffic areas. The units must be designed of materials that will withstand cleaning and sanitizing solutions without becoming excessively corroded and drain pans and lines should be made of inert material that will not be affected by these same solutions. Freezers,

freezing tunnels or spirals, and their components must also be constructed to appropriate hygienic design criteria, and properly cleaned and maintained to avoid potential contamination (Moerman and Fikiin 2016).

Improved hygienic design for ancillary environmental equipment listed above is available, however, many older facilities do not have the luxury of using this more expensive technology. In addition, there are unique traditions of concern in certain industry segments. For example, in the fresh produce industry, it has been traditional for packing sheds to be open to the environment, and these sheds are not used on a continuous basis. Sealing these buildings might be a goal, but sealing alone would not be a complete solution. It must be done with careful considerations of environmental surfaces of ancillary equipment, including appropriate HVAC systems to avoid condensation issues. Condensate may be a source of bacterial aerosols as well as increasing the risk of corrosion of equipment, components, and piping systems.

12.7.2 Environmental Monitoring

A discussion of non-product surfaces would not be complete without a brief discussion about *Environmental Monitoring*. In response to several serious outbreaks in the mid-1980s, the FDA instituted a product and environmental sampling study for identification of pathogens (Lecos 1986). The Dairy Safety Initiative (the first time that the regulatory agency proactively looked for environmental pathogens in food facilities) was followed by a broader FDA environmental pathogen testing program in other commodities, as well as an FSIS pathogen testing in meats and poultry. Under the FDA Preventive Controls Rules (FDA 2015a, b), environmental pathogen monitoring is required for facilities manufacturing ready to eat food (or food consumed without treatment to significantly minimize pathogens).

While environmental pathogen testing is an in-exact science, the procedures and reliability of testing methods have improved over the past 30 years. Most reputable food companies have rigorous environmental testing programs. Concern for environmental pathogens has stimulated improvements in the hygienic design of facilities, and non-product contact surfaces, as well as improvements in environmental cleaning and sanitizing programs.

12.8 Hygienic Design Implications of Location, Installation, and Operation of Food Equipment

12.8.1 Location of Food Equipment

The old adage “location, location, location” describing the 3 most important factors in real estate, also applies to location of food equipment in a food facility. Locating

food equipment should be a planned activity, with input from all personnel involved including management; production and operation; cleaning and sanitizing; and maintenance. The goal is that food equipment for high-risk food processing functions be located to minimize risks of contamination from the facility and surroundings. Consideration should be given to potential harborages for vermin or insects, overhead contamination, and distance from sources of contamination (e.g., floor drains; waste bins; sources of aerosol contamination). Extreme care must be exercised to avoid locating equipment for packaging of ready to eat foods near equipment involved in handling of raw product. Finally, equipment should be located in a logical sequence for processing and cleaning operations. Consideration should be given to the sequencing of cleaning and sanitizing operations to avoid situations where one line is being cleaned while the other line is still in production.

12.8.2 Installation and Operation of Food Equipment

Food equipment shall be installed with proper orientation (leveling, drainage considerations), and to allow sufficient space under, around and between equipment for adequate cleaning, inspection and maintenance (Graham 1992a, b). According to 3-A Standards criteria:

- Equipment shall be installed to allow adequate free drainage;
- Machine leveling feet shall be sealed to the floor at installation; and
- There shall be no exposed threads in equipment unless the threads are sanitary acme threads.

The *NSF Manual on Sanitation Aspects of Installation of Food Service Equipment* (NSF 1968) provides recommendations for equipment installation in food service and retail food facilities. The FDA Food Code provides the following criteria for installation of food equipment in retail food facilities:

- Unless sealed to walls, food equipment should be kept at least 4 inches from walls.
- Floor mounted equipment should be sealed to the floor, platform, or pedestal or should be kept no less than 6 inches from the floor.
- Table mounted equipment should be sealed to the table or be kept at no less than 4 inches from the counter top.

Recommendations and guidelines regarding adequate space required between and around equipment are varied, and are dependent upon the size and type of equipment. For large equipment (4–8 feet in diameter), a minimum distance of 12 inches from walls and between and around equipment is often recommended. For very large equipment (8 feet diameter or greater), a minimum of 18 inches from walls and between equipment is recommended.

Food equipment shall be operated according to the manufacturer's recommendations and so that it does not create an unsanitary condition. Consideration should be

given to the impact of a specific piece of equipment on processing lines entering and exiting, shields and covers, and other equipment.

12.9 Future Concerns and Implications in Hygienic Design

Implementation of regulatory requirements will result in increased demand for environmental cleaning and sanitizing programs as well as emphasis on environmental pathogen monitoring. Therefore, it is anticipated that more emphasis will be placed on hygienic design, especially with regard to non-product contact surfaces, in the future. This emphasis will focus on those food processing and handling facilities considered to be high risk due to increased prevalence with food borne illness outbreaks.

The development of novel materials for use as components of food contact materials, and engineering of food equipment will continue to evolve. Considerable research is being done on antimicrobial food contact surfaces through the use of materials (e.g., copper; titanium oxide; colloidal silver or silver nanoparticles; bioactive polymers) that have antimicrobial activity when used under certain conditions, or the use of these antimicrobial agents and others (e.g., quaternary ammonium compound sanitizers; light activated materials; electrostatic repulsive materials; others) as surface coatings (Moerman and Partington 2016). Use of any antimicrobial materials requires acceptance by regulatory authorities due to potential toxicity issues, as well as efficacy considerations (Warriner and Murray 2018). Many antimicrobial surfaces are finding application in non-product contact surfaces (e.g., refrigerator trays and shelves; dishwasher surfaces).

12.10 Conclusions

For the production of safe food, food manufacturers must implement and document a food safety plan which includes the principles of hygienic equipment design. Adhering to the principles of hygienic design through requiring conformance to specific standards, where available, provides a solid foundation for any food safety program. Consideration must be given to the many variables involved in handling and processing different foods. Intimate knowledge of the process being considered, as well as foresight into addressing future usage and applications, will allow all the involved parties the ability to choose equipment materials and construction that will provide for manufacturing the safest product. Materials and construction/fabrication features of equipment, along with consideration to installation, operation and maintenance for all product contact and non-product contact surfaces in the manufacturing facility will aid in creating a solid foundation for a complete overarching food safety program.

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Chapter 13
Equipment Cleaning, Sanitation, and Maintenance

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13.1 Introduction

A clean and sanitized food processing equipment is the very first step toward ensuring the quality and safety of the final food product. It is imperative to maintain the hygiene of the food processing equipment and food processing plant, and to monitor the operations during food production. During the cleaning and sanitation of food processing equipment, the processing protocols and the physical, chemical, and biological properties of the final product should all be taken into consideration. A combination of Hazard Analysis Critical Control Point (HACCP), Good Manufacturing Practices (GMP), and Sanitation Standard Operating Procedures (SSOP) forms the very foundation of food safety system in a processing plant. While GMP offers recommendations for general food processing aspects, SSOP is developed based on GMP but is applicable to specific operations; both of which are necessary for a smooth HACCP implementation and are essential to the success of HACCP. Therefore, in this chapter, the mechanisms of cleaning and sanitation is introduced, followed by the cleaning, sanitation, and maintenance of representative food processing equipment groups, and concluding with some novel and environmentally friendly approaches that are gaining popularity and being adopted recently.

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13.2 Cleaning, Sanitation, and Maintenance (CSM) Mechanisms and Corresponding Agents and Protocols

To achieve satisfactory cleaning and sanitation, an in-depth understanding of the soil is the first and foremost. As defined by Jennings (1965), soil is a 'matter out of place', with distinct nature from food (and the surrounding environment involved) with different phases and conditions. All, from dirt on the excavated potatoes to bacteria in the processing milk, can be referred to as soil. In a food processing plant, the particulates and visible dirt are most easily detected and removed, via flushing, air filtration, or vacuum cleaning. For undesirable and/or unwanted food soil components such as carbohydrates, proteins, lipids, and minerals, they are commonly categorized into either organic and inorganic soils, or water-soluble and water-insoluble soils. Another type of soil commonly found in food processing plant is biofilm. Biofilm is formed by microorganisms as a survival strategy to overcome adverse environmental changes (Stepanović et al. 2003). After the initial attachment and early development, when mature, microbial cells adhere to the surfaces to form biofilm, which cannot be easily removed thereafter. Developed biofilms are typically more resistant to sanitation than non-adhered microorganisms and therefore more attention is needed to reduce biofilm formation in food processing equipment and plants (Coughlan et al. 2016).

In addition to understanding the type and nature of soil in a food processing plant, it is imperative to have a comprehensive understanding on (i) the type of equipment involved, especially the food-contact surface properties; (ii) the expected outcome of the cleaning and sanitation procedures; and (iii) a knowledge of the function, application and possible availability of cleaning agents and sanitizers. A well-established HACCP plan in the processing plant, which is thoroughly discussed in another chapter of this book, could provide guidance to the plant managers and equipment operators.

The removal of soils, i.e., cleaning, as a starting procedure, is affected by many factors, including the nature of the soil, temperature, and concentration of the cleaning solution, the frequency and duration of the cleaning implementation, and whether turbulence or any other dynamic and mechanical forces are additionally applied. Typically, with higher chemical concentration, longer cleaning duration, and higher temperature, a better cleaning performance can be achieved, especially when the temperature exceeds the melting point of target lipid. However, as noted in Table 13.1, with increased temperature, occurrence of significant polymerization or carbohydrate caramelization increases, leading to hard-to-remove patches of soils. Therefore, the appropriate cleaning protocols must be selected to avoid such situations. Turbulence can be achieved by the applications of spray balls or rotating arms to enhance cleaning performance. It can also be introduced via air pulsation, which is discussed in the following section.

Removal of soil with cleaning solutions includes both physical and chemical reactions. Sometimes biological reactions are included. The chemical functions of cleaning solutions can be summarized as emulsification of fats and oils, wetting on

Table 13.1 Food soil characteristics[a]

Type of soil	Solubility	Heat induced changes	Recommended cleaner
Carbohydrates	Water soluble	Caramelization formation: harder to clean	Ammonia or nonionic detergent, tepid water
Lipids	Water insoluble	Decomposition and polymerization: harder to clean	Anionic or nonionic basic detergent
Proteins	Water insoluble, alkaline and slight acid soluble	Denaturation: harder to clean	Highly alkaline detergent containing -ortho and -metasilicates
Minerals	Water solubility differ, most are acid soluble	Unless interacting with other components, generally easy to clean	Acid detergent containing chelating agents and a corrosion inhibitor
Biofilm	Water solubility differ, depending on the formation stage	Destroyed in most cases	Chlorinated solutions, depending on the microorganisms and formation stage

[a]Adapted from Troller (1993)

soil surfaces and lowering the surface tension of the solution, penetration into the porous media, deflocculation or dispersion of aggregates, suspension of insoluble particles, sequestrating metal cations, buffering strong acidic or alkaline environments, etc. (Tamime 2008). Common cleaning agents include detergents (such as trisodium phosphate, sodium carbonate, sodium metasilicate pentahydrate, tretrasodium pyrophosphate, and sodium tripolyphosphate), sequestrants and chelators, surfactants (nonionic and anionic), etc.

The major function of sanitation procedures in food processing plants is to destroy, remove, or inhibit the microorganisms (Mokgatla et al. 2002). The ultimate purpose of sanitation is to control the possible threats to human health and the food spoilage. Sanitizers, on most occasions, are relatively expensive and therefore, the sanitation procedures should be done timely and wisely. Sanitation duration, solution concentration and temperature, solution surface tension and pH, and the possible estimation of the microbial load and locations, along with an estimation of the amount of interfering organic soils and mineral residuals; all these factors affect the use of sanitizers and thus the performance of sanitation. Table 13.2 is a summary of commonly used commercial sanitizers.

Cleaning and sanitation can be performed by hand or using equipment and devices (Holah 2003). Manual cleaning and sanitation are not used when dealing with large quantity, high productivity, and highly automated food processing plants; therefore, this approach is not the focus of this chapter. For advanced cleaning and sanitation procedures involving mechanical equipment, the chemicals used in cleaning and sanitation are usually delivered via either foams and gels, pressurized mists (low pressure), water jets (low or high pressure), electrical scrubbing brushes, air-injected slugs, etc. These methods have respective fields of applications and have been well documented. Cleaning-in-place (CIP) is one of these highly automated protocols. Cleaning and sanitation in CIP are conducted within the equipment in its assembled form during the circulation/recirculation of rinse water, wash solution,

Table 13.2 Advantages and disadvantages of various sanitizers[a]

Compound	Advantages	Disadvantages
Hypochlorites	Broad spectrum of activity Inexpensive Easy to use	Corrosion and lipid oxidation Possible discoloration Inactivated by organic matter Odor and skin irritation
Quaternary ammonium sanitizers	Non-corrosive Non-irritating No flavor/odor	Ineffective against Gram negative bacteria and possible resistance development Film formation
Iodophors	Non-corrosive Easy to use Non-irritating Broad spectrum of activity	Flavor/odor Forms purple compound with starch Moderately expensive
Peracetic acid	Broad spectrum of activity Particularly effective against spores	Hazardous to use Usually restricted to CIP, cold temperature and CO_2 environment
Acid-anionics	Broad spectrum of activity against vegetative cells Low toxicity to mammals	Effective at very low pH levels (1.9–2.5), hence corrosive

[a]Adapted from Troller (1993); Holah (2003)

and sanitizing solution (Wang et al. 2016). Typical CIP includes alkaline detergent washes, acidic detergent washes, several rounds of water rinses, and sanitation cycles. The implementation of CIP in a food processing plant accelerates the turnover of processing and cleaning/sanitation, thus improving the production efficiency. However, CIP implementation in food processing plants must be considered with the target soil type, the food products being processed, and the available space and other resources. In addition, possible installation factors and economic benefit should be evaluated prior to implementation. Detailed studies of CIP with an example on dairy processing are introduced in the following section.

Evaluation of the cleaning and sanitation procedures largely depends on what activity is being monitored and what outcomes are expected (Holah 2003). The evaluations can be undertaken either before, during, and/or after a certain procedure; but a typical and cost-effective approach is to compare before and after CIP to better evaluate the cleaning and sanitation performance. Food contact surfaces with high-risk locations such as turns, dead corners and angles, etc. are of major concerns (Cordier 2002). Simple evaluation could be conducted by an inspector to see, hear, and touch the contact surfaces, and even by smelling for unusual odors. Microbiological approaches are also widely applied, including swabbing, flooding, rapid sampling and detection using enzyme linked immunosorbent assay (ELISA), etc. An accurate and fast approach widely used nowadays is the application of adenosine triphosphate (ATP) bioluminescence method to evaluate the performance of cleaning and sanitation procedure (Carrick et al. 2011). The principle of ATP bioluminescence method is that the bioluminescence is produced by the luciferase via the oxidative decarboxylation of luciferin in the presence of ATP, which indicates the existence of living organisms, in addition to some non-microbial

ATP (Osimani et al. 2014). In this method, the amount of light emitted is measured with a luminometer, which is expressed as relative light units, RLU, is used to represent the existence and amount of ATP, indicating the level of cleanliness of the surface of interest. For example, a RLU of 0 indicates perfectly cleaned, with absolute no ATP detected. However, for most food contact surfaces, a RLU of 0 is difficult to achieve; therefore, some rule-of-thumb acceptable numbers are provided based on experience and practices. For example, a generally accepted cleaned stainless steel surface is supposed to reach a RLU below 1000 (Wang et al. 2013).

13.3 Cleaning, Sanitation, and Maintenance (CSM) of Food Processing Equipment

Food processing equipment vary with specific functions and processing purposes. In this chapter, the CSM is discussed from two perspectives: (1) CSM of the generic equipment and (2) CSM of some representative food processing equipment.

13.3.1 Generic Food Processing Equipment

The CSM of processing equipment used for transport, separation, mechanical processing, evaporation, and dehydration are included in this section along with a brief mention of the CSM of non-thermal processing equipment.

13.3.1.1 Transport and Separation Equipment

Transport equipment are one of the most widely used food processing equipment in plants. They can be classified based on the physical properties of materials being transported. Solid and powder materials are typically transported via various conveyors and pneumatic transportation devices (Bioakina et al. 2016). For liquids, pumps are the most widely used equipment (Stahle et al. 2016).

Belt conveyor are often used for bulk, granular materials in horizontal direction and has a small tolerance in inclination, for selection, inspection, and packaging. Rubber, plastic, or steel belts are the key components in belt conveyors, with rubber belts being the most common (Bajda and Hardygora 2018). However, rubber is a porous material which easily ages harboring foreign particles and microorganisms. Therefore, rubber needs constant check and replacement (Gebler et al. 2016). For vertical or large inclination, bucket conveyor is often used instead of belt conveyor. The hopper is the main food-contact surface and is typically made of robust metal and is relatively durable. Screw conveyor is usually used for the transportation of powdery, granular, and small-sized loose aggregates with low friction. The spiral

blades are made from thin steel sheets then welded to the shaft; therefore, the spiral blades and welding connections of shaft are of cleaning and sanitation focus. Regular inspection of spiral blades for possible broken pieces is also needed (Owen and Cleary 2009; Roos et al. 2016).

Pneumatic conveyor uses high-speed airflow, typically generated by a blower, to transport the bulk materials from one location to another along a pipeline. Compared with above-mentioned transportation approaches, pneumatic conveyor has the advantage that, the transportation is carried out within the pipeline, thereby reducing the dust pollution in the plant, improving the environment hygiene, and reducing the transportation losses (Wypych and Yi 2003). The structure is rather simple; only one-direction pipes with no backhaul are used, leading to a convenient management, improved production efficiency, and high-level of automation. However, to make sure that the blower is working reliably and to reduce the wear of parts, air introduced into the system must be pre-dusted or filtered. The separator and the discharging devices with spiral structures, as well as the impellers need routine inspection and cleaning.

For the CSM of solid and powder material transportation equipment, considerations must be taken as to the characteristics of the materials transported and the subsequent processing procedures needed for the product. For materials that require further cleaning and disinfection, the standards of cleaning and sanitation of the transportation equipment are relatively low, and more attention could be paid to ensure that no foreign objects (such as broken spiral blade fragments or metals) or undesirable microorganisms are introduced during transportation/operation. It is good practice to make sure that the mechanical transmissions, such as drive units, traction devices, shafts and bearings, springs, and balances are regularly adjusted and calibrated. For the transportation of materials that are in the midst of processing, it is essential and critical to ensure the cleanliness and sanitation of all food-contact surfaces. These contact surfaces require high level of hygiene and need frequent cleaning and sanitation, and therefore the materials used in food-contact surfaces need to exhibit high resistance to oxidation and corrosion (Yebah and Hung 2005) and the equipment needs to have as few angles and dead corners as possible (Wang et al. 2014). Consumable surfaces (such as rubber) need regular inspection and replacement when necessary.

The liquid material transportation is mainly pump-based, such as gear (positive displacement) pumps, centrifugal pumps, roots pumps, vane pumps, etc. All the components in a centrifugal pump used to transport low to medium-viscous liquids are made of corrosion-resistant stainless steel (Martynenko et al. 2015). Closed impeller with a few blades are typically used and both the pump cover and the impeller should be easy to disassemble. Vacuum pump is usually used for conveying jam, ketchup, and other food materials with bigger particles (Singh and Heldman 2008). The advantage of liquid transportation equipment is that they can be easily cleaned and sanitized with liquid detergents and sanitizers, but from the maintenance point of view, inspection focus is possible corrosion.

Sedimentation, filtration, and pressing are the three main approaches in solid-liquid separation. The filter cloth and pads used in filtration system need to be

thoroughly cleaned and sanitized before re-used (Jackson et al. 2008). For the pressing separation, the thread rods (used in manual pressing), the claw cups (used in claw pressing), and the cutters (used in centrifugal pressing) that are in direct contact with the food require routine cleaning and sanitation (Singh and Heldman 2008). The detergent and sanitizer selection, and the duration and frequency of cleaning and disinfection depend on the equipment, the contact surface properties, and the physicochemical properties of the material being processed. For parts like the spiral blades used in spiral centrifuges, discs used in disc centrifuges, and scrapers used in horizontal scraper discharge centrifuges, they should be manually disassembled, cleaned and sanitized on a regular basis (Troller 1993). For single-stage or multi-stage leaching tanks used in extraction, all surfaces in direct contact with the liquid during leaching require proper cleaning and sanitation.

There is also an increasing trend toward using membrane separation (Fig. 13.1). The separation size of membrane separation could range from 10 nm all the way up to 10 μm (Tamime 2008). The major advantage of membrane separation is that the process does not require any heat, therefore a conservation of heat-sensitive nutrients and flavor could be achieved. In membrane separation, permeate and retentate are separated physically in membrane separation. Therefore, both fractions could be used if needed. The efficacy of membrane separation (reverse osmosis or microfiltration) depends on size, flow direction limitation, and the pressure difference (Walstra et al. 2006). Fouling is a typical phenomenon in membrane separation due to the constant accumulation of components onto the membrane surface (Hamza et al. 1997). Membrane fouling can be classified into inorganic (Ravanchi et al. 2009), organic, biological/microbial or particulate/colloidal fouling (Liu et al. 2001). To reduce the rate of fouling and avoid colloidal particle aggregations, the quality of water used in cleaning and sanitation needs to be monitored and controlled (D'Souza and Mawson 2005). The efficacy of membrane cleaning and sanitation depends on the type and amount of foulant and type of polymers, which form the membrane surface and induce the surface roughness (Shang et al. 2012). Typical cleaning regimes include a minimal round of alkaline wash and acid wash, in addition to several rounds of rinse and possible membrane preservation (Tamime 2008). Other cleaning agents, in addition to alkaline and acid washes, such as chlorinated solutions, organic solvents, and enzymatic detergents, might be needed depending on the fouling condition and membrane composition (D'Souza and Mawson 2005).

13.3.1.2 Mechanical Processing Equipment

This section focuses on the mechanical equipment used during process such as crushers, peelers and mixers. Other mechanical equipment used in extrusion, forming, screening, and grading share similar principles for cleaning, sanitation and maintenance, and therefore will not be explicitly described.

Food pulverizer utilizes the integrated effects of impact, extrusion, shearing and friction to pulverize the material; most pulverizers have the basic crushing and grinding functions, using either stress from two surfaces (such as roll crushers or

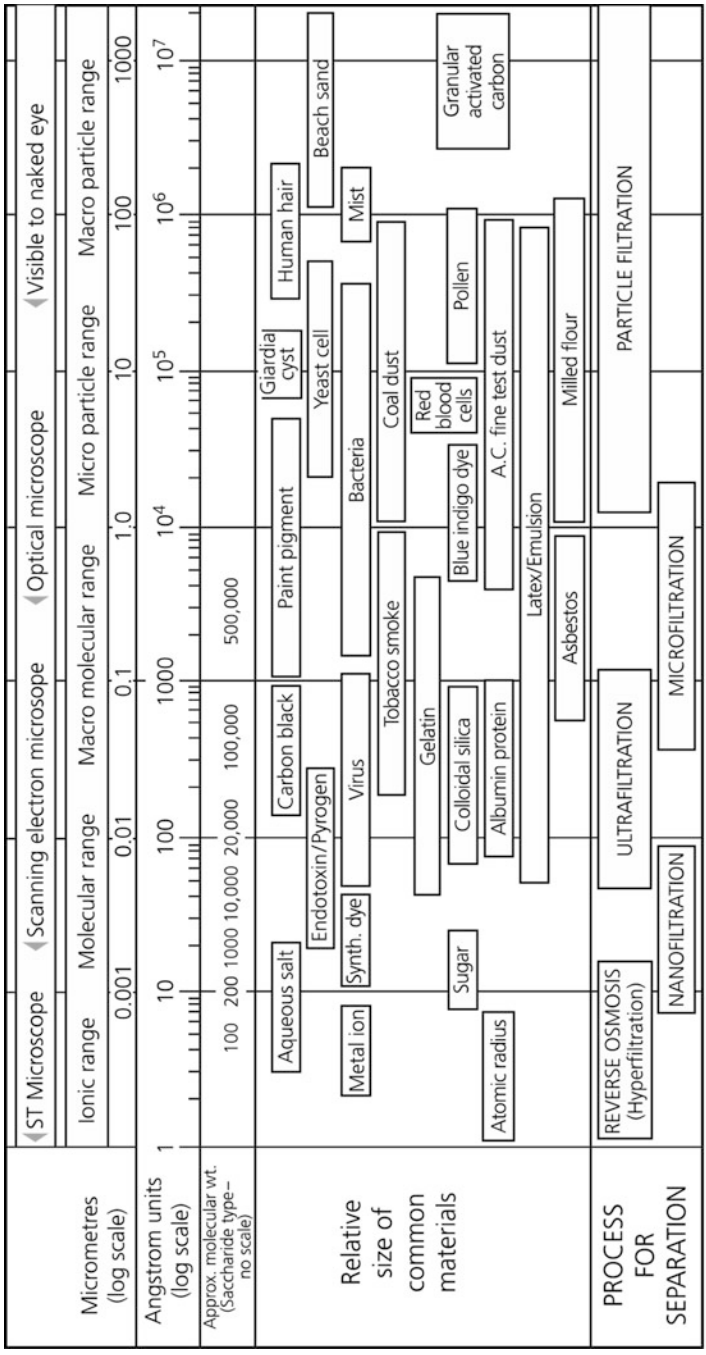

Fig. 13.1 Spectrum of membrane filtration in the food and other industries. (Reproduced by permission Tamime 2008)

pan mills) or by the attrition between particles (such as hammer mills or rotary grinders). Pressure, impact or shear forces are the commonly applied force during the process. For impact crushers, broken metals and/or any other foreign objects mixing in the food are major concerns (Singh and Heldman 2008). For pulverizers using high-speed airflow (such as jet mills), it is necessary to ensure the purity and sterility of the air; and due to its intrinsic high-wear disadvantage, a regular inner surface inspection of all channels is needed from a maintenance point of view.

For peeling and shelling equipment, if the raw materials need to be further cleaned and disinfected after peeling and shelling (such as peanut shelling and brown rice peeling), the cleanliness requirements of the corresponding equipment are relatively low (Güzel et al. 2005). However, if fresh produce such as vegetables and fruits are peeled via cutting, the cleanliness of the blades must be guaranteed; and if by mechanical friction, the friction belts (such as rubber sheets) should be cleaned, inspected, and replaced routinely (Doyle and Erickson 2008).

Mixers help to mix different ingredients, and the mixing shafts and impellers should be corrosion-resistant and easy to disassemble for cleaning and sanitation. Homogenizers utilizes shear forces generated in the flow to refine the material and break fat globules in some cases (Walstra et al. 2006); and in this case, the feeding chamber, suction and discharge valves, plungers, and pumps that are in direct contact with the liquid, need to exhibit strong resistance to corrosion with high rigidity (He et al. 2005; Gruetzmacher and Bradley 1999).

13.3.1.3 Evaporation and Dehydration Equipment

For liquid food products, water is mostly removed by evaporation process, a physical separation. Due to the high thermal efficiency of evaporators, as compared to dryers, evaporation is widely used as a pre-concentration process. During evaporation process, viscous liquid foods tend to become higher in viscosity due to the removal of water, which results in adhesion on the food contact surfaces (Tanglertpaibul and Rao 1987; Nindo et al. 2005). Moreover, if not handled properly for products with heat sensitive components, proteins might denature and polysaccharides might aggregate then caramelize with over-heating, causing fouling (Ozden and Puri 2010). Fouling reduces heat transfer and evaporation rate, and also pose potential damage to the products. In these cases, increasing the flow rate of the solution possibly with agitation can reduce fouling, especially with non-Newtonian fluids whose apparent viscosities decrease with increased shear rates; and a more frequent cleaning and sanitation of the surfaces also help. There are also foaming occurrences, especially in vacuum evaporation; in these cases, both operation and the cleaning processes need to be foam-controlled, typically with the addition of surfactants (Singh and Heldman 2008). For equipment used in evaporation, the selection of surface materials is of utmost importance and the application of steam and vapor could greatly reduce the energy consumption for heat-insensitive products. With fixed volume, the heated surface area needs to be maximally enlarged with simple and easy-to-clean structure

(Singh and Heldman 2008), and mechanical cleaning involving spray balls and rotating arms can enhance the cleaning and sanitation performance.

Dehydration is the removal of water typically used for reducing the water activity for longer preservation and reducing deteriorative reactions (Beuchat et al. 2013). The most efficient dehydration systems need to maintain the maximal vapor-pressure gradient as well as temperature gradient between the air and the interior parts of the product. These conditions lead to different systems such as tray or cabinet dryers, tunnel dryers, puff-drying, fluidized-bed drying, spray drying and freeze drying. In most cases, the non-uniform cabinet drying requires regular inspection for dead corner soils; rotated trays are better for maintenance purposes. For other types of dehydration systems with the assistance of air recirculation, the hygiene of circulated air should be strictly controlled. Special attention should be paid to the vacuum system of freeze drying.

13.3.1.4 Other Emerging Food Processing Equipment

Non-thermal processing emerges in response to the consumers' call for minimally-processed food, with minimal heat generation and retaining sensory attributes; the above-mentioned membrane separation is one of them. Several novel non-thermal processing technologies have been introduced in the other chapters of this book, such as high hydrostatic pressure processing, pulsed electric field, irradiation, etc., The cleaning and sanitation of novel technologies may not be the major concern as most of them effectively inactivate microorganisms. Their maintenance however, needs to be regularly checked and inspected (including the electrodes and chamber feeding ports in the pulsed electric field processing, the piston and vessels, any part of the pressure vessel that might have incidental contact with the food in high pressure processing, the lamps and reflectors in the pulsed light processing, and the food sample conveyors in irradiation facilities, etc.) (Ortega-Rivas 2012).

13.3.2 Various Food Processing Examples

13.3.2.1 Fresh Produce Processing

During the harvest and pre-processing of fresh produce, foreign objects and mechanical damages are most commonly observed (Zhang et al. 2016). Chemical concern, especially unnecessarily excessive use of pesticides, is most seen as direct contamination (Krol et al. 2000; Biehl and Buck 1987). In the field, the indirect contamination mostly comes from the environment, such as the surrounding air, soil and feeding water (Yadav et al. 2015). For most fresh produced harvested from the field, the first and foremost matter, apart from removing as much as possible visible dirt and soil and foreign objects, is to cool the product temperature in order to maintain the quality and extend shelf life. The cooling medium thus becomes really important

to prevent cross-contamination that might occur during transportation (Nerín et al. 2016). If any air circulation is applied during cooling, either in a room or simply using forced air, the hygiene of the air needs to be strictly monitored, to prevent possible pathogenic microorganisms being transmitted with air. If any water is involved in the cooling process, such as hydro-cooling, icing or vacuum cooling, after checking that the produce's suitability of using water cooling (which might not be the case for some soft tissue, delicate products with large water-bearing surfaces such as berries), the dripping of either condensed or evaporated water is prohibited and needs continuous monitoring (van Ginkel et al. 2005; Oh and Logan 2005). During these processes, the water (or ice) used must be potable and microorganism-free and stored properly in hygienic conditions before use; otherwise, these methods pose great risk to the safety of the produce (Bullerman and Bianchini 2007; Kabak 2009). Sometimes cooling water is circulated to save energy (Beuchat 1996). In these cases, the cooling water is typically chlorinated to prevent cross-contamination. The concentration of effective sanitizers in the recirculated water therefore needs to be constantly monitored.

For equipment involved in harvest, such as conveyor belts and dump tanks, the food-contact surfaces need to be cleaned and sanitized using approved compounds on a regular basis. Trucks for transportation should be low-temperature controlled and are exclusively used for fresh produce transportation, instead of having transported live animals (or products) or other toxic materials before. It must be bear in mind that this low temperature (typically below 4 °C) cannot 'inactivate' microorganisms; *Listeria monocytogenes* for example, can proliferate at low temperature then contaminate the produce and causing poisoning. Therefore, equipment/parts such as refrigeration coils, drip pans, air cooling fans, drain tiles and inner walls all need to be cleaned and sanitized on a regular basis.

For packing lines and packages of fresh produce, good GMPs are the key to prevent equipment from microbial and chemical contamination. An utmost important consideration that needs to be pointed out is a complete separation between packing and storage, facility and personnel. This is essential in preventing cross-contamination. General recommendations also include that the strict control of temperature, humidity and regular clean of packing areas (Ritchie et al. 2009; Martínez-Romero et al. 2007), produce-contact equipment surfaces need to be cleaned and sanitized, proper function of equipment with no loose parts, avoidance of possible oil leaks on machinery and appropriate application of food-grade lubricants, a thorough clean and sanitation of containers and boxed before use as well as keep them properly stored and off the floor, etc.

13.3.2.2 Meat Processing

Theoretically speaking, the freshly harvested meat is free from microorganisms; however, due to microbial colonies on the skin and in the gastrointestinal tract, in addition to inappropriate meat processing procedures, potential hazards still occur. The bio-hazard during meat processing includes both microbiological hazard and

parasite hazard (Bolton et al. 2002). Pathogenic microorganisms during slaughter and on raw meat typically include *Salmonella*, *Escherichia coli* O157:H7, and for thermally processed meat products, *Clostridium botulinum* and *Clostridium perfringens* are the biggest concern for their heat-resistant spores. *L. monocytogenes* is particularly seen in the highly productive poultry processing which involves scalding and mechanical feather removal procedures passing pathogens from one carcass to another (Hugas et al. 2002). Bio-hazard could be reduced by strictly implementing plant SSOP including personnel proper dressing and tool disinfection (knives, brisket saw and splitting saw for instance) during and between processes, as well as a good record keeping (Eisel et al. 1997; Boland et al. 2001).

During meat processing, one of the most imperative tasks is to prevent cross-contamination. Separations between meat processing procedures, especially between raw and cooked processing areas are needed; and all meat contact surfaces are suggested to employ high quality, non-toxic, non-absorbent, and anti-corrosive stainless steel materials. All equipment and parts, tables and counter tops, which have direct contact of meat, should be cleaned and sanitized throughout the day as needed. Cold chain needs to be maintained to prevent re-contamination after treatment from mesophilic pathogens which had capability to survive low temperature and proliferate in mild temperature. Multiple hurdle systems might be implemented for microbiological interventions if necessary. In-line detection of metals and other possible foreign objects during processing is favored (Mousavi et al. 2002; Lundén et al. 2003; Fritzson and Berntsson 2006; Giaouris et al. 2014). Considering the large water usage during slaughter, the hygiene of cold and hot water needs to be strictly controlled to prevent possible microorganisms from growing and forming biofilm. From an environmentally friendly perspective, the exhaust should undergo mechanical and biological treatment to reduce to required biochemical oxygen demand (BOD) level (Bustillo-Lecompte and Mehrvar 2015).

13.3.2.3 Dairy Processing

Raw milk needs to be collected then immediately cooled below 4 °C before being further handled (Cullor 1997); with the help of a stirrer installed in the insulated storage tank to maintain the low temperature. The pipe filters (before milk flows into the storage tank) used to remove impurities differ based on the scales and sizes of the processing plant and serve as the very first step of milk processing. Plate exchangers are usually used to cool down the milk after the collection, and also heat up the milk during heat treatment (such as pasteurization) (Jun and Puri 2005; Balasubramanian and Puri 2008; Balasubramanian and Puri 2009). In a typical milk house, the number of microorganisms could reach to 10^3–10^4 CFU/L in the air, and much higher in the feed and feces, with the feces microorganisms reach to even 10^9–10^{11} CFU/g (Husu 1990; Hayes et al. 2001). Therefore, it is recommended to feed the cows after milking to maintain the hygiene of the milk house. All milk-contact surfaces, such as the milk pails (for manual milking), milk claws and cups, milk pumps and transfer pipelines, cooling tanks and stirrer(s), valves and pipes should

be thoroughly cleaned and disinfected each time after use and disinfected again before next milking (Wang et al. 2019).

A major task of cleaning and sanitation in milk processing is the cleaning and sanitation of processing pipelines and storage units with well-organized procedures. Dairy Practices Council (DPC) recommended a typical CIP for the milking equipment as shown in Table 13.3 (DPC 2010).

Milking system CIP usually starts with a tepid water rinse at the completion of the milking, then a heated, alkaline wash (sometimes chlorinated) is conducted to remove the organic soils such as milk proteins and lipids. Followed by the alkaline wash, an acid wash is conducted to remove the residual minerals (inorganic) and leaving the pipeline inner surfaces a low pH and bacterial-inhibited environment. Before the start of the next milking, a sanitizing rinse through the pipelines is applied to ensure the sanitation of the milk contact surfaces. To reduce water consumption, and enhance the CIP performance with better cleaning effectiveness, especially for the alkaline wash and acid wash cycles, an external mechanical force is commonly applied as in air 'slugs'. The introduction of air on a set interval during the wash cycles greatly enhance the interactions between chemical wash solutions and the milk soils and achieve better CIP performance. For milk storage units such as milk pails and milk tanks, similar CIP is conducted using spray balls instead. As indicated earlier in this chapter, the evaluation of the surface cleanliness can be achieved using ATP bioluminescence method (with a recommended cutoff RLU value indicating cleanliness for different materials), and the evaluation of sanitation performance can be achieved using bacterial swabbing and incubation. There are some drawbacks of conventional milking system CIP, especially the potential hazard of chemical solutions. The chemicals used in alkaline and acid washes are highly concentrated and could pose potential threat to the dairy farmers and plants operators. Moreover, these chemicals are not environmentally friendly and need to be properly disposed after use, which in-turn increase the entire cost. A novel CIP using electrolyzed oxidizing (EO) water is therefore proposed and tested. EO water is generated from salt solution in an electrified chamber with a selective membrane in the middle. With the driving force of electric currency, two types of EO water solutions are generated; from the anode side, alkaline EO water is generated, and simultaneously from the cathode side, acidic EO water is generated (Dev et al. 2014). The only chemical used in EO water generation is salt, therefore EO water is relatively environmentally friendly

Table 13.3 Conventional CIP for parlor milking systems[a]

Cleaning cycle	Conventional CIP
Warm water rinse	2 minutes; 43.3–48.9 °C
Alkaline wash	8–10 minutes; start: 71.1–76.7 °C; finish: 48.9 °C; pH >12.0; 120 ppm chlorine; 1100 ppm alkalinity; >20 slugs
Acid wash	3–5 minutes; pH ~ 3.0
Sanitizing rinse	EPA registered dairy sanitizer solution

[a]DPC (2010)

and cost effective; also, less harmful to the farmers and operators. Moreover, the cleaning and sanitation of EO water solutions, both alkaline wash/acid wash separated CIP approach and alkaline wash/acid wash combined one-step CIP approach, have exhibited comparable cleaning and sanitation performance on lab scale milking systems (Dev et al. 2014; Wang et al. 2015a), with the corresponding kinematic processes being mathematically modelled (Wang et al. 2015b, c). The cleaning and sanitation performance of alkaline wash/acid wash separated CIP approach has also been proved to be comparable with conventional CIP on a real-world dairy farm (Wang et al. 2013).

13.3.2.4 Beverage Manufacturing

Chlorine gas and sodium hypochlorides are the most commonly used disinfectants in water treatment due to their high biocide efficacy and low price. It is well known that with lower pH, hypochlorous acid works 80–100 times better than hypochlorites (Kumar and Margerum 1987). Despite its high efficacy in destroying bacteria and viruses, it must be noted that it is relatively weak in killing protozoa such as Giardia and Cryptosporidium, and with distribution differences in pH, the disinfection efficacy varies (Ono et al. 2012; Driedger 2000; Pereira et al. 2008).

Ozone on the other hand, is a better zero-residue disinfectant in water treatment. Due to its short halftime, ozone gas could only be generated and used in-line for sanitation (Wade et al. 2003). Ozone in aqueous form has shown its effectiveness in killing food-related microorganisms, such as *E. coli* O157:H7 on alfalfa seeds and sprouts (Kim and Yousef 2000; Sharma et al. 2002), strawberries and raspberries (Bialka and Demirci 2007a), etc. Ozone is now more widely used in bottled water in beverage industries, but not in carbonic beverages such as sodas. The short half-life of ozone greatly hurdles its application, but this will be introduced in a later chapter of this book, however, for more details reader is referred to Prabha et al. (2015).

Ultraviolet (UV) is also a novel approach in water treatment before used for beverages. UV can be divided into different categories based on wavelength, including UV-A (399–315 nm), UV-B (314–280 nm) and UV-C (279–100 nm). The most effective UV wavelength is 245 and 285 nm within the UV-C range (Sharma and Demirci 2003; Ozer and Demirci 2006; Bialka and Demirci 2007b; Bialka et al. 2008). UV dosage not only depends on the target microorganisms, but also depends on the frequency of application. Demirci and Krishnamurthy (2006) showed that with pure water inoculation, *Bacillus subtilis* could not be enriched with flow rate from 2 to 14 L/min; and when inoculated with municipal waste water effluent, significant reduction of microorganisms (*B. subtilis* and *E. coli*) and removal of turbidity, suspended solids (SS), chemical oxygen demand (COD) and total organic carbon (TOC) could be achieved.

Due to the special process of filling during beverage processing, it is important to guarantee the cleanliness and sanitation of circulating air; the application of high efficiency filters (HEPA) and regular replacement helps (Manfredi and Vignali 2015; Mazzuckelli et al. 2007). In addition to the standard cleaning and sanitizing

Table 13.4 Cleaning suggestions on food processing plants[a]

Cleaning objects	Detergents	Cleaning devices
Plant floor	Detergents with self-foaming agents	High pressure foam projector
Plant inner wall and ceilings	Detergents with self-foaming agents	Foam projector
Processing equipment and conveying belt	Chlorinated solution or detergents	High pressure rotary hydraulic spray
Closed equipment	Alkaline solutions then regular acidic detergents	CIP devices

[a]Adapted from Troller (1993)

procedures, there are some additional notes for regular inspections (and could also be applied to other processing plants). One is the accumulated sediments of tire-print and conveying tracks (Marriott and Gravani 2006). Alkali is typically effective for tire-prints and indentations but for conveying tracks, they are more easily cleaned via high pressure foam cleaning to remove the oil and grease from overflow of containers, tracks, and bearings, etc. (Gibson et al. 1999). Another is the formation of films. Inactivation surfaces could be attributed to additives in containing tank, transfer lines or filers, and biofilm. For hard surfaces, the cleaning of these film requires regular routine for intervention. A general practice of cleaning procedures is listed in Table 13.4.

13.4 Food Packaging Equipment

Various food packaging materials such as glass, metal, plastics, and paper-based materials are used in food industry (Marsh and Bugusu 2007). Glass is chemically inert to foods, and impermeable to gases and vapors, making it perfect packaging material to maintain food freshness. It is capable to withstand high temperature during sterilization, as well as foods with high acidity. Moreover, most of them is visually transparent, leading to a visual inspection of the cleanliness. Tinted/colored glasses could protect the food from light. Nowadays, more glass packaging is made with thinner glass with higher resistance to pressure, impact, and thermal shock. Aluminum and steel are the two most commonly used metal packaging materials. They are easier to be formed into different shapes and exhibit excellent physical protection of the food and can be recycled after use with minimal adverse environmental impact. They are resistant to corrosion and could be cleaned and sanitized with varieties of agents. Plastic materials are also widely used in food packaging and they can be either thermosets or thermoplastics (Marsh and Bugusu 2007). Thermosets contain irreversible cross-link chemical bond and therefore exhibit improved mechanical properties and are more resistant to high temperature; the curing process of thermoplastics on the other hand, is completely reversible with no chemical bonding, making thermoplastics ideal materials for remolding and reshaping.

With different packaging materials, the selection of cleaning and disinfection agents vary. For example, as stated above, for milk processing lines, alkaline and acidic solutions could be used, for the stainless-steel milk contact surface; however, it is not recommended to use with harsh chemicals such as sodium hypochlorite to repeatedly clean polycarbonate materials due to possible leaching of bisphenol A, a known environmental estrogen (vom Saal and Hughes 2005). A major trend in food packaging is the manufacturing of lightweight, reusable, and refillable packages (Duncan 2011). Care must be taken when reusing and recycling these materials – adequate cleaning and disinfection must be achieved to remove contaminants, and adhesive microorganisms (and possible biofilms). In addition, cleaning and sanitation solutions used in these harsh processing procedures need to be carefully and properly handled to avoid unnecessary incident to the operators.

Aseptic packaging is a packaging approach during which both the food and the packaging materials are thoroughly cleaned and commercially sterilized to achieve a conservation of sterility during packaging. To this end, food usually undergoes ultra-high temperature (UHT) treatment and the packaging materials are heated up to 80 °C in 30% concentration of hydrogen peroxide for up to 15 seconds for inline sterilization (Ansari and Datta 2003). With these, aseptic packaged foods can maintain fresh and original taste without refrigeration or the addition of preservatives for at least 6 months. More detailed information on this topic can be found in another chapter of this book.

13.5 Conclusions and Future Trends

As discussed in this chapter, the cleaning, sanitation, and maintenance of food processing equipment are essential procedures. Factors such as processing requirements, food product being treated, and the available facilities and conditions all affect what method to use, which detergent and sanitizer to select and how the cleaning, sanitation, and maintenance proceed. Looking forward, by using different algorithms (machine learning for instance) for system design and control, more effective and intelligent realizations might be achieved with more dynamic characteristics. Of no doubt, especially for food equipment cleaning and sanitation purposes, computational simulation and higher-level automation are always desirable. Computational simulations not only facilitate equipment design, but also with the assistance of more rapid analysis approaches (such as the rapid quantitative assessment of soil concentration and composition from critical point sampling), they possess the potential of cleaning status prediction building upon the above-mentioned supervised learning methodologies. Continuous effort should always be put into the equipment design and manufacture, robotic/automatic cleaning, development of non-thermal processing that causes less soiling etc. For those that do need complicated cleaning and sanitation procedures, development of green, environmentally friendly detergents and sanitizers are the main foci of future direction, along with processing procedures of reduced energy cost and increased efficiency.

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Part IV
Modeling and Process Design

Chapter 14
Microbial Growth Models

Ehsan Mahdinia, Shaowei Liu, Ali Demirci, and Virendra M. Puri

14.1 Introduction

Foodborne illness, or foodborne disease, is a growing public health issue around the world, primarily resulting from contaminated or toxic food. In the United States alone, it was estimated that 48 million cases of foodborne diseases occurred in 2016 and approximately 128,000 people were hospitalized, and 3000 people died from the ingestion of contaminated food, in the same year (FDA 2018). The most common foodborne pathogens, including *Salmonella serotypes*, *Staphylococcus aureus*, *Campylobacter coli*, *Escherichia coli* O157:H7, *Bacillus cereus*, and *Listeria monocytogenes*, frequently cause illness in the United States and all over the world (FSIS 2018). Therefore, it is of greatest importance to examine food raw or ready-to-eat materials or final products for the existence of pathogenic bacteria and their growth during the storage. In general, conventional detection methods including traditional microculture, molecular biology, immunological, and metabonomic methods, etc. are extensively employed to test food products' safety to ensure the public human health and reduce the risk of infection by pathogens (Cho and Ku 2017).

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Studying microbial physiologies and predicting their behavior under different circumstances are a constant need for securing microbial food safety (Bazin 2018). The demand for microbial growth modeling began to be realized with the critique that food quality control based on the challenge tests of the final products are expensive, laborious, and time-consuming (Baranyi and Pin 2001; Ross and Mcmeekin 2003). As early as 1980s, microbial growth modeling was reported as an interdisciplinary research area that combined microbiology, statistics, mathematics, and computer science, either in food safety or microbial natural habitats in ecosystems or in bioprocessing applications, where microbial growth is beneficial for productions of the value-added products (Widder et al. 2016; Zwietering et al. 1990; Mitchell et al. 2004).The risks of foodborne pathogens are extremely vast. Even astronauts have not been safe from these risks as the pathogens may find their way into the space stations and threaten the health of astronauts, and thus there is a definite need to experimentally study and mathematically model the growth of these pathogens in such peculiar environments (Van Houdt et al. 2018). Fundamental principles and methods from aforementioned fields are commonly employed to describe and predict microbial growth in specific foods under defined conditions (Baranyi and Pin 2001; Esser et al. 2015). Additionally, these growth models have also been used to predict the shelf life and assess risks in food safety programs such as Hazard Analysis and Critical Control Point (HACCP) and Quantitative Microbial Risk Assessment (QMRA). Furthermore, ComBase Predictor (CDPM 2018) serves as a repository for data to estimate microbial growth in different food environments, and helps to define data gaps, and standardize the work and results of different risk assessors, which plays a significant role in international trade (Baranyi and Tamplin 2004).

Therefore, microbial growth models are widely used as tools for process optimization in food safety control systems (Skinner et al. 1994). In this chapter, it was aimed to summarize and provide an update for existing microbial growth models, including primary predictive models, secondary predictive models, and tertiary models. In addition, some representative models are described in detail covering basic assumptions, limitations, a summary of parameters, possible enhancements, and the needed improvement. The reason for this is that complete framework and knowledge of microbial growth models can assist research or modify existing models; meanwhile, more typical models can be employed in food safety engineering for enhancing public health.

14.2 Compilation of Current Literature

Compared to large animals, microorganisms have a high rate of growth and reproduction. Depicting, understanding, and predicting microbial growth is of great concern for food safety engineering (Esser et al. 2015). To perform assessment studies, different models of microbial growth have been proposed, which can be

classified by a systematic analysis of their final purpose, the types of microorganism, and their impact on food spoilage or food safety (Pérez-Rodríguez and Valero 2013; Whiting 1995).

Standard terminology and classification of models with specific functions make predictive models more precise and simpler to use (Baranyi and Roberts 1992). Several different model classification schemes related to microbial growth models have been used in food safety research, including empirical, mechanistic, and kinetic and probabilistic models. Notably, the classification method proposed by Whiting and Buchanan (1993) is often used that groups most model types into primary, secondary, and tertiary models (Table 14.1):

(i) Primary models: describe the kinetic processes of microbial growth and inactivation phases using only a few parameters and record the increase (or decrease) of population density over time.
(ii) Secondary models: characterize the environmental factors on the parameters of a primary model, such as temperature, moisture, pH, and concentration of preservatives.
(iii) Tertiary models: combine one or more primary and secondary models through computer software and present a model system that establishes a user-friendly interface.

This chapter summarizes several typical sub-models contained in primary, secondary, and tertiary models, and introduces the microorganisms, materials, conditions, verification, validation, advantages and disadvantages of each model.

Table 14.1 Classification of microbial growth model

Primary models	Secondary models	Tertiary models
Gompertz model Jefferies and Brain (1984)	ANNs (artificial neural networks) Gruenreich (1995)	Pathogen Modelling Programme Buchanan (2010)
Logistic model Jason (1983)	Bayesian network models Adcock (2010)	Food MicroModel McClure et al. (1994)
The Rosso model Rosso et al. (1993)	The square root model Ratkowsky et al. (1982)	Growth Predictor Baranyi et al. (1999)
Baranyi and Roberts model Baranyi et al. (1993)	Response surface model (polynomial model) Draper (2006)	Pseudomonas Predictor Neumeyer et al. (1997)
Monod model Monod (1949)	Arrhenius model Labuza and Riboh (1982)	ComBase Baranyi and Tamplin (2004)
Compartmental model Vanier and Bower (1999)		Sym'Previus Leporq et al. (2005)
Weibull model Farewell (1982)		IPMP 2013 Huang (2014)

14.2.1 Primary Models

The kinetic parameters related to primary models have been developed for predicting the growth of microorganisms on food, including environmental factors, food ingredients, and the growth stage of microorganisms. Primary models predominantly estimate the changes in population density versus time during the lag phase, exponential phase, stationary phase, and death phase (Oscar 2005; Ross and Mcmeekin 2003) (Fig. 14.1).

Primary models and their modifications were developed using different theoretical bases and hypotheses (Table 14.1). For instance, the Baranyi and Roberts model assumes that during the lag phase, bacteria need to synthesize substrate(s) for further growth (Baranyi et al., 1993; Bursova et al. 2017; Kowalik and Lobacz 2015), Weibull model (Eq. 14.1) assumes that every microorganism has its own resistance to a lethal agent; as a simple model, bacteria can be divided into two sub-populations: growing or non-growing (Farewell 1982; Mishra and Puri 2013; Ngnitcho et al. 2018).

$$\lg N = \lg N_0 - \left(\frac{t}{\delta}\right)^p \tag{14.1}$$

where N_0 is the initial number of the microbial population; N represents the number of microorganisms that survived after the different treatments have been applied; t is the treatment time; δ is the characteristic time scale parameter, and p is the dimensionless shape parameter. When p is less than 1, the survivor curve displays upward concavity; when p is greater than 1, the survivor curve possessed a downward concavity; and when p equals 1, it represents a linear curve.

Monod model (Fig. 14.2 and Eq. 14.2) can be used under the condition that microbes grow in limited nutrient(s) (Koch et al. 1998; Monod 1949).

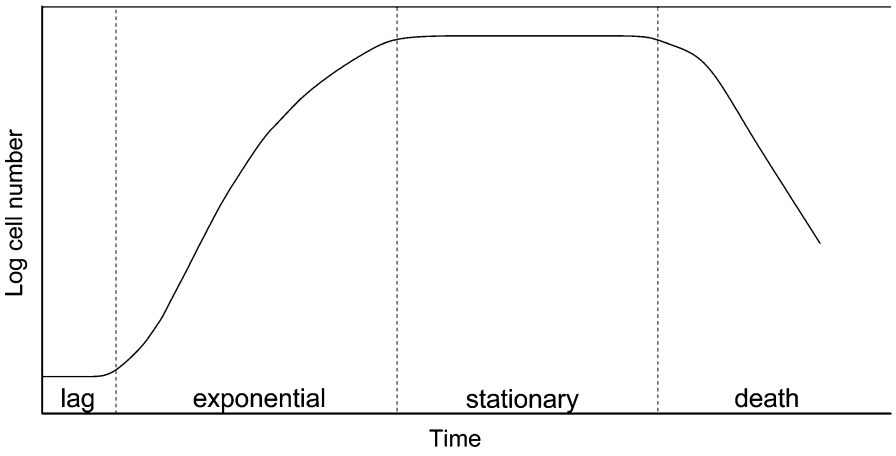

Fig. 14.1 Four-phase kinetics in a microbial growth curve

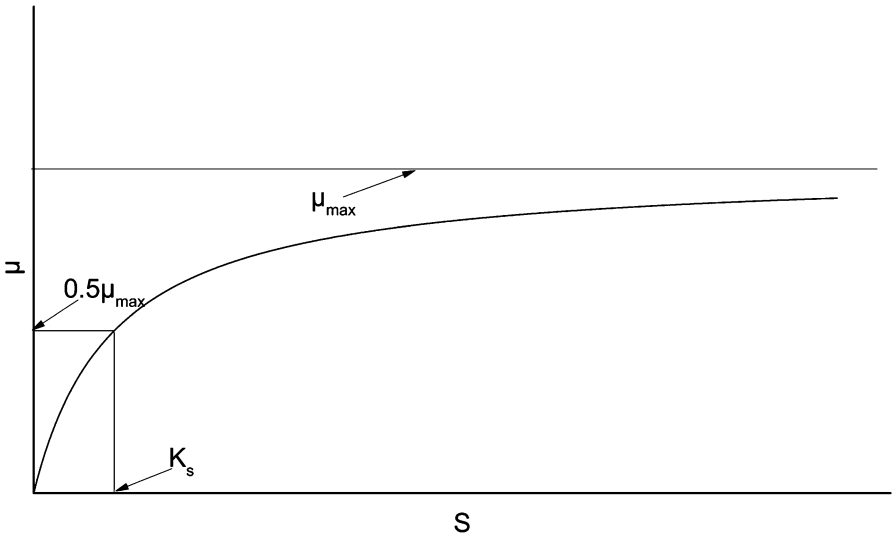

Fig. 14.2 The Monod model illustration

$$\mu = \mu_{\max} \frac{S}{K_s + S} \tag{14.2}$$

where μ is the specific growth rate; μ_{max} is the maximum specific growth rate;S is the concentration of the limiting substrate for growth; and K_s is the half-velocity constant, the value of S when $\mu/\mu_{max} = 0.5$.

Sometimes, researchers start with statistical models to first identify the effective factors on microbial growth and then use these effects and outcomes of the statistical models in primary mathematical models to better picture the effects (Carrascosa et al. 2014, 2016). Furthermore, each primary model has its own specifications and advantages in different applications. For example, the Logistic model is perhaps the simplest primary model and thus is most convenient and therefore preferable to use in most occasions; or the Weibullian model is best to fit in non-linear behaviors (Franco-Vega et al. 2015). The most important and typical primary models are presented in subsequent subsections.

14.2.1.1 Logistic Model

The logistic function model (Fig. 14.3 and Eq. 14.3), a common sigmoid curve first proposed in 1845 (Verhulst 1845), is increasingly used to describe microbial growth as a function of initial microbial density, time, growth rate, and final microbial density (Volterra 1928; Wachenheim et al. 2003). Subsequently, it was applied to food, ecology, demography, biology, medicine applications for predicting the growth of microorganisms, tumors, animals or plants, as well as in economy for

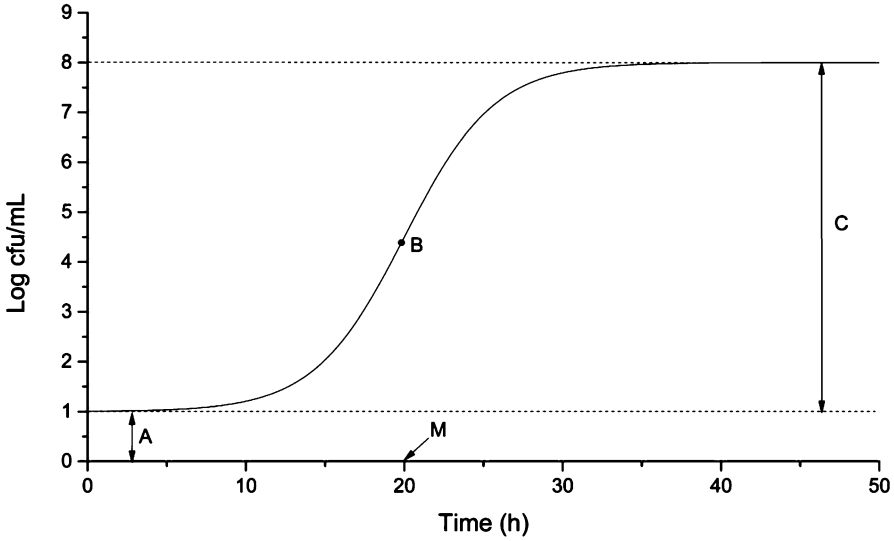

Fig. 14.3 The modified logistic function – A typical illustration

the illustration of how innovation spreads (Giovanis and Skiadas 2007; Román-Román and Torres-Ruiz 2012; Tsoularis and Wallace 2002).

$$y(t) = A + \frac{C}{1 + \exp\left[-B(t - M)\right]} \tag{14.3}$$

where y(t) is the cell concentration at time t; A is the lower asymptotic line of the growth curve as t decreases to zero (initial population level, N_0); C is the difference between the upper asymptotic line of the growth curve (maximum population level, N_{max}) minus the lower asymptotic line; B is the relative maximum growth rate at time M; and M is the time at which the growth rate is maximum.

Numerous modifications of the logistic model have been extensively employed to describe microbial growth in food systems. For example, a log-logistic model was employed to predict the survival of *Y. enterocolitica* and achieved an excellent agreement with the observed survival behavior in mayonnaise and milk (Little et al. 1994; Stern et al. 2010). Similarly, a log-logistic model was proposed for the deli meat industry to select optimum processing conditions of near infrared (NIR) heating through investigation of inactivation kinetics of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in ready-to-eat sliced ham (Ha and Kang 2014). With the improved logistic models, the growths of *E. coli*, *S. aureus*, *V. parahaemolyticus*, and *P. fluorescens* at various temperatures in food have been researched, which is becoming a prototype of an alert system for microbial food safety (Fujikawa 2011; Fujikawa et al. 2004; Fujikawa et al. 2009; Kahraman et al. 2016; Walter et al. 2016). *L. monocytogenes* as a significant food-borne pathogen has a high mortality rate among the high-risk populations (Kuan et al. 2017). Several

models for *L. monocytogenes* growth have been developed with the logistic function as their basis (Fang et al. 2013; Hassan et al. 2001; Pal et al. 2008). For example, a molecular predictive model was developed for rapid detection of *L. monocytogenes* growth in vacuum-packaged chilled pork through appropriate real-time polymerase chain reaction (PCR) detection technology (Ye et al. 2013). Similar to *L. monocytogenes*, many logistic models concentrated on *C. perfringens* which as an anaerobic Gram-positive pathogen has a history of a serious threat to human health (Corradini et al. 2006; Dors et al. 2016; Huang et al. 2017; Juneja et al. 2001). For instance, a probability model was developed to define the threshold of *C. perfringens* growth and was validated using experiment data, suggesting that the combination of sodium tripolyphosphate (STPP), sodium lactate (NaLA),and sodium chloride (NaCl) could prevent microbial growth in meat and poultry and thus food poisoning outbreaks (Huang et al. 2017). In addition, controlling microbial quality of food plays a critical role in proper sensory quality and food safety. A logical background-dependent non-dimensional model was provided to estimate aerobic bacterial growth in pan-fried meat patties at various temperatures and was verified by experimental data (Sojung and Dongsun 2015).

14.2.1.2 Gompertz Model

In recent years, the Gompertz equation (Fig. 14.4) for modelling the asymmetrical sigmoid shape of microbial growth curves has been widely and successfully used to describe and predict nonlinear responses, which was originally employed in humans to record the mortality (Jefferies and Brain 1984). Gibson et al. (1987) first modified

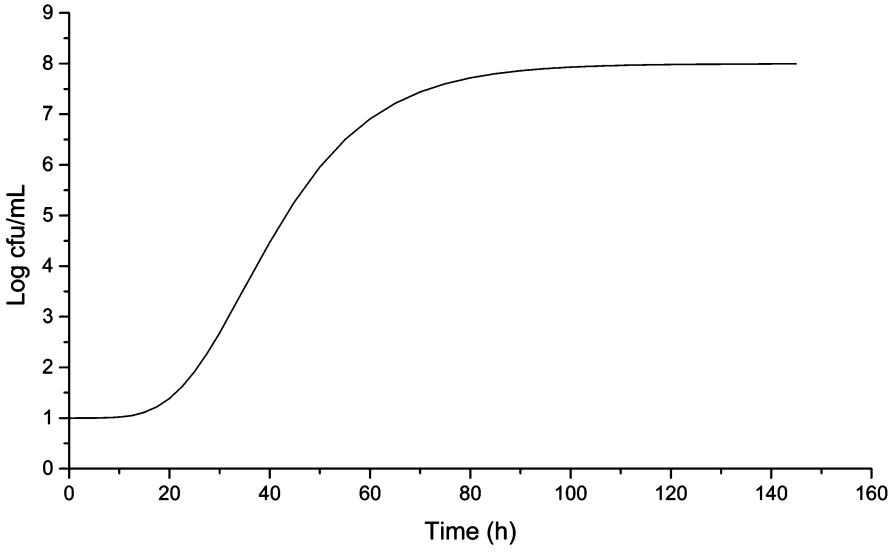

Fig. 14.4 The modified Gompertz model – A typical illustration

the Gompertz model to fit the growth curve of *C. botulinum* in pork in the presence of natural spoilage organisms, and calculated the lag times, growth rates, generation times, and time to maximum growth rates.

To some extent, microbial growth data are not sufficiently accurately described by the standard Gompertz model due to the fixed values of reliability at the inflection points (Kececioglu et al. 1994). To evaluate the accuracy, the Gompertz model was often used with some modifications such as those by Zwietering et al. (1991), e.g., *S. aureus* growth in Feta cheese, mold growth in long-grain rough rice during storage, *P. fluorescens* in fresh meat in different temperatures and pH, *Salmonella spp.* in processed meat products and microbial inactivation with high-pressure processing (Atungulu et al. 2016; Gonçalves et al. 2017; Jimyeong et al. 2016; Serment-Moreno et al. 2017; Zhu et al. 2011). Specifically, the microbial (aerobic plate counts, total coliforms, and lactic acid bacteria) growth in salted cabbages at different temperatures was investigated, and a modified Gompertz model was developed to determine the shelf-life, which provided proper guidance for food quality control (Kim et al. 2018). In addition, the effect of silver nanoparticles on the growth kinetics of *E. coli* and *S. aureus*, was evaluated by a modified Gompertz model, and it was found that the modified Gompertz model (Fig. 14.4 and Eq. 14.4), incorporating cell death, was useful for microbial growth kinetics research under the influence of antimicrobial agents (Chatterjee et al. 2015).

$$y(t) = y_0 + C * \exp\left\{-\exp\left\{\left[(2.7182 * \mu_{\max}) * \frac{LPD - t}{C}\right] + 1\right\}\right\} \qquad (14.4)$$

where y(t) is the cell concentration at time t; C is the asymptotic increase in population density; μ_{max} is the maximum specific growth rate; LPD is the lag phase duration; and t is the storage time.

14.2.1.3 Baranyi and Roberts Model

Besides the logistic and Gompertz models, a semi-mechanistic biologically-based growth model was developed by Baranyi and Roberts to describe microbial growth under dynamic time-varying temperature conditions. The empirical primary models were developed at isothermal conditions, in which the physiological state of the microorganism is represented by a single variable, and during lag phase bacteria need to synthesize an unknown substrate (Baranyi and Roberts 1994; Baranyi et al., 1993; Gospavic et al. 2008).

Yersinia enterocolitica, as a foodborne pathogen, which can cause acute intestinal tract diseases in humans, is easily observed in foods during production and storage (Stern and Pierson 2010). Accordingly, a large number of Baranyi and Roberts models were developed to model growth of *Y. enterocolitica* (Divya and Varadaraj 2015; Geeraerd et al. 2000; Sarka et al. 2017). For detailed examination, a model was developed to investigate the behavior of *Y. enterocolitica* in Camembert cheese under refrigerated conditions, serving for the consumers who are interested in using

cheese to prepare salads and sandwiches (Kowalik and Lobacz 2015). Since the storage temperature cannot prevent the proliferation of *Y. enterocolitica*, the growth dynamics of *Y. enterocolitica* during storage temperatures (8 °C and 24 °C) were studied with a modified Baranyi and Roberts model for assessing the potential risk to consumers (Bursova et al. 2017). In addition, Baranyi and Roberts model was also employed to estimate *L. monocytogenes* growth in fresh-cut romaine lettuce (Alavi et al. 2001), cantaloupe and sterilized whole milk (Guzel et al. 2017) and *S.* Enteritidis growth in chicken juice (Noviyanti et al. 2018), leading to useful risk assessment methods. Most recently, a novel rearrangement of the Baranyi and Roberts model was used to fit the growth of *E. coli* and *S.* Typhimurium under mild conditions of temperature (25–37 °C), salt concentration (0.086, 0.51 and 1.03 mol·L^{-1}), and pH (4.5–6.85), which showed a great compatibility with standard data and highly accurate growth rates and lag phase duration (Mytilinaios et al. 2015). To explore the effect of oregano essential oil on the shelf-life of vacuum-packed cooked sliced ham, lactic acid bacteria growth at various temperatures was evaluated. It was concluded that the Baranyi and Roberts model accurately fitted to microbial growth curves with R^2 and RMSE values ($R^2 \geq 0.884$, RMSE $\leq$0.270) better than Gompertz model (Menezes et al. 2018). Similarly, the growth of *Pseudomonas spp.* on sliced mushrooms stored between 4 °C and 28 °C were also fitted to Baranyi and Roberts models with the lowest MSE and highest R^2 compared to the modified Gompertz and logistic models (Tarlak et al. 2018). Overall, Baranyi and Roberts model and its modifications have been widely used in food microbiology, and have become a significantly important member of the most popular models applied in daily life (Acai et al. 2016; Kim et al. 2016; Liu and Puri 2007; Mai and Huynh 2017; Vadasz and Vadasz 2007).

14.2.2 Secondary Models

Secondary models are mainly used to predict how environmental factors (e.g., temperature, moisture, pH, concentration of preservatives and initial bacterial count) affect the parameters (e.g., growth rate and lag time) in primary models. With the advancement of mathematics and computer science, various secondary models, including response surface models, Arrhenius models, and square root models, are established and developed (Table 14.1). In many studies, primary models were first utilized by researchers to investigate the effective factors and then the results were used in suitable secondary models to further investigate the individual effects of every factor (Nyhan et al. 2018; de Oliveira Elias et al. 2018). Three of the most commonly applied secondary models, e.g. artificial neural networks, square root models and response surface methodology models, are introduced below.

14.2.2.1 Artificial Neural Networks

Artificial neural networks (ANNs) are computing systems known as analogous mechanisms of the biological neural networks, relying on a batch of nodes called artificial neurons (Fig. 14.5). Generally, microbial growth, inactivation, and probability of growth under complicated environmental conditions can be predicted and described by ANN models (Najjar et al. 1997; Pérez-Rodríguez and Valero 2013). Since decades ago, ANNs models, as an alternative and powerful technique, present high accuracy and generalization ability in modeling, leading to the extensive application in predicting the non-linear relationship between input (e.g. temperature, pH, and initial bacterial) and output in food microbial systems (Kavuncuoglu et al. 2018; Lou and Nakai 2001a, b; Ozturk et al. 2012; Zheng et al. 2017).

For instance, AANs were applied to predict residual pathogenic bacteria such as coliforms and *E. coli* on tomato fruits and lettuce leaves for more realistically assessing the risk of fresh produce consumption (Keeratipibul et al. 2011). *S.* Typhimurium is a harmful pathogenic bacteria contained in intermediate product or final product during processing and storage; a great many of ANNs are used to control it (Ozturk et al. 2012; Raoufy et al. 2011; Siripatrawan et al. 2006). For example, to extend the shelf-life of surimi, citric acid was used to control *S.* Typhimurium growth, combined with the models of back-propagation ANN and particle swarm optimization-based back-propagation artificial neural network (PSO BP-ANN) for ensuring food safety (Qin et al. 2018). Meanwhile, emphasis was also put on *L. monocytogenes* (Ramosnino et al. 2010; Rebuffo et al. 2006). An

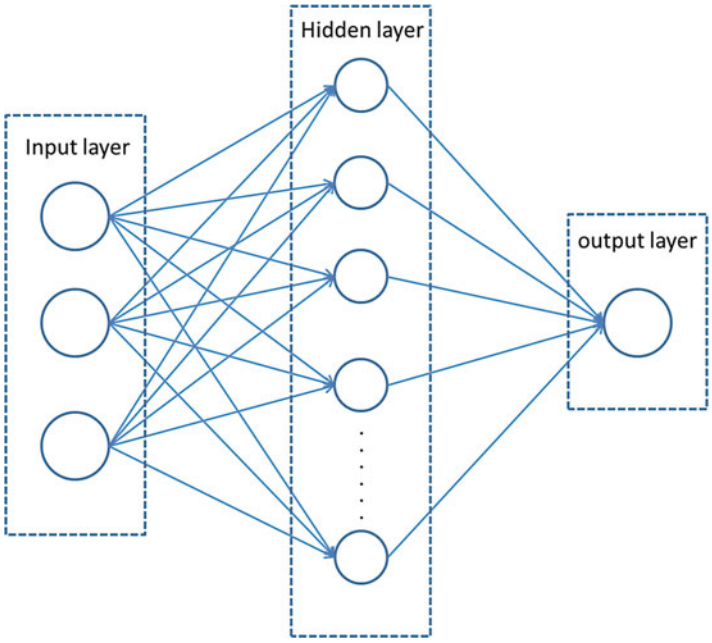

Fig. 14.5 Input, hidden and output layer in ANNs

autoregressive network with an exogenous input (NARX) model was developed to perform real time *E. coli* growth prediction with high accuracy with an emphasis to find hidden neurons and delays selection in the prediction process, which was possible only using ANN (Shamsudin et al. 2017).

Due to the excellent fault tolerance, ANN models are more suitable for modelling complex relationships in uncertainties and variations of conditions in predictive microbiology. However, it is still limited in use because of its complexity and high-cost of learning.

14.2.2.2 The Square Root Model

The square root model was proposed by Ratkowsky et al. (1982), which has been used to describe a linear relationship between the square root of growth rate and temperature. Some commonly used models are also called Ratkowsky models or Huang square root models (Huang et al. 2011).

The combination of Baranyi model with the Ratkowsky square root model has been used to quantify the influence of temperature on the growth of bacteria, such as *B. cereus* and *E. cloacae* in liquid whole egg products (Grijspeerdt and De Reu 2005). *V. vulnificus*is is a Gram-negative bacterium responsible for food-borne illnesses related to the consumption of oysters (Hald et al. 2016). By using a square root model, a predictive model for *V. vulnificus* in postharvest oysters as a function of temperature to minimize the risk against consumers was developed (DaSilva et al. 2012). The *L. monocytogenes* growth in sterilized whole milk for a range of temperature values (4–35 °C) was calculated by the Zwietering square root model with maximum relative error of 10.42% and the RMSE of 0.28 log CFU/ml (Alavi et al. 1999). In addition, the growth parameters of *L. monocytogenes* on vacuum packed sliced Mortadella and the growth and survival models for *S. enterica* and *L. monocytogenes* in leafy greens were modeled by the square root model, as well as the effect of storage temperature on growth rate of *L. monocytogenes* (RMSE = 0.014–0.099) (Bolivar et al. 2018; Daminelli et al. 2014; Mishra et al. 2017). Furthermore, Ratkowsky square root and Huang square root are models widely used to study the effect of temperature on *Salmonella* growth (Fujikawa et al. 2015; Sabike et al. 2015; Sakha and Fujikawa 2012). Fang et al. (2015) showed that the Huang square root model was more applicable to predict the effect of temperature on *Salmonella* growth, while the model of Ratkowsky square root was usually more suitable for the background microorganisms with a wider temperature range.

14.2.2.3 Response Surface Model (Polynomial Model)

Response surface model (RSM) is a mathematical-statistical method established by Box and Wilson (1951) that can be used to research the relationships between one or more response variables and factors (e.g., pH, temperature, pressure, etc.). RSM is a

powerful practical tool widely used in food science and technology, not only in food microbial predictions but in other fields of study (Huang et al. 2016; Mohammadi et al. 2016; Xu et al. 2016). In response surface analysis, a regression equation should be obtained first, and then the optimal value can be obtained by reasonable value of the independent variable (Baş and Boyacı 2007; Bezerra et al. 2008). The regression may be a curve or surface relationship; hence this model is referred to as the response surface model.

Basically, RSM utilizes statistically precision to design experiments in most efficient way in order to minimize the number of experiments required to ensure the desired efficiency. RSM designs are mostly used to optimize procedures where the experiments are costly or time-consuming and thus not easily replicable (Box and Wilson 1951). RSM models use various experimental designs, each with certain advantages, to create such efficiency in the experiments. For instance, the most commonly used design is a Central Composite Design (CCD) where a second order (Full quadratic) model is used based on orthogonality without needing to use a complete three-level factorial experiment, where the number of experiments can be significantly more (Mahdinia et al. 2018a). Furthermore, a Box-Behnken design can be used instead of a CCD and the modeling precision can be preserved (if cross-effects permit) with even fewer number of experiments (Mahdinia et al. 2018b). For example, for three continuous variables and each variable with three levels, a complete three-level variable design requires 27 experiments to cover all combinations whereas, a CCD design comes with 20, and a Box-Behnken design with only 15 (Mahdinia et al. 2018c). Most of the times, prior to an RSM design, there are a number of candidate factors that are hypothesized to affect response(s) and researchers need to screen through them to determine effective ones from ineffective ones. In these situations, researchers use screening methods such as the Plackett-Burman design to reduce the number of experiments and therefore save time and money (Izmirlioglu and Demirci 2015).

The inactivation effect of high-pressure processing in combination with mild heat on *L. monocytogenes*, the influence of UV-C light and trans-cinnamaldehyde on mesophiles and yeasts in grapefruit juice and the effect of electrolyzed oxidizing water based clean-in-place technique for cleaning milking system inoculated by four common microbial in milk were modeled by RSM (Ates et al. 2016; Dev et al. 2014; Ochoa-Velasco et al. 2018).

The RSM makes it possible to understand the interactions between experimental variables, and helps to determine and adjust operating conditions in the parametric amplification process in food microbiology (Buchanan and Bagi 1994; Han et al. 2001; Jha et al. 2017; Krishnamurthy et al. 2008; Yoon et al. 2014); however, optimization is often a compromise among variables since one response variable impacts the other variables (Pinzi et al. 2010). Obviously, the application of RSM is not just limited to food technologies or food safety. These days, RSM designs are also applied in fermentation technologies and synthetic biology, even in human psychology (Berenjian et al. 2011; Izmirlioglu and Demirci 2016; Coban and Demirci 2014; Ercan and Demirci 2014; Mahdinia et al. 2017a, 2019c).

14.2.3 Tertiary Models

The tertiary models are powerful, user-friendly microbiological prediction tools that include one or more primary and secondary models. Based on the informative database, the effects of different conditions on microorganisms can be expediently calculated and compared, and the inaccuracy of predictions in microbiology can be reduced. Scientific research institutions can not only input their own data, but also exchange data with other institutions.

14.2.3.1 ComBase (The Combined Database for Predictive Microbiology)

A large amount of data on the effects of various factors on microorganism lays the foundation of microbial predictive model packages, such as the Pathogen Modeling Program (PMP) and the former Food MicroModel (FMM), which was gradually replaced by the ComBase (Baranyi and Tamplin 2004; Koseki 2009; Mcmeekin et al. 2006). ComBase was established by the University of Tasmania and the United States Department of Agriculture, Agricultural Research Service (USDA-ARS), encompassing ComBase database and ComBase models. The goal is to help companies reduce the investment of time and money in testing, and contains over 60,000 growing records, for describing how environmental factors (e.g. temperature, pH, a_w, preservatives, and atmosphere) affect the food microbial growth (CDPM 2018).

A large number of researchers use ComBase individually or combined with other tertiary models (Aaslyng et al. 2014; Doona et al. 2005; Kapetanakou et al. 2017; Madden et al. 2017; Marc et al. 2005; Garre et al. 2017). For instance, a multiple food predictive model systems was developed to study the effect of food (micro) structure on microbial dynamics via ComBase, and further compared the results with the maximum specific growth rate values of *S. Typhimurium* and *S. aureus* estimated by the Baranyi and Roberts model at different temperature (4, 8 and 12 °C) in fish products (Baka et al. 2017). Meanwhile, the growth rate and lag-phase of *S. aureus* in fresh Minas cheese from Brazil at different pH (5.0, 5.5 and 6.5), salt concentrations (1.1, 2.1, and 4.5%) and temperatures (7.5, 10, 12.5, 15 and 17 °C) were evaluated by PMP and ComBase for improving the risk assessment in food security (Nunes and Caldas 2017). Additionally, Lobacz et al. (2013) modified the Gompertz and Ratkowsky square root models used to predict and validate the *L. monocytogenes* growth during the ripening and cold storage in mold-ripened cheeses. The results were compared with PMP and ComBase, which offered a typical example for the extensive use and applications of microbial predictive models in the food processing industry.

14.2.3.2 Integrated Pathogen Modelling Program

The Integrated Pathogen Modeling Model (IPMP 2013) was developed by USDA-ARS (USDA 2018). Though most researchers prefer to use MATLAB, SPSS, or R to analyze data, it is rather difficult to master them for common users without programming knowledge. Fortunately, IPMP 2013 is a free program convenient for researchers to analyze microbial data and develop the knowledge of predictive microbiology, where the logistic model, Baranyi model, re-parameterized Gompertz model, Weibull model, Ratkowsky square root model, Huang square root model, and Arrhenius model are included (Huang 2014).

The growth of *S. aureus* under various storage temperatures (10, 15, and 25 °C) in raw pork was predicted via IPMP. Based on a comparative study, the re-parameterized Gompertz model was assessed as the most accurate model at 10 and 15 °C, and the Baranyi model at 25 °C, in which the critical control points for storage temperature in the HACCP can be set up to improve product safety for meat (Lee et al. 2015). In addition, the growth of *C. botulinum* in ground beef under different temperature conditions under anaerobic conditions were also analyzed by IPMP with the Huang model and cardinal parameters model, validated by Laplace distribution showing a high accuracy (60% of the residual errors are ±0.5 log CFU/g) (Huang 2018). Similarly, the specific growth rates, lag times, and minimum temperature for growth of nonpathogenic *E. coli* at different incubation temperatures (10, 15, 22, and 30 °C) in ground chicken meat was analyzed by IPMP with the Huang primary and secondary square root models. Approximately 83.9% of the residual errors of ±0.5 log CFU/g suggested the accuracy in predicting the growth of uropathogenic *E. coli*. (Sommers et al. 2018).

14.2.4 Summary of Predictive Models

Predictive models in food safety engineering describe not only the growth but the survival or inactivation of microorganisms in foods under various conditions, giving the opportunity to minimize the risk of pathogenic outbreaks (Ross and Mcmeekin 2003). While microbial growth modeling is not limited to food safety applications, perhaps the vast number of applications are in the food industry (Mitchell et al. 2004). On the basis of their structure, the models and their modifications are introduced in three categories; i.e. primary, secondary, and tertiary models. Logistic and Gompertz models tend to fit isothermal growth curves with 3–4 intuitive parameters, such as the maximum growth rate and the asymptotic population size (Esser et al. 2015). Different from logistic and Gompertz models, the Baranyi and Roberts model is a mechanistic model. Due to the advantages (i.e., accurate, simple and practical), the Baranyi and Roberts model has become the most used primary model in food microbial prediction. Furthermore, the parameters in the model have physiological significance (Baranyi and Roberts 1994; Mytilinaios et al. 2012).

Secondary models aim to describe the microbial growth and the effect of external factors such as temperature and pH; serving as prediction tools for risk assessment in foods. Typically, the square root model describes a linear relationship between the square root of growth rate and temperature (Ratkowsky et al. 1982). Because of the fewer parameters, user-friendly processing and accurate prediction, many researchers tend to use this model in food research. Even so, the efforts of improvement are always continuing. Huang et al. (2011) reported a new secondary square root model, which can accurately estimate the minimum and maximum growth temperatures of bacteria. With the significant progress made in the past few decades in ANNs, our understanding of interacting parameters has been considerably enhanced. Compared with traditional models, ANNs often show better characteristics in regard to food microbial prediction and parameter optimization; moreover, the accuracy of prediction by ANNs can be further improved by algorithm optimization.

Tertiary mathematical models such as ComeBase and IPMP 2013 are derived from the primary or secondary models or their combinations. The IPMP 2013 is one of latest predictive microbiology tools (Huang 2014). It offers a user-friendly interface with high-accuracy in microbial prediction. Like other tertiary models, the IPMP 2013 has been used for predictive microbial data and to develop predictive models.

In summary, all of the models mentioned are very practical and significant in food safety engineering. To make them more user-friendly for novice modelers, the typical Baranyi and Roberts model, square root model, RSM, and ANNs, and the software IPMP 2013 are selected for further detailed description and comparison in the next section.

14.3 Examples of Specific Growth Models

14.3.1 Baranyi and Roberts Model

14.3.1.1 Basic Assumptions

Baranyi and Roberts model (Baranyi and Roberts 1994; Baranyi et al. 1993a, b) is a typical semi-mechanistic growth model for the microorganism's growth. The lag time is determined by the initial variable value at inoculation and post-inoculation. With the standardized cultivation methods, the growth state of microorganism including the lag parameter and maximum specific growth rate of microorganisms are relatively constant and independent on the subsequent growth conditions. Srivastava and Volesky (1990) proposed that the microorganisms do not grow under the conditions when the bottleneck-substance titer is lower than the minimum level, and the accumulation rate changes with temperature. Combining previous studies (Baranyi et al. 1993a, b, 1995) with the aforementioned theory, the model successfully predicted the growth of *Brochothrix thermosphacta* at temperatures ranging from 5 °C to 25 °C.

Below are the equations of the Baranyi and Roberts model, and the entire derivation of the model is available in Baranyi and Roberts (1994) and Baranyi et al. (1995).

$$\begin{aligned}\frac{d}{dt}q &= vq \\ \frac{d}{dt}x &= \mu_{\max}\frac{q}{1+q}\left(1-\frac{x}{x_{\max}}\right)x\end{aligned} \tag{14.5}$$

The variable y(t) denotes the natural logarithm of the cell concentration x(t). The solution of the above differential equations is:

$$y(t) = y_0 + \mu_{\max}A(t) - \ln\left(1+\frac{e^{\mu_{\max}A(t)}-1}{e^{y_{\max}-y_0}}\right) \tag{14.6}$$

where $y_0 = \text{In } x_0$, $y_{\max} = \text{In } x_{\max}$

$$\begin{aligned}A(t) &= t + \frac{1}{v}\ln\left(e^{-vt}+e^{-h_0}-e^{-vt-h_0}\right) \\ h_0 &= -\ln\left(\frac{q_0}{1+q_0}\right) = -\ln(\alpha_0) = \mu_{\max}\lambda\end{aligned} \tag{14.7}$$

where q is a measure of the initial state of cells, μ_{max} is maximum specific growth rate, v is the rate of increase of the limiting substrate, assumed to be equal to μ_{max}, y (t) is the cell concentration at time t, y_{max} is maximum cell concentration, λ is lag-phase duration.

14.3.1.2 Limitations and Possible Enhancements

There are some shortcomings and limitations in the Baranyi and Roberts model, even though it has been extensively used in various microorganisms and environments of the food safety engineering (Alavi et al. 2001; Bursova et al. 2017; Liu and Puri 2007; Lobete et al. 2017; Longhi et al. 2016; Tarlak et al. 2018).

Michaelis-Menten constant (K_p), assumed to be independent of actual environment (E_2), is one of the most important assumptions of the Baranyi and Roberts model. Based on that, the equation of $q(t) = P(t)/ K_p$ was applied to predict the physiological state of the microorganism by only one variable, which appears to be an oversimplification (Baranyi and Roberts 1994; Li et al. 2007). Additionally, the assumption of q_0 is a constant is only suitable for the positive temperature changes, and q_0 actually decreases with the reduction of incubation temperatures (Alavi et al. 1999; Swinnen et al. 2004; Yilmaz 2011).

For nearly two decades, great efforts had been made to enhance the Baranyi and Roberts model (Mytilinaios et al. 2015). Under some circumstances, the

non-autonomous form of the Baranyi and Roberts model may impede drawing accurate conclusions. Vadasz and Vadasz (2007) developed a more biologically meaningful autonomous version of Baranyi and Roberts model to keep the accuracy, leading to a meaningful interpretation for the physiological state of the cells after inoculation.

The modification of the model has been of urgent concern since the traditional models cannot fit the real conditions especially in some extreme environments (Julio et al. 2016; Mellefont and Ross 2003; Robinson et al. 1998). For example, Zhou et al. (2011) explored the growth of *Salmonella* Enterica under a range of osmotic stress conditions, critical to the growth or no-growth regions, to propose that microorganism may build a protection against harsh environments. Once the protection reached the minimum level, the microorganisms start growing rather than dying. This suggested that the classical definition of the lag via inoculum level is not suitable, resulting in an extension of the Baranyi and Roberts model. At a constant temperature, the Baranyi and Roberts model can be formulated as a series of coupled equations with analytical solution. However, under dynamic temperature conditions, the equations do not have an analytical solution and were usually solved using the Runge–Kutta method (Gumudavelli et al. 2007; Koseki and Isobe 2005; Singh et al. 2011; Velugoti et al. 2011; Zhu and Chen 2015). With the aim to simplify the Baranyi and Roberts model, Zhu and Chen (2015) derived a numerical equation to estimate model parameters through combining numerical solution with simulated microbial growth data. In addition, these equations can be easily used in computer programs or commercial software.

14.3.1.3 Comparison with Other Models

The Baranyi and Roberts model is well-known and extensively applied in many aspects of biology, and inevitably led to comparisons with Gompertz and Logistic models. Numerous studies suggested that the mechanistic growth models like Baranyi and Roberts model, were more precise than empirical models, such as Gompertz model and Logistic model (Baty et al. 2002; Huang 2008; Li et al. 2014; Longhi et al. 2014; Menezes et al. 2018). But it is not always the case since the Baranyi and Roberts model appears to be more time-consuming than other models in some specific conditions. For example, the growth data of *Staphylococcus aureus* in sandwich fillings at different temperatures was determined by the Gompertz model, Logistic model, and Baranyi and Roberts model. The Gompertz model showed the best performance in coefficient of determination (R^2), the standard deviation (Sy.x), and the Akaike's information criterion (AIC) (Ding et al. 2010). Additionally, the Baranyi and Roberts model, modified Gompertz model, Logistic model and Huang model were used to evaluate the effect of essential oils on the growth of *Salmonella* Typhimurium in rainbow trout stored under aerobic, vacuum and modified atmosphere conditions. Based on comparisons, the empirical models (modified Gompertz model, the Logistic model) were better than the other two mechanistic models (Baranyi and Roberts model and Huang model) (Yilmaz 2011).

14.3.2 Square Root Model

14.3.2.1 Basic Assumptions

Even though Arrhenius equation was modified and applied to describe bacterial growth, the modified law relationship was not suitable for the complex microbial growth processes related to numerous substrates and enzymes, and thus Ratkowsky et al. (1982) proposed a linear relationship between the square root of the growth rate constant (r) and the temperature (T) (Eq. 14.4). Initially, Ota and Hirahara (1977) discovered empirically that a plot of the square root of the rate of nucleotide breakdown in cool-stored carp muscle versus temperature was nearly linear. Unfortunately, there is no theoretical foundation, but it has an excellent fit to the data.

$$\sqrt{r} = b(T - T_0) \tag{14.8}$$

where b is a regression coefficient and T_o is a conceptual temperature-independence of metabolic rate, which is an intrinsic property of the organism. However, at a higher temperature, the previous equation does not work because of the inactivation or denaturation of proteins and other factors . Therefore, Ratkowsky et al. (1983) modified the equation and named the optimized equation as "Ratkowsky Square Root model", suitable for the description of bacterial growth throughout the entire temperature range (Eq. 14.5). Moreover, it fits data well and has meaningful statistical properties, for instance, the least-squares estimators of the parameters were almost unbiased and normally distributed.

$$\sqrt{r} = b(T - T_{\min})\{1 - \exp[c(T - T_{\max})]\} \tag{14.9}$$

where T_{min} and T_{max} are the minimum and maximum temperatures, respectively, at which the growth rate is zero, b is the regression coefficient of the square root of growth rate constant below the optimal temperature and c is an additional parameter to enable the model to fit the data for temperatures above the optimal temperature.

14.3.2.2 Limitations and Possible Enhancements

The Ratkowsky Square Root model is not suitable for predicting positive values of bacterial growth rate if the temperature is above T_{max}. Zwietering et al. (1991) modified the traditional model so that above the maximum growth temperature, T_{max} predicts no positive values of the growth rate (Eq. 14.6).

$$r = [b(T - T_{\min})]^2\{1 - \exp[c(T - T_{\max})]\} \tag{14.10}$$

Considering the temperature and water activity, a_w, and that temperature and pH independently affect microbial growth rate, McMeekin et al. (1992) proposed a

modified Square Root model (Eq. 14.7) to describe the rate in response to a combination of temperature, water activity, and pH values. However, the interactions of a factor are inevitable, e.g., in the case of acid potentiated ions such as nitrite.

$$\sqrt{r} = c\sqrt{(a_w - a_{w,\min})(pH - pH_{\min})}(T - T_{\min}) \tag{14.11}$$

Later, Zwietering et al. (1996) proposed an enhanced version of the Square Root model, which was also named as the Gamma model:

$$r = c(a_w - a_{w,\min})(pH - pH_{\min})(pH_{\max} - pH_{\min})(T - T_{\min})^2 \tag{14.12}$$

where T_{min} is defined as a hypothetical temperature, which is the point at which the line of the square root of growth rates intercepts the temperature axis (Heitzer et al. 1991; Huang et al. 2011; Ratkowsky et al. 2005). Many researches showed that T_{min} estimated by the Square Root model is lower than the true minimum growth temperature (Baranyi et al. 1995; Huang 2011; Juneja et al. 2009; Stannard et al. 1985). Therefore, it is necessary to close the gap between the model-calculated T_{min} and measured T_{min}. Huang (2010) used a Bělehrádek-type model (Eq. 14.9) to develop a nonlinear regression equation to describe the relationship between growth rate of *Escherichia coli* O157:H7 in beef and growth temperature and the results demonstrated that the T_{min} estimated by the new model was better than Ratkowsky Square Root model. However, Ross et al. (2011) disagreed that this new model is more suitable than the traditional Square Root model, so more research is needed in the future to make this model more encompassing of and truer to the real world conditions.

$$r = b(T - T_{\min})^{1.5} \tag{14.13}$$

Furthermore, Huang et al. (2011) developed an updated model covering a wider range of temperatures to describe the growth of *L. monocytogenes* in beef frankfurter and the T_{min} was also closer.

$$r = b(T - T_{\min})^{1.5}\{1 - \exp[c(T - T_{\max})]\} \tag{14.14}$$

However, the Square Root model is relatively weak in predicting the conditions beyond the extreme values of the environmental parameters, which has been viewed as the bottleneck of the square root function.

14.3.2.3 Comparison of the Models

Various square root models and their modifications have been used to predict the microbial growth and were compared with other models. Non-linear Arrhenius model (Schoolfield model) (Eq. 14.11) and Square Root model are available to

describe the effects of temperature and other environmental factors on lag phase duration and growth rate (Ratkowsky et al. 1983; Schoolfield et al. 1981). Additionally, the dependent variables expressed as ln *rate* and sqrt *rate* are involved in Schoolfield model and the Square Root model. However, there are incompatibilities between the aforementioned typical models. Through predicting the effect of temperature on the growth of bacteria in foods, Adair et al. (1989) evaluated the ability of the two models with the mean squared error (MSE) among the observed generation, lag time and the predicted data. Based on their study, they proposed the Schoolfield model was a more reliable description of the experimental data than the Square Root model for the two more parameters involved in the Schoolfield model. While Ratkowsky et al. (1991) held the view that the two models performed almost equally via the MSE criterion with the theoretical foundation and published data. The increased variability of data affects the Schoolfield model much more than the Square Root model because sqrt *rate* is constant, but ln *rate* increases progressively with response time. Therefore, the parameter values can vary widely at low temperatures and long times.

$$r = \frac{\rho\left(25^{\circ}\mathrm{C}\right)\frac{T}{298}\exp\left[\frac{\Delta H_A^{\neq}}{R}\left(\frac{1}{298}-\frac{1}{T}\right)\right]}{1+\exp\left[\frac{\Delta H_L}{R}\left(\frac{1}{T_{1/2L}}-\frac{1}{T}\right)\right]+\exp\left[\frac{\Delta H_H}{R}\left(\frac{1}{T_{1/2H}}-\frac{1}{T}\right)\right]} \tag{14.15}$$

where T is the temperature in Kelvin; R is the gas constant; ρ (25 °C) is a constant; $\Delta H \neq A$ is the heat (enthalpy) of activation of the growth rate-controlling reaction; ΔH_L is enthalpy of low temperature denaturation of the rate-controlling enzyme; ΔH_H is enthalpy of high temperature denaturation of the rate-controlling enzyme; $T_{l/2L} = \Delta H_L/\Delta S_L$ and is the temperature at which half of the population of the rate-controlling enzyme is active and the other half has been inactivated by low temperature; ΔS_L is entropy of low temperature denaturation of the rate-controlling enzyme; $T_{l/2H} = \Delta H_H/\Delta S_H$ and is the temperature at which half of the population of the rate-controlling enzyme is active and the other half has been inactivated by high temperature; and ΔS_H is entropy of high temperature denaturation of the rate-controlling enzyme.

Numerous researchers have considered the Square Root model as more accurate than other models (Fernandez-Piquer et al. 2011; Koutsoumanis and Nychas 2000; Martins et al. 2015). Several predictive models such as Square root, Polynomial, and Arrhenius models (Eq. 14.12) have been used for the description of *L. monocytogenes* growth states in different food materials (e.g. meat, fish, egg, milk, dairy products, cheese, vegetables), and the Gamma model (Eq. 14.8) is evaluated as good as other models via MSE, R^2, bias factor and accuracy factor (Ross 1996; te Giffel and Zwietering 1999). Similarly, the impacts of temperature on *L. monocytogenes* growth in salmon roe were modeled by the Ratkowsky Square Root model, Huang Square Root model, and an Arrhenius model, respectively. The Ratkowsky Square Root model was more suitable to describe the effect of temperature on the specific growth rates in unsalted salmon roe because the nominal

minimum temperature was close to the real minimum growth temperature, and Huang Square Root model was more suitable in salted salmon (Cornu et al. 2006; Li et al. 2016).

$$ln\,(r) = ln\,(b) - \left(\frac{E_a}{RT}\right) \tag{14.16}$$

where b is pre-exponential factor; Ea is activation energy for bacterial growth; R is the gas constant; T is the temperature in Kelvin.

However, some researchers held the opposite conclusions (Cayré et al. 2003; Fernandez-Piquer et al. 2011; Giannuzzi et al. 1998). For instance, Mataragas et al. (2006) evaluated the spoilage of cooked cured meat products by an Arrhenius model and Square Root model and proposed that both of them fit well, but the Arrhenius model was more adaptable than the Square Root model. Analogously, Kreyenschmidt et al. (2010) assessed the shelf life of sliced cooked ham based on the growth of lactic acid bacteria and showed that the Arrhenius equation gave a better result.

14.3.3 Response Surface Methodology (RSM)

14.3.3.1 Basic Assumptions

The variables in an RSM model can be continuous (e.g. temperature or length) or categorical (number of participants) in nature. The model is essentially a second-degree polynomial approximation. The effectiveness of each variable is also tested in the model and the model is usually reiterated to only include the most effective variables to ensure parsimoniousness.

Since RSM utilizes statistical estimation to explain the effect of the variables, it is easy to apply the method to any set of variables in the process without the need to profoundly study these variables or the process beforehand. In other words, the flexibility and the fact that the model can be reiterated to great extents make RSM approaches ideal to oversophisticated processes where mathematical models are unable to operate (Myers and Montgomery 1995). The RSM designs employ several features including orthogonality (the property that allows RSM to estimate individual effects without confounding with other effects), rotatability (the property of rotating points of the design about the center of the factor space) or uniformity (used to control the number of center points in the design) (Box and Wilson 1951).

14.3.3.2 Limitations and Possible Enhancements

RSM experiment designs come on different levels of applicability and therefore complexities. The simplest design is a 2- level factorial experiment or a fractional

Table 14.2 An example for a 2 level 3-factor factorial design

	Factors		
Treatment	A	B	C
(1)	−1	−1	−1
a	1	−1	−1
b	−1	1	−1
ab	1	1	−1
c	−1	−1	1
ac	1	−1	1
bc	−1	1	1
abc	1	1	1

factorial design. Fractional designs take on all possible combinations of the factor levels. Therefore, a factorial design is a fully crossed design and thus does not leave out any combinations. But sometimes scientists cannot afford to investigate all the possible combinations. The experiments may be too expensive or too much time-consuming that may take weeks to complete each of them which may be the case in many microbial studies (Mahdinia et al. 2017a, b, 2018d, 2019a, b). In those cases, a fractional factorial design may be used that dismisses a large number of the combinations (possibly more than half). Table 14.2 shows a simple s-level factorial design with 3 factors.

The precision may be preserved, yet the potency of the model to address complex cross-effects is definitely reduced. In this fashion, other more complex and more capable designs were fabricated (Montgomery 2017).

14.3.3.2.1 Central Composite Design (CCD)

A CCD uses a second-order quadratic model. Similar to a 3-level factorial design, the CCD uses three levels for the factors and replicates over the middle levels of treatment to probe for the sensitivity and replicability of the experiments (Yolmeh and Jafari 2017). Table 14.3 shows a circumscribed design (CCC) for a three-factor model.

As seen in Table 14.3, the CCC uses factor settings outside the range of the factors in the factorial part and thus provides high quality predictions over the entire design space. A central face-centered design (CCF) uses the same number of runs but do not require settings outside the range. As a result, a CCF design is usually unable to provide precision for estimating pure quadratic coefficients. Nevertheless, both of CCDs require 20 runs for a 3-factor experiment while a full factorial design would require 27, of course.

Table 14.3 A coded CCC for a 3-factor experiment

	Factors		
Experiment	A	B	C
1	−1	−1	−1
2	+1	−1	−1
3	−1	+1	−1
4	+1	+1	−1
5	−1	−1	+1
6	+1	−1	+1
7	-1	+1	+1
8	+1	+1	+1
9	−1.682	0	0
10	+1.682	0	0
11	0	−1.682	0
12	0	+1.682	0
13	0	0	−1.682
14	0	0	+1.682
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0

14.3.3.2.2 Box-Behnken Design

When scientists would like to use as few number of runs as possible and are not really expecting to run into convoluted cross-effects between factors; they turn to Box-Behnken designs. Table 14.4 shows the coded design for it.

As seen in Table 14.4, the Box-Behnken design does not require over or under-range settings and only requires 15 runs. As a result, it does not provide as high resolutions over a vide space of prediction like a CCC does. However, its missing corners may be useful when we should avoid combined factor extremes; which prevents a potential loss of data in those cases (Montgomery 2017).

Usually, the CCD models end up giving in a full quadratic model to explain the effects of all variables (A, B and C) on the response (Y) (Yolmeh and Jafari 2017):

$$Y = \beta_0 + \beta_A A + \beta_B B + \beta_C C + \beta_{AB} AB + \beta_{AC} AC + \beta_{BC} BC + \beta_{AA} A^2 + \beta_{BB} B^2 + \beta_{CC} C^2 \quad (14.17)$$

Table 14.4 A coded Box-Behnken design for a 3-factor experiment

	Factors		
Experiment	A	B	C
1	−1	−1	0
2	+1	−1	0
3	−1	+1	0
4	+1	+1	0
5	−1	0	−1
6	+1	0	−1
7	−1	0	+1
8	+1	0	+1
9	0	−1	−1
10	0	+1	−1
11	0	−1	+1
12	0	+1	+1
13	0	0	0
14	0	0	0
15	0	0	0

14.3.3.3 Comparison of Models

Above, we talked about how in theory RSM designs vary for different applications and how they may help us model sophisticated effects without usual difficulties of mathematical models. But what does that mean when it comes to microbial growth modeling? Cole et al. (1990) were one of the pioneers using RSM factorial design to investigate the simultaneous effects of pH, salt and temperature on *L. monocytogenes* survival and growth. They found out that survival at low pH and high salt concentrations is strongly temperature dependent and hence *L. monocytogenes* is the only species that poses great consumer health threats over refrigeration periods. Also, their polynomial model helped understand and develop better preservation conditions to minimize *L. monocytogenes* survival. In another study, García-Gimeno et al. (2002) used RSM to investigate the effects of NaCl concentration, pH and storage temperature on the growth curve of *Lactobacillus plantarum* in comparison with an ANN model. Their findings indicated that the RSM was a more precise model despite the fact that ANN models were more vastly used. As another example, effects of temperature, pH, and sodium chloride on growth of *Staphylococcus attreus* was predicted using a quadratic model in comparison with the modified Gompertz model with high precision by Sutherland et al. 1994.

As the RSM can be used for studying and modeling the effects of factors on microbial growth and survival, further post-modeling optimization techniques can be used to maximize a deactivation method efficiency. In this fashion, Han et al. (2002) used a Box-Behnken design to optimize *E. coli* O157:H7 deactivation using ozone treatment on green peppers. The variables were ozone gas concentration, humidity

and treatment time. The finding of the optimum conditions not only helped reduce the risk of the foodborne microorganisms, but ozone itself as a hazardous gas. Similarly, Skandamis and Nychas (2000) used RSM to obtain a quadratic model to predict the effects of temperatures, pH and oregano essential oil concentrations on the survival of an *E. coli* O157:H7 strain in eggplant salad. In conjunction with a Baranyi and Robert model, they accurately predicted the survival kinetics of the *E. coli* strain, which led to coherent predictions with viable-count measurements.

14.3.4 Artificial Neural Networks (ANNs)

14.3.4.1 Basic Assumptions

Artificial neural networks (ANNs) are an empirical non-linear method based on a set of mathematical equations to imitate the function of the human brain (Zupan and Gasteiger 1991). Basic ANNs contain three layers, the input layer, hidden layer, and output layer. The input layer is made up of the environmental influencing factors. The hidden layer is composed of numerous neurons and links between the input layer and the output layer, which may have one or multiple layers. The number of nodes (neurons) in the hidden layer is variable, and the nonlinearity of the neural network increases over the number of nodes, resulting in a more robust neural network (Gevrey et al. 2003). The output layer consists of the dependent variables (e.g., maximum specific growth rate and lag phase duration). Notably, ANNs do not assume the previous hypothesis of normality and independency between independent factors which are inevitable constraints for other methods. Instead, ANNs derives nonlinear functions directly from experimental data. Meanwhile, from one neural network model, different output can be obtained by various multi-equation models resulting in a smaller estimation error. (Pérez-Rodríguez and Valero 2013).

14.3.4.2 Limitations and Possible Enhancements

ANNs as a black box model stress their flexible behavior and prefer to describe the unknown relationship between microbial growth parameters and environmental influencing factors (Geeraerd et al. 2004; Khayet et al. 2011). However, this advantage brings about a side-effect, though ANNs hold high accuracy and great ability when multiple variables are described, a lack of interpretability limits the application in practical settings (Nelofer et al. 2012).

Back-propagation (BP) technique is the most common training algorithm for fee-forward neural network. It has the advantages of ANNs such as good prediction performances and easy to master (Jiang et al. 2016; Sadrzadeh et al. 2008; Wang et al. 2017a); however, it also has some issues including the local minima, overfitting, and slow convergence rate (Chen et al. 2014). The accuracy and efficiency of traditional ANNs can be improved by modifications. Several evolutionary techniques, such us genetic algorithm (GA) and particle swarm optimization (PSO)

algorithm are regularly used to solve the shortcomings of BP-ANN (He and Zhang 2018; Sun and Zhang 2018; Wang et al. 2017b; Zhang et al. 2016). The particles in PSO follow the trend that bird flocking and fish schooling share information for better living. Based on that, the nonlinear problem involving multiple variables can be solved more effectively (Kennedy and Eberhart 2011). A genetic algorithm is a randomized search method derived from Darwin's evolutionary theory (survival of the fittest) with an efficient and parallel global searching ability (Goldberg 1989). In addition, to enhance BP-ANN, Pruning algorithms and two hidden layers are frequently used to remove the unnecessary node and increase accuracy, respectively (Huang 2003; Reed 1993). Moreover, radial basis function neural networks (RBF-ANN) are another kind of models different from BP-ANN. For any BP-ANN, there is always an RBF-ANN that can replace it, and vice versa; however, the RBF-ANN is superior to BP-ANN in terms of approximation ability, classification ability, and learning speed (Marini 2009). A great many types of ANNs are not mentioned in this part. With the aim to apply ANNs in food microbial growth prediction, much more professional books and papers are strongly recommended to be investigated (Bishop 2006; Haykin 1994; Huang et al. 2007; Mitchell 1997).

14.3.4.3 Comparison of Models

As an unconventional microbial growth model, ANNs are always compared to the traditional microbial growth models such us Response Surface Methodology model (RSM) (Baş and Boyacı 2007; Garcíaa-Gimeno et al. 2003; Huang et al. 2007). RSM requires the order of the model to be stated, while ANNs implicitly match the growth conditions to the kinetic parameters. A further advantage of ANNs is that they allow the inclusion of non-growth data (García-Gimeno et al. 2005). For instance, ANNs and RSM were both used for predicting bacterial growth in a simulated medium of modified-atmosphere-packed cooked meat products. The results showed that the accuracy of ANNs was higher than RSM (Lou and Nakai 2001a, and b). Similarly, ANNs provided better predictions for the maximum specific growth rate of the fungus *Monascus ruber* than RSM (Panagou et al. 2010).

Compared to the Arrhenius model, ANNs offered several advantages in its non-linearity, parallelism, noise tolerance, learning, and capability for generalization (Gosukonda et al. 2015). For example, the Arrhenius model, BP-ANN, and RBF-ANN were used to predict the freshness of brined bream fillets stored at different temperatures, respectively. The RBF-ANN exhibited a great ability in function approximation, learning speed (compared to BP-ANN), and multi-output ability and self-learning (compared to the Arrhenius model) (Wang et al. 2015). Meanwhile, the ANNs were more effective than the Arrhenius model in predicting the quality of rainbow trout fillets during storage at different temperatures (Liu et al. 2015). Furthermore, Panagou et al. (2011) compared the partial least squares modelling (PLS) with ANNs for the rapid detection of the microbial spoilage of beef fillets on the basis of Fourier transform infrared spectral fingerprints via bias factor, accuracy factor and root mean square error. They concluded that PLS models

presented better correlation of total viable counts on meat surface with FTIR spectral data (Panagou et al. 2011).

14.3.5 Integrated Pathogen Modeling Program (IPMP2013)

14.3.5.1 Basic Assumptions

As mentioned in the last section, IPMP2013 is a microbial data analysis tool developed by USDA-ARS containing both primary and secondary models (USDA 2018). Twenty-one models are available to describe incomplete growth curves, complete growth curves, microbial survival, and inactivation, as well as the effect of temperature on microbial specific growth rates. The software includes the data window, model window, plot window, and report window. In particular, IPMP 2013 provides an interface allowing the users to adjust the initial guess values of each parameter (Fig. 14.5). The estimated parameters, the associated standard errors, t-values and p-values, and lower and upper 95% confidence intervals are shown in the analysis results. The sum of squared errors (SSE), mean and root mean of squared errors (MSE and RMSE), residual standard deviation, and Akaike information criterion (AIC) are all belong to error analyses (Huang 2014). It is an easy-to-use microbial data analysis tool which can be directly used without any programming knowledge (Fig. 14.6).

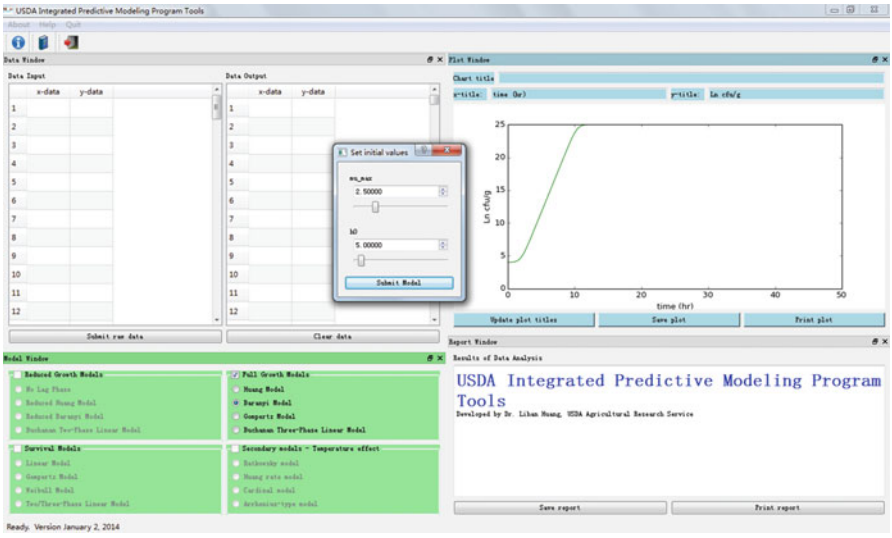

Fig. 14.6 Interface of IPMP2013 (USDA 2018)

14.3.5.2 Comparison of the Models

It is important to note that IPMP2013 is a fitting software. Whereas, Food MicroModel (FMM), Pathogen Modelling and Programme (PMP, and ComBase model are databases (Fig. 14.7).

There are other fitting software programs for microbial prediction. Many researchers use commercially available mathematical tools, such as MATLAB, SAS, and SPSS, while others use open-source (free) statistical analysis tools, such as R, for data analysis. However, these software packages are unfriendly to those individuals who lack in programming knowledge. For instance, R packages can offer considerable flexibility only for the users equipped with R's command line interface and script writing (Kahm et al. 2010). Later, some user-friendly tools have been developed such as two typical free Excel add-in packages (DMFit and GInaFit). The DMFit includes both primary models (reparameterized Gompertz model and the Baranyi model) and secondary models (Gamma model, Ratkowsky model, Cardinal model and polynomial model) (Combase, 2018). Specifically, the DMFit not only fits a primary curve to log CFU counts versus time data, but estimates the kinetic parameters such as growth/death rate, lag time, and maximum population density, the GInaFit includes nine different types (log-linear model, Weibull model, Biphasic model and their modifications) of microbial survival (inactivation) models, if the user does not have a clear idea of the general shape of their survival curves yet, different model types available can be tested and compared (Geeraerd et al. 2005).

Compared with DMFit and GInaFit, IPMP 2013 provides sufficient models, and the analysis results obtained from IPMP 2013 are identical to those from either R or SAS (Huang 2014). Even so, few shortcomings in IPMP2013 were also presented by researchers. For example, it lacks a local regression option and supports a limited number of data points. Additionally, it can only analyze one growth curve at a time

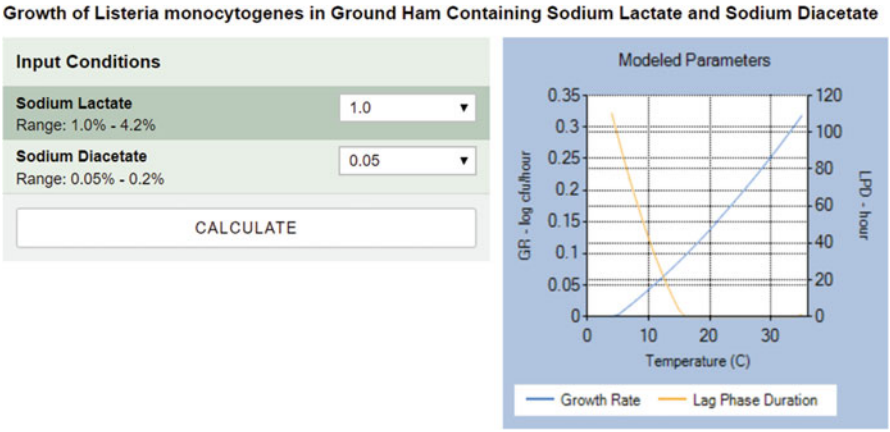

Fig. 14.7 Interface of PMP online (Hwang and Tamplin 2010; USDA 2018)

which limits its use in analyzing high-throughput microtiter plate-based data (Bukhman et al. 2015). Nevertheless, IPMP 2013 remains to be a good microbial growth fitting software.

14.4 Concluding Remarks and Future Trends

In this chapter, the most widely used traditional and novel food microbial predictive models were summarized. Most of these food microbial predictive models have been widely applied in the food industry for estimating risk, identifying critical control points, evaluating reformulations, and education (Whiting 1995). It is helpful for the researchers in the field of food safety engineering to understand and master the fundamentals of microbial growth modeling to enable the development of more reasonable and accurate models.

Microbial growth modeling has progressively become an indispensable part of food engineering. Recognizing its importance and significance, researchers and practitioners are working towards addressing the grand challenge of developing a first-principle-based universal growth model, which is applicable for all microorganisms and foods under all environmental conditions. To that end, significant time and effort have been invested in expanding the scope of and generalizing the current and new models to achieve a universal model. For example, ComBase integrates numerous microbial growth data from many different models, which can be used to assist researchers and food companies in developing new food products, reformulating foods, produce food safety plans, reducing food waste, and helping public health organizations in developing science-based food policies (ComBase, 2018). Currently, the available data is not sufficient for the varieties of food materials and microorganisms. To fill the data gap, a team-effort involving food engineers, food scientists, and microbiologists is urgently needed.

With the increasingly robust and ever-improving microbial models, especially with the constant updating of the database, the food microbial predictive models have been widely used with confidence in HACCP and QMRA programs. However, some limitations restrict their application in food safety and engineering (Amézquita et al. 2005; Halder et al. 2010; Plaza-Rodríguez et al. 2015). For instance, different models and their modifications fit different circumstances; meanwhile, even small discrepancy in the environment (e.g., nutritional ingredient, processing method, etc.) may lead to different results. Moreover, some pathogenic microorganisms such as *C. botulinum* are not allowed in food, suggesting that preventing pathogenic bacterium is just as, if not more, important than predicting its growth (Collins 2010). Additionally, since microbial prediction is mostly used in the safety field, the model should overestimate the microbial growth rate for those special situations requiring a greater margin for error. Accordingly, three guiding principles are recommended in applications:

(i) The real conditions should be in the usable range of the model;
(ii) The model should be used for conservative estimations;

(iii) The widely used and proven models should be slightly modified, if necessary, to fit the actual situation.

In summary, although models are convenient to use in both scientific research and practical applications, they should be combined with traditional or novel and emerging microbial testing in concert with the indispensable experience of experts or practitioners rather than lieu of them.

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Chapter 15
Microbial Inactivation Models for Thermal Processes

Simen Akkermans, Cindy Smet, Vasilis Valdramidis, and Jan Van Impe

15.1 Introduction

A variety of microorganisms can come into contact with food products during various stages of the food chain. A fraction of these microorganisms is subdivided into food pathogens and spoilage causing microorganisms. Pathogens cause consumers to get ill upon consumption and are therefore related to food safety. Spoilers can grow to such an extent that the product is no longer considered edible and is therefore spoiled. As such, spoilage microorganisms are mainly linked to food quality. Whether discussing microbial pathogens or spoilers, it is inevitable that these microorganisms can come into contact with food products. It is thus essential to prevent that the presence of these microorganisms will lead to food safety or quality issues.

The behaviour of microorganisms in food products is determined by the intrinsic and extrinsic properties. The intrinsic properties are inherent to the food itself, including pH, water activity, composition, and preservatives. The extrinsic properties, on the other hand, are rather related to the storage conditions such as temperature and humidity, among others. This is the topic of another chapter of this book. Please refer to that chapter for more information. However, all these properties can

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influence the microbial behaviour such as survival, growth, or inactivation rate. The intrinsic and extrinsic properties themselves are determined through the design of the food product and the production process. As such, food products and processes need to be designed in such a way that the microbial quality and safety of a food product can be guaranteed during its shelf life.

In many food products, the population of microorganisms can be too high during the production or could become too high during the desired shelf life, given the extrinsic and intrinsic properties. In those cases, additional treatments have to be applied to inactivate these microorganisms. Heat treatments are among the most commonly used treatments to reduce the microbial load. A wide range of thermal treatments exists such as high-temperature short-time (HTST) pasteurisation (Grant et al. 2002), canning, steam surface pasteurisation (McCann et al. 2006), ohmic heating (Knirsch et al. 2010), infrared heating (Krishnamurthy et al. 2008), microwaves (Tang 2015), and radio frequency waves (Luechapattanaporn et al. 2004), etc. For all of these thermal treatments, the inactivation is caused mostly by the temperature to which the microorganisms are subjected, which should be at least above the maximum temperature that allows growth.

For the design of heat treatments, models from the field of predictive microbiology can be applied. Predictive microbiology is a scientific discipline that deals with making mathematical descriptions of the behaviour of microorganisms, specifically with an application to food safety and quality. Kinetic models describing the growth or inactivation of a microbial population are typically classified as either primary or secondary models. Primary models describe the evolution of a population of cells with time. The secondary models, on the other hand, describe the effect of the environmental conditions (e.g., temperature) on the parameters of the primary models (e.g., inactivation rate). These two types of models will be explained in Sects. 15.2 and 15.3 of this chapter with respect to describing the microbial inactivation due to a thermal treatment.

15.2 Primary Models for Thermal Inactivation

As mentioned before, the primary inactivation models describe the decrease of the microbial population as a function of (treatment) time. The most general mathematical equation for thermal microbial inactivation as a function of time can be written as:

$$\frac{dN(t)}{dt} = -k(t, \boldsymbol{e}) \cdot N(t) \tag{15.1}$$

This differential equation expresses the change of the total number of survival cells or the concentration of cells $N(t)$ with time t. k is a model parameter for the inactivation rate as a function of time under a set of environmental conditions $\boldsymbol{e}$. The initial condition for this differential equation is that the number of cells at the time

point equal to zero ($t = 0$) is N_0. A variety of different models can be derived from this generic equation, depending on the assumptions and desired parameterisation. The most basic models assume a linear (or first order) relationship between the logarithm of the microbial population and time. These are probably still the most commonly used models. However, in some cases, this linear relationship may be a potentially dangerous oversimplification. Therefore, some models have been proposed that can describe nonlinear inactivation kinetics as well. These two types of models are explained in the following sections.

15.2.1 Linear Primary Model

Given the model in Eq. (15.1), the differential equation for a linear primary model for microbial inactivation is easily derived by proposing that the inactivation rate k is a constant. As such, the following equation is obtained:

$$\frac{dN(t)}{dt} = -k \cdot N(t) \tag{15.2}$$

This equation was first proposed by Chick (1908), based on the observation that inactivation curves appeared to be similar to unimolecular reactions. Solving this differential equation leads to the following exponential decrease of the microbial population:

$$N(t) = N_0 \cdot e^{-k \cdot t} \tag{15.3}$$

Using this model to describe the inactivation of a population on a logarithmic scale results in the expression:

$$n(t) = n_0 - k \cdot t \tag{15.4}$$

where, $n(t)$ and n_0 are the logarithm of the number of cells as a function of time and of the initial number of cells, respectively. In this case, from Eq. (15.3), the natural logarithm should be used. However, in the literature it is seen that the logarithm of base 10 is more commonly used for describing microbial inactivation. Care should be taken when using an inactivation rate that is obtained from literature, since it is essential to know whether it was based on the natural logarithm or the base 10 logarithm. These inactivation rates can be converted to a different logarithmic scale using the following formula:

$$k_{ln} = ln\,(10) \cdot k_{log} \tag{15.5}$$

where k_{ln} is the inactivation rate given a natural logarithm and k_{log} the inactivation rate for a base 10 logarithm.

Most commonly, the inactivation as a function of time is not described by the inactivation rate but rather by a D-value of a specific microorganism at a given set of conditions. This D-value expresses the time that is required for a single decimal reduction of the microbial population. This decimal reduction corresponds to a decrease with a value of one on a logarithmic scale of base 10 or with a reduction of the population by 90%. The calculations using the D-value generally rest on the assumption that the relative inactivation rate of the population is a constant. As such, it is not seen as a time- or population-dependent process. The D-value itself is a function of the specific environment, most importantly of the temperature, and of the microorganism. As such, the number of cells in the population can be calculated as:

$$n(t) = n_0 - \frac{t}{D} \tag{15.6}$$

where $n(t)$ represents the number of cells as a function of time on a logarithmic scale of base 10. The time t is commonly represented in minutes or seconds, depending on the rate of the inactivation kinetics. n_0 is the initial number or concentration of cells in the food product before the treatment. D is expressed in the same time units as t. As can be seen when comparing Eq. (15.4) with Eq. (15.6), D is the inverse of k_{log}. This linear model for microbial inactivation is illustrated in Fig. 15.1, for the parameterisations in Eq. (15.4) and Eq. (15.6).

15.2.2 Nonlinear Primary Models

For the purpose of providing models that are compatible and have sufficiently broad applicability, the current overview focusses primarily on models that have two important characteristics. Firstly, the mathematical models should be formulated based on a (set of) differential equation(s). Thermal processing of food products is

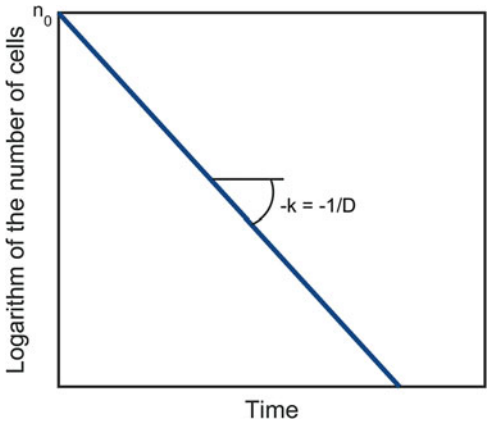

Fig. 15.1 The linear inactivation model represented with model parameter for the logarithm of the initial number of cells (n_0) and the inactivation rate (k) or D-value (D)

always a dynamic process. There is a heating step during which the temperature increases, followed by a hold time, during which a specific temperature is maintained and finally there is again a cooling step. This dynamic property requires to take the influence of the temperature profile into account. This leads to the need to formulate the models as differential equations. Secondly, the equations should be parameterised using either the D-value or the inactivation rate. Since secondary models for inactivation kinetics are commonly built to describe the effect of environmental conditions on the D-value or the inactivation rate, the primary models should rest on these parameters as well. As explained in Sect. 15.2.1, the D-value is the inverse of the inactivation rate, so it does not matter which of these two parameters is used.

15.2.2.1 Biphasic Model

A first nonlinear primary model that complies with the two requirements stated above is the biphasic model proposed by Cerf (1977). This model is based on the assumption that the microbial population consists of two different subpopulations with a different inactivation rate or initial concentration. One of these subpopulations has a higher initial population size and a faster inactivation rate than the other. This leads to the so-called biphasic inactivation dynamics, which is illustrated in Fig. 15.2. The difference in inactivation rates is explained as a difference in heat resistance of the two different strains. It should be noted that this biphasic behaviour due to mixed populations is not seen if the strain that has the largest initial population size has a slower inactivation rate than the other strain. In case of large differences in the population sizes, the two individual inactivation curves may not intersect during experimentation. As such, biphasic behaviour will often not be observed, even when subpopulations with a different thermal resistance are present. Humpheson et al. (1998) proposed that the biphasic behaviour can also occur in the presence of a single microbial strain due to the production of heat shock proteins, which protect the cells from the heat treatment and lower the inactivation rate. They demonstrated the validity of this assumption by adding chloramphenicol to the medium, which inhibits protein synthesis. The addition of 100 μg chloramphenicol per mL was seen to increase the inactivation rate of the second phase with about 40%.

The biphasic model was developed based on the assumption of two subpopulations and is represented by the following set of differential equations (Xiong et al. 1999):

$$\frac{dN_1(t)}{dt} = -k_1 \cdot N_1(t) \tag{15.7}$$

$$\frac{dN_2(t)}{dt} = -k_2 \cdot N_2(t) \tag{15.8}$$

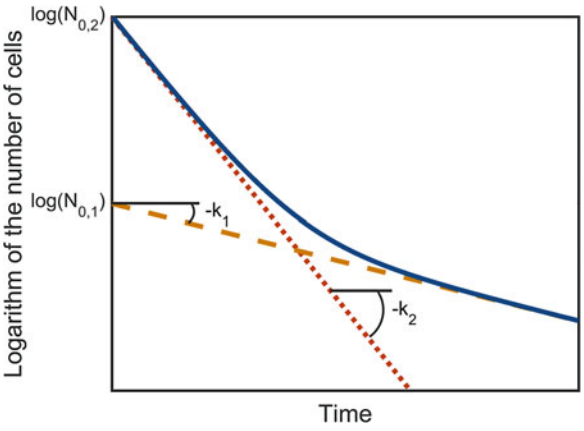

Fig. 15.2 The biphasic model that is based on two subpopulations, each with a different initial number of cells ($N_{0,1}$ and $N_{0,2}$) and inactivation rate (k_1 and k_2). The total population (–) is the sum of population 1 (---) and population 2 (•••)

This model is a simple extension of the model in Eq. (15.2), with two populations, $N_1(t)$ and $N_2(t)$, that have different inactivation rates, k_1 and k_2, and different initial quantities of cells, $N_{0,1}$ and $N_{0,2}$. The total population size $N(t)$ is then defined as $N_1(t) + N_2(t)$. This model using differential equations is also applicable when the environmental conditions change with time, and thus, k_1 and k_2 would be time dependent. For static conditions, $N(t)$ can be calculated:

$$N(t) = N_{0,1} \cdot e^{-k_1 \cdot t} + N_{0,2} \cdot e^{-k_2 \cdot t} \tag{15.9}$$

Again, this formula is easily derived from the model for a homogeneous population, which is in Eq. (15.3).

15.2.2.2 Geeraerd Model

Another commonly used nonlinear model for thermal inactivation is the model proposed by Geeraerd et al. (2000). This model was designed to describe microbial inactivation behaviour that has a shoulder effect and/or tailing behaviour. This model can be derived from Eq. (15.1) by writing the growth rate $k(t)$ based on three factors:

$$\frac{dN(t)}{dt} = -f_{shoulder}(t) \cdot k_{max}(t) \cdot f_{tail}(t) \cdot N(t) \tag{15.10}$$

Each of these three factors is basically related to a specific phase of the inactivation curve, which is illustrated in Fig. 15.3a. As such, there is a factor for the

shoulder, one for the log-linear inactivation and one for the tail. The second factor, for the log-linear inactivation is simply equal to the maximum specific inactivation rate $k_{max}(t)$. During the log-linear inactivation, the inactivation rate achieves a maximum. This is demonstrated in Fig. 15.3b, which illustrates the inactivation rate as a function of time. The factors $f_{shoulder}(t)$ and $f_{tail}(t)$ each result in values between zero and one, describing the reduction of the inactivation rate due to the mechanisms in the shoulder and tail phase. These factors are calculated as follows in the Geeraerd model:

$$\frac{dN(t)}{dt} = -\left(\frac{1}{1+C(t)}\right) \cdot k_{max}(t) \cdot \left(1 - \frac{N_{res}}{N(t)}\right) \cdot N(t) \tag{15.11}$$

For the factor related to the shoulder phase, an intracellular component $C(t)$ is defined. This is assumed to be a component that is critical for the survival of the microorganism and is inactivated following first-order inactivation kinetics:

$$\frac{dC(t)}{dt} = -k_{max}(t) \cdot N(t) \tag{15.12}$$

As such, the concentration $C(t)$ decreases exponentially. For inactivation dynamics with a shoulder effect, the initial value of $C(t=0) = C_0$ would be relatively large compared to 1. As such, the fraction in the first factor has a value close to 0 at $t = 0$. This causes the inactivation rate (multiplication of the first three factors in Eq. (15.10) or (15.11)) to be close to 0. This can also be seen in Fig. 15.3b and c, which illustrate the growth rate and the value of $f_{shoulder}(t)$, respectively. As the value of $C(t)$ decreases with time following Eq. (15.12), the value of this fraction approaches one, and the inactivation rate approximates k_{max}. The duration of the shoulder phase t_s can be calculated as follows:

$$t_s = \frac{ln\,(C_0)}{k_{max}} \tag{15.13}$$

This equation only holds when the maximum specific inactivation rate k_{max} is a constant, i.e., independent of time. The tailing effect of a microbial inactivation curve can be due to a resistant population, i.e., a part of the population that resists the treatment. This resistance can be due to the presence of different species or different phenotypes (Humpheson et al. 1998). The size of the resistant population N_{res} defines the modelled tailing behaviour. During the initial phases of the microbial inactivation the $f_{tail}(t)$-factor is approximately one, since the value of N_{res} is significantly smaller than the value of $N(t)$. As the value of $N(t)$ decreases, the $f_{tail}(t)$-factor approaches zero and so does the growth rate. This behaviour of the $f_{tail}(t)$-factor and its impact on the growth rate can be seen in Fig. 15.3d and b, respectively. Modifications of this model can easily be made by removing the factors for the shoulder or tail. The model can also be expanded and describe biphasic inactivation curves with shoulder based on the same principles discussed above (Geeraerd et al. 2005).

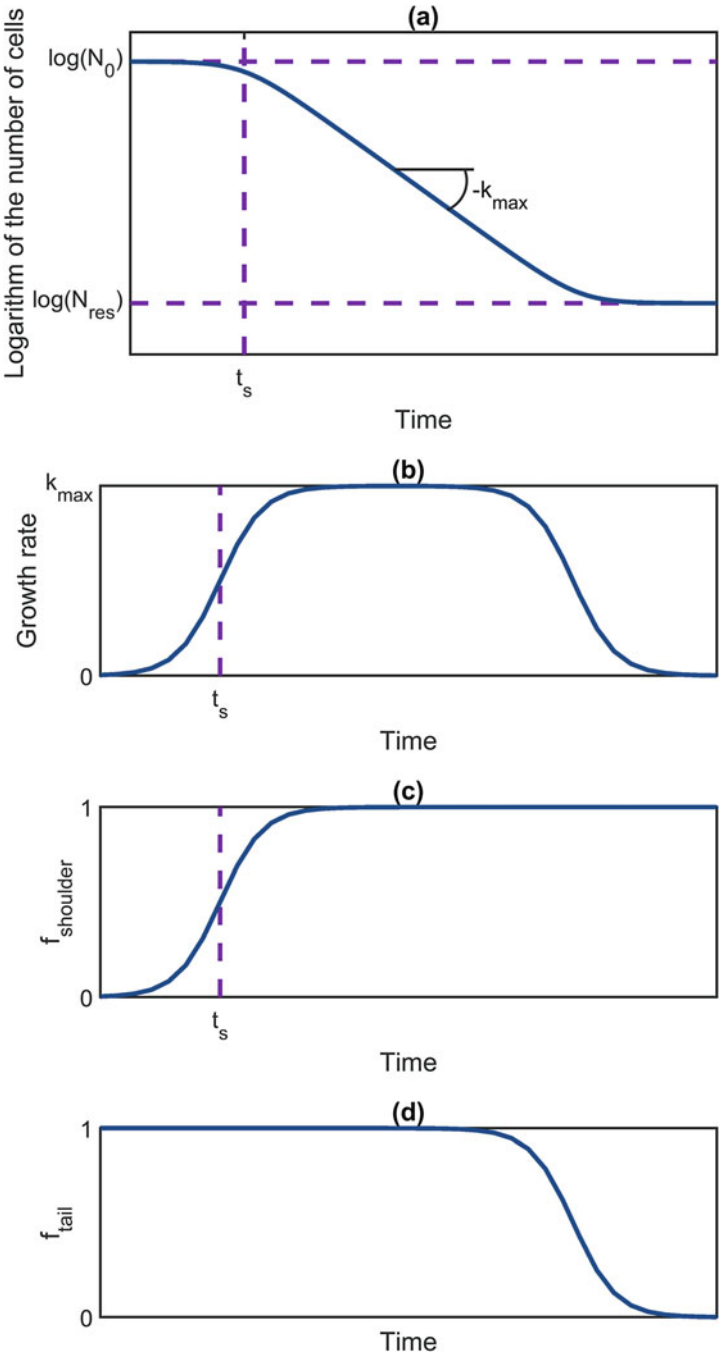

Fig. 15.3 Inactivation model proposed by Geeraerd et al. (2000). (**a**) the inactivation curve with the model parameters N_0 the initial number of cells, t_s the duration of the shoulder, k_{max} the maximum inactivation rate, and N_{res} the residual number of cells; (**b**) the evolution of the growth rate with time; and (**c**) and (**d**) the evolution of the factors $f_{shoulder}$ and f_{tail} with time

15.2.2.3 Weibull Model

The Weibull model is based on a model for the probability distribution of microbial death during heat inactivation. In fact, it is the cumulative asymmetric probability density function of the Weibull distribution. This model was first postulated by Peleg and Cole (1998) and has frequently been used since, thanks to its good fitting capacity for many applications. The following formulation is used for this model on a logarithmic scale:

$$n(t) = n_0 - \left(\frac{t}{\delta}\right)^p \tag{15.14}$$

where the parameter p is a shape parameter and δ is related to the inactivation rate. This model is illustrated in Fig. 15.4 for three different values of p. The inactivation curve is (a) linear for p equal to 1, (b) concave for p larger than 1 and (c) convex for p smaller than 1. In the former case, δ corresponds to the D-value. By taking the derivative of Eq. (15.14) to time, the differential equation for this model can be obtained:

$$\frac{dn(t)}{dt} = -\frac{p \cdot t^{p-1}}{\delta^p} \tag{15.15}$$

It should be noted that the right-hand side of this differential equation is dependent directly on the time. This means that the number of cells is dependent on the absolute time instance (Geeraerd et al. 2000). Logically speaking, this will not be the case and it is an indication of the more black-box nature of the Weibull model (i.e., that it does not rely on the mechanism for microbial inactivation). This can also be seen from the parameterisation, since the parameters p and δ have little interpretation. Another problem with this model is the lack of the possibility to add a tailing

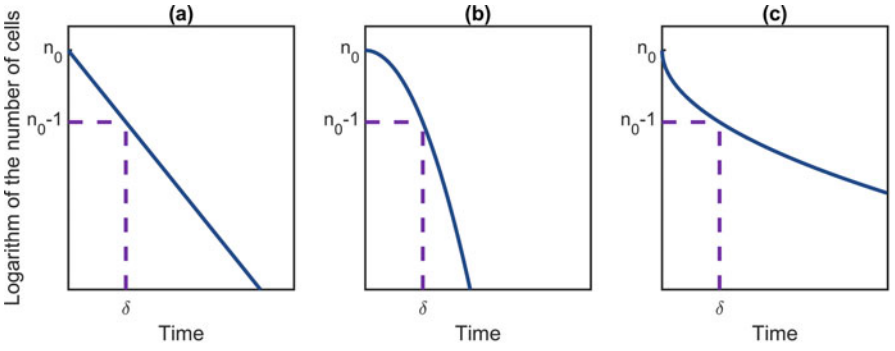

Fig. 15.4 Weibull inactivation models for which the initial number of cells (n_0), population level after a one decimal reduction ($n_0 - 1$), and the rate parameter (δ) are indicated. The subfigures represent cases with a different shape parameter p: (**a**) a linear model with $p = 1$, (**b**) a concave model with $p > 1$, and (**c**) a convex model with $p < 1$

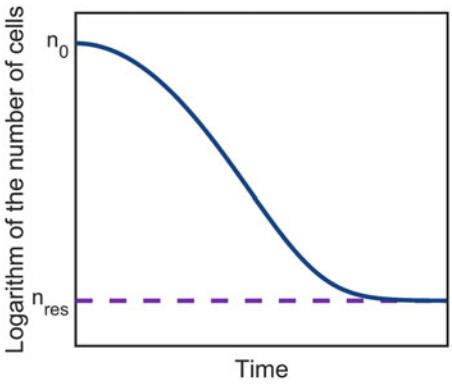

Fig. 15.5 Weibull model with tailing effect. The initial and residual number of cells are indicated (n_0 and n_{res})

effect. Therefore, Albert and Mafart (2005) included the description of the tailing effect into the Weibull model. When differentiating their model, this results in the following equation (Cappuyns et al. 2012):

$$\frac{dn(t)}{dt} = -\frac{p \cdot t^{p-1}}{\delta^p} \cdot \left(1 - 10^{n_{res} - n(t)}\right) \tag{15.16}$$

This model is illustrated in Fig. 15.5. When comparing this extended Weibull model with the Geeraerd model, Albert and Mafart (2005) found no noticeable difference in the fitting capacity of the two models.

15.3 Secondary Models for Thermal Inactivation

Secondary models are intended to describe the effect of the environment on the parameters of the primary models elaborated in the previous section. As such, they provide a mathematical description of, e.g., the effect of temperature on the inactivation rate or the effect of temperature and pH on the parameter δ of the Weibull model. A wide range of relatively accurate secondary models is nowadays available for many different microorganisms and food products to describe microbial growth. However, for microbial inactivation, there are far fewer accurate secondary models. The reasons for this include: (i) the relatively high experimental uncertainty within each inactivation experiment, (ii) the relatively high experimental uncertainty between different experiments, and (iii) the occurrence of different types of inactivation behaviour (e.g., occurrence and absence of shoulder and/or tail) (Abe et al. 2018). These properties of inactivation experiments make it difficult to build mathematical models based on their results.

An overview of some available examples for secondary models for microbial inactivation is given below. It provides a general framework that can be applied to build secondary models. Each model structure is followed by one or more secondary

models that have been built for specific microorganisms under specific treatments. The model structures with identified model parameters are given separately in Table 15.1.

15.3.1 The Arrhenius Equation

The Arrhenius equation was originally proposed to describe the effect of temperature on the rates of chemical reactions (Arrhenius 1889). The basic form of this model is commonly written as:

$$ln\,(k) = ln\,(A) - \frac{E_a}{RT} \tag{15.17}$$

where, k is the inactivation rate, A is a constant, E_a is the activation energy and R is the ideal gas constant, which is equal to 8.314 J · mol^{-1} · k^{-1}. Since the constant A has little interpretation, it can also be circumvented by writing the Arrhenius equation as:

$$ln\,(k) = ln\,(k^*) + \frac{E_a}{R} \cdot \left(\frac{1}{T^*} - \frac{1}{T}\right) \tag{15.18}$$

where k* a reference inactivation rate at the reference temperature T^*. Based on data of Ball and Olson (1957), Simpson and Williams (1974) determined that E_a and k of the spores of *Clostridium botulinum* are respectively $2 \cdot 10^{40}$ s^{-1} and $3.1 \cdot 10^5$ J · mol^{-1}. This corresponds to using, e.g., a value of 0.158 s^{-1} for k* at a T* of 121.1 °C (Mafart et al. 2012).

Some authors have also extended the Arrhenius equation to include the effect of other environmental conditions on thermal inactivation kinetics. Based on the data of Xezones and Hutchings (1965), Davey et al. (1978) proposed the following equation for the effect of pH on the inactivation kinetics of *Clostridium botulinum*:

$$ln\,(k) = a_0 + \frac{a_1}{T} + a_2 \cdot pH + a_3 \cdot pH^2 \tag{15.19}$$

where a_0, a_1, a_2, and a_3 are model fitting parameters. This model was extended by Cerf et al. (1996) to include the effect of water activity, and was applied to the inactivation kinetics of *E. coli* based on the data reported by Reichart (1994). The following equation was used:

$$ln\,(k) = a_0 + \frac{a_1}{T} + a_2 \cdot pH + a_3 \cdot pH^2 + a_4 \cdot a_w^2 \tag{15.20}$$

where a_w represents the water activity and was only included as a quadratic term. The models of Eq. (15.19) and Eq. (15.20) are listed in Table 15.1.

Table 15.1 Overview of secondary models for thermal inactivation, listed alphabetically by author

Microorganism	Environment	Model	Source
Bacillus weihenstephanensis KBAB4 spores	Lab medium	$\delta\left(T_{sp}, pH_{sp}, T\right) = 7.9 \cdot \gamma_2\left(T_{sp}\right) \cdot \gamma_1\left(pH_{sp}\right) \cdot 10^{-\frac{T-90}{9.0}}$ $\gamma_2\left(T_{sp}\right) = \frac{\left(T_{sp}-2.4\right)^2 \cdot \left(T_{sp}-37.4\right)}{484.0 \cdot \left(T_{sp}-24.4\right)+286.0 \cdot \left(26.8-2T_{sp}\right)}$ $\gamma_1\left(pH_{sp}\right) = \frac{\left(pH_{sp}-5.2\right) \cdot \left(pH_{sp}-10.8\right)}{\left(pH_{sp}-5.2\right) \cdot \left(pH_{sp}-10.8\right)-\left(pH_{sp}-8.0\right)^2}$	Baril et al. (2012)
Bacillus licheniformis AD978 spores	Lab medium	$\delta\left(T_{sp}, pH_{sp}, T\right) = 6.0 \cdot \gamma_2\left(T_{sp}\right) \cdot \gamma_1\left(pH_{sp}\right) \cdot 10^{-\frac{T-100}{7.3}}$ $\gamma_2\left(T_{sp}\right) = \frac{\left(T_{sp}-5.7\right)^2 \cdot \left(T_{sp}-50.0\right)}{1953.6 \cdot \left(T_{sp}-49.9\right)+4.4 \cdot \left(55.6-2T_{sp}\right)}$ $\gamma_1\left(pH_{sp}\right) = \frac{\left(pH_{sp}-5.6\right) \cdot \left(pH_{sp}-11.4\right)}{\left(pH_{sp}-5.6\right) \cdot \left(pH_{sp}-11.4\right)-\left(pH_{sp}-8.5\right)^2}$	Baril et al. (2012)
Bacillus cereus ATCC7004 spores	Lab medium	$\delta\left(T_{sp}, pH_{sp}, T\right) = 0.2 \cdot \gamma_2\left(T_{sp}\right) \cdot 10^{-\frac{T-T^*}{z}}$ $\gamma_2\left(T_{sp}\right) = \frac{\left(T_{sp}-11.8\right)^2 \cdot \left(T_{sp}-47.0\right)}{670.8 \cdot \left(T_{sp}-37.7\right)+240.9 \cdot \left(49.5-2T_{sp}\right)}$	Baril et al. (2012)
Escherichia coli B200	Lab medium	$\ln k = 90.58 - \frac{0.3028 \cdot 10^5}{T} - 0.5470 \cdot pH + 0.0494 \cdot pH^2 + 3.067 \cdot a_w^2$	Cerf et al. (1996)
Clostridium botulinum 62A spores	Spaghetti, tomato sauce and cheese	$\ln k = 105.1 + \frac{37.39}{T} - 2.325 \cdot pH + 0.1634 \cdot pH^2$	Davey et al. (1978)
Clostridium botulinum 62A spores	Macaroni creole	$\ln k = 104.4 + \frac{36.49}{T} - 2.615 \cdot pH + 0.1878 \cdot pH^2$	Davey et al. (1978)
Clostridium botulinum 62A spores	Spanish rice	$\ln k = 107.8 + \frac{37.60}{T} - 2.795 \cdot pH + 0.2032 \cdot pH^2$	Davey et al. (1978)
Clostridium sporogenes PA 3679 spores	Mushroom extract, citric acid	$\log k = -11.16 + 0.1000 \cdot T - 0.7876 \cdot pH$	Fernández et al. (1996)
Clostridium sporogenes PA 3679 spores	Mushroom extract, glucono δ-lactone	$\log k = -10.94 + 0.0972 \cdot T - 0.0384 \cdot pH$	Fernández et al. (1996)
Bacillus stearothermophilus ATCC12980 spores	Mushroom extract, citric acid	$\log k = -10.55 + 0.1039 \cdot T - 0.3140 \cdot pH$	Fernández et al. (1996)

Bacillus stearothermophilus ATCC12980 spores	Mushroom extract, glucono δ-lactone	$\log k = -12.05 + 0.1156 \cdot T - 0.2990 \cdot pH$	Fernández et al. (1996)
Bacillus cereus CNRZ 110 spores	Lab medium	$\log D = -\frac{T-100}{9.15} - \left(\frac{pH-7.50}{3.70}\right)^2$	Gaillard et al. (1998a)
Bacillus cereus CNRZ 110 spores	Lab medium	$\log D = \log 0.676 - \frac{T-100}{9.28} - \left(\frac{pH-7.50}{4.08}\right)^2 - \frac{a_w-1}{0.164}$	Gaillard et al. (1998b)
Clostridium botulinum spores cocktail (6 strains)	Turkey	$\ln D = -9.9161 + 0.6159 \cdot T - 2.8600 \cdot pH - 0.2190 \cdot Cl + 2.7424 \cdot P + 0.0240 \cdot T \cdot pH - 0.0041 \cdot T \cdot Cl - 0.0611 \cdot T \cdot P + 0.0443 \cdot pH \cdot Cl + 0.2937 \cdot pH \cdot P - 0.2705 \cdot Cl \cdot P - 0.053 \cdot T^2 + 0.1074 \cdot pH^2 - 2.7678 \cdot P^2$	Juneja et al. (1995)
Salmonella cocktail (8 serotypes)	Chicken	$\log D = 11.54 - 0.1832 \cdot T + 0.00819 \cdot F$	Juneja et al. (2001)
Salmonella cocktail (8 serotypes)	Turkey	$\log D = 10.39 - 0.1632 \cdot T + 0.00203 \cdot F$	Juneja et al. (2001)
Salmonella cocktail (8 strains)	Ground chicken	$\ln t_7 = 17.655 - 0.240 \cdot T - \frac{283.88 \cdot T}{c_a} + 2.582 \cdot c_a^2 - 0.397 \cdot c_i$	Juneja et al. (2013)
L. monocytogenes cocktail (5 strains)	Ground beef	$\ln\delta = 2.815 - 0.338 \cdot (T-60) - 0.008 \cdot (T-60)^2 + 0.031 \cdot Cl \cdot (T-60) - 0.815 \cdot P \cdot (T-60) + 0.038 \cdot Cl \cdot L$ $\ln p = 0.010 - 0.002 \cdot (T-60)^2 + 0.096 \cdot Cl - 0.083 \cdot (T-60) \cdot P$	Juneja et al. (2014)
Escherichia coli OH157:H7	Apple juice	$\log D = 5.736 - 0.037 \cdot T - 0.022 \cdot B - 0.001 \cdot T^2 - 10^{-6} \cdot B + 3 \cdot 10^{-5} \cdot T \cdot B$	Splittstoesser et al. (1995)
Salmonella cocktail (4 strains)	Whey protein solutions	$\ln\delta = 9.91 - 0.10 \cdot T \pm 4.34 \cdot a_w$ $\ln p = -0.006 \cdot T$	Santillana Farakos et al. (2013)
Salmonella Enteritidis PT 30	Almond kernels	$\sqrt{\delta} = 137.25 - 3.19 \cdot T - 54.37 \cdot a_w + 0.02 \cdot T^2 + 0.74 \cdot T \cdot a_w$ $\frac{1}{p} = 2.98 + 0.03 \cdot T - 2.88 \cdot a_w$	Villa-Rojas et al. (2013)

The following symbols are used: *B* benzoic acid (mg/L), c_a carvacrol (%), *Cl* sodium chloride (%), c_i cinnamaldehyde (%), *D* D-value (min), *δ* parameter of the Weibull model related to the inactivation rate, *F* adjusted fat level (%), *k* inactivation rate (min^{-1}), *L* sodium lactate (%); *p* shape parameter of the Weibull model (−), *P* sodium pyrophosphate (%), *S* sorbic acid (mg/L), and *T* temperature (°C)

As reported by Ross and McMeekin (1994), several studies have found the Arrhenius equation to be inappropriate for modelling the experimental data on thermal inactivation as deviations from the model existed at high and low temperatures. As a result, several alterations have been made to the Arrhenius equation to make it more suitable under different conditions. The most widely known alteration of the Arrhenius model is that of Schoolfield et al. (1981). However, during the recent decades, models related to the Arrhenius equation have fallen in disuse.

15.3.2 The Bigelow Model

Bigelow (1921) established that there exists a linear relationship between the temperature and the logarithm of the time required to kill all microorganisms. This leads to the following equation:

$$\log D = \log D^* - \frac{T - T^*}{z} \tag{15.21}$$

The parameters D and z in this equation are commonly referred to as the D-value and z-value. As explained in Sect. 15.2.1, the D-value represents the time needed for a single decimal reduction in the size of the microbial population. The z-value is the temperature increase that is required for a ten-fold decrease in the D-value. The parameters T^* and D^* are a reference temperature with corresponding D-value. The reference temperature can be chosen freely in the range of applicable temperatures. The relationship between the D-value and z-value is represented in Fig. 15.6.

It should be noted that significant variability can exist in the D-value and z-value for different strains of the same species. Lemaire et al. (1989) studied the D-value of *Listeria monocytogenes* at a typical pasteurisation temperature of 72 °C for a set of 38 different strains. They found that the D-value ranged from 0.06 to 1.50 seconds.

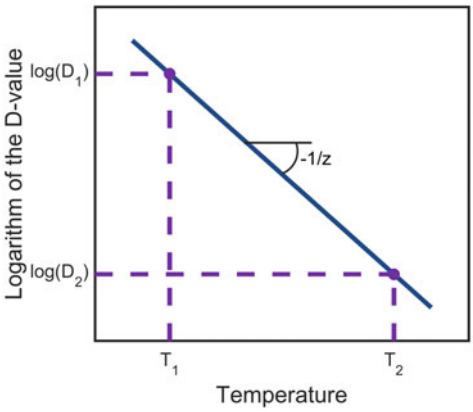

Fig. 15.6 Graphical representation of the relationship between the z-value and D-values. The z-value (z) can be calculated as the opposite of the inverse of the slope between two logarithms of D-values (D) at two different temperatures (T)

Table 15.2 An overview of D-values at four different temperatures for eight different microorganisms (Sörqvist 2003)

	Temperature (°C)			
	55	60	65	72
	D-values (seconds)			
Enterococcus faecium	3813	1150	347	65
Enterococcus faecalis	1393	415	123	23
Listeria innocua	1625	162	16	0.6
Listeria monocytogenes	643	87	12	0.7
Escherichia coli	266	39	5.6	0.4
Yersinia enterocolitica	168	30	5.4	0.5
Salmonella spp.	222	24	2.6	0.1
Campylobacter jejuni	50	8.2	1.3	0.1

Also, the z-value was strain dependent, with a range of 3.1 to 6.5 °C. This indicates that experimental testing should be performed with strains which are known to be the most heat resistant or with strain cocktails.

An overview of the D-values and z-values of eight different microorganisms is given in Table 15.2 for the temperatures 55, 60, 65 and 72 °C (Sörqvist 2003). From these eight microorganisms, *Enterococcus faecium* is the most heat resistant since it has the highest D-values with almost 6 minutes for a single decimal reduction at a typical pasteurisation temperature of 72 °C. *Campylobacter jejuni* on the other hand has the fastest inactivation kinetics with just 1.3 seconds needed for a decimal reduction at the same temperature. These data demonstrate the wide difference in inactivation kinetics over different species at the same temperatures. The z-values of these microorganisms can be calculated based on the given dataset to be able to calculate the inactivation rate at any temperature between 55 and 72 °C by using Eq. (15.17).

Mafart and Leguerinel (1998) proposed to extend the Bigelow model with the effect of pH on the thermal inactivation by making a simple combination of a term for the temperature-effect and a term for the pH-effect:

$$\log D = \log D^* - \frac{T - T^*}{z_T} - \left(\frac{pH - pH^*}{z_{pH}}\right)^2 \tag{15.22}$$

where z_T is defined as the z-value for temperature and z_{pH} is the z-value for pH. Gaillard et al. (1998a) used this model to describe the effects of temperature and pH on the thermal inactivation of *Bacillus cereus* and compared the results with a model that included an interaction term. The authors found that the additional term can in fact be neglected since it has little effect on the quality of the fit. The model without interactions is reported in Table 15.1. A further extension of this model was made by Gaillard et al. (1998b) to include the effect of water activity as well. This resulted in the following equation:

$$\log \mathrm{D} = \log \mathrm{D}^* - \frac{\mathrm{T} - \mathrm{T}^*}{\mathrm{z_T}} - \left(\frac{\mathrm{pH} - \mathrm{pH}^*}{\mathrm{z_{pH}}}\right)^2 - \frac{\mathrm{a_w} - 1}{\mathrm{z_{a_w}}} \tag{15.23}$$

where, z_{a_w} is the z-value for the water activity and the reference water activity is taken equal to one. This model was identified for *Bacillus cereus* spores as well (Table 15.1). By identifying the z_{pH}-values at different temperature and water activities, it became clear that z_{pH} was a function of the temperature and water activity. As such, interactions between the effects of the different environmental conditions were clearly present but not taken into account in the model. The authors justified the absence of interactions based on the fact that less than 2.4% of the total variation of the data would be explained by the interactions.

Baril et al. (2012) extended the concept of the Bigelow model significantly for the common application of heat resistance of bacterial spores. The model was not a classical application of the Bigelow equation based on the D-value, but based on the parameter δ of the Weibull model according to the work of Mafart et al. (2002). The value of δ was calculated as:

$$\delta\left(T_{sp}, pH_{sp}, T\right) = \delta^*_{max} \cdot \gamma_2\left(T_{sp}\right) \cdot \gamma_1\left(pH_{sp}\right) \cdot 10^{-\frac{T-T^*}{z}} \tag{15.24}$$

with δ^*_{max} the value of δ at the optimum sporulation temperature and pH for heat treatment at the reference temperature T^*. The two γ-factors, $\gamma_2(T_{sp})$ and $\gamma_1(pH_{sp})$, represent the effect of the sporulation temperature and pH on the value of δ. These factors are calculated according to the cardinal parameter model published by Rosso et al. (1995):

$$\gamma_n(x) = \frac{(x - x_{max}) \cdot (x - x_{min})^n}{\left(x_{opt} - x_{min}\right)^{n-1} \cdot \left[\left(x_{opt} - x_{min}\right) \cdot \left(x - x_{opt}\right) - \left(x_{opt} - x_{max}\right) \cdot \left((n-1) \cdot x_{opt} + x_{min} - n \cdot x\right)\right]} \tag{15.25}$$

where, n is a shape parameter, which is 1 for the effect of pH and 2 for the effect of temperature and x_{min}, x_{opt}, and x_{max} are respectively the minimum, optimum and maximum temperature or pH for sporulation. The value of γ_n is zero for values lower than x_{min} or higher than x_{max}. This was the first model to include the effect of sporulation temperature and pH in a model for heat inactivation. The model with the effect of sporulation temperature and pH was applied to new data on *Bacillus weihenstephanensis* and *Bacillus licheniformis*. Moreover, a simplified version of this model that only contained the effect of sporulation temperature was applied to data of González et al. (1999) on the heat inactivation of *Bacillus cereus* spores (Table 15.1).

15.3.3 Polynomial Models

Polynomial response surface models are very commonly used for different engineering applications. In the context of microbial inactivation, the polynomial models are described by an equation similar to the following:

$$\ln k(x_1, x_2) = a_0 + a_1 \cdot x_1 + a_2 \cdot x_2 + a_3 \cdot x_1^2 + a_4 \cdot x_2^2 + a_5 \cdot x_1 \cdot x_2 \qquad (15.26)$$

This exemplary model describes the effect of two influencing factors x_1 and x_2 on the natural logarithm of the inactivation rate. The parameters a_0 until a_5 are model parameters to be estimated using experimental data. The current equation contains linear effects (terms of a_1 and a_2), quadratic effects (terms of a_3 and a_4), and interactions between the influencing factors (term of a_5). Depending on the complexity of the microbial responses, some of these terms can be left out of the model. The same type of model can also be constructed for more than two influencing factors. In a way, the polynomial models used for microbial inactivation are not that different from the Arrhenius-type models for multiple effects discussed in Sect. 15.3.1, as both rely on the description of the logarithm of the inactivation rate based on a set of terms with fitting parameters. However, the Arrhenius-type models typically contain a term with the inverse of the temperature.

A model with two influencing factors of the same structure as Eq. (15.20) was developed by Fernández et al. (1996). This model was applied to the heat inactivation of the spores of *Bacillus stearothermophilus* and *Clostridium sporogenes*. However, the authors found that the data obtained could also be described with a simple linear model for the combined effect of temperature and pH, without interactions. As such, a model of this form was proposed for their application:

$$\ln k(T, pH) = a_0 + a_1 \cdot T + a_2 \cdot pH \qquad (15.27)$$

The models with appropriate model parameters for the two bacterial species are presented in Table 15.1. Another example of such a polynomial model was suggested by Juneja et al. (1995) for the effect of temperature (70–90 °C), pH (5.0–6.5), sodium chloride concentration (0.0–3.0%), and sodium pyrophosphate concentration (0.0–0.3%) on the inactivation rate of *C. botulinum* spores. Experiments were carried out with a mixture of six strains in lab medium. The model equation that was obtained is listed in Table 15.1. Based on this equation, Juneja et al. (1995) did find it necessary to include interaction and quadratic terms where Fernández et al. (1996) omitted them.

Models with a variety of different influencing factors have been proposed as well. Splittstoesser et al. (1995) produced two models for the thermal inactivation of *Escherichia coli* in apple juices. In one model, the addition of benzoic acid was included and in the other the addition of sorbic acid (Table 15.1). The results showed the significant influence of sorbic acid on reducing the D-value, and an even stronger effect was found for benzoic acid. Juneja et al. (2001) also proposed a model for the

effect of temperature and adjusted fat level on the D-value of *Salmonella* in poultry. The model was constructed to include a parameter that made the distinction between the poultry species. Omitting this factor results in the following equation:

$$log\, D = a_0 + a_1 \cdot T + a_2 \cdot F \tag{15.28}$$

with F the adjusted fat level. This model is reported for both chicken and poultry (Table 15.1). Also the effect of antimicrobials on the thermal heat resistance of *Salmonella* has been modelled. Juneja et al. (2013) studied the effect of cinnemaldehyde and carvacrol concentrations on the heat resistance of *Salmonella* in ground chicken. The research was able to demonstrate the effect of these plant-derived antimicrobials to inhibit the thermal resistance of *Salmonella*. When modelling these effects, the authors found that predictions based on the D-value greatly overestimated the inactivation that was achieved due to the presence of the shoulder and tailing effect. Therefore, the time needed to achieve a 7.0-log inactivation (t_7) was modelled instead. The following model structure was constructed:

$$ln\, t_7 = a_0 + a_1 \cdot T + a_2 \cdot \frac{c_a}{T} + a_3 \cdot c_a^2 + a_4 \cdot c_i \tag{15.29}$$

with c_a and c_i the concentrations of carcvacrol and cinnemaldehyde in percentage (Table 15.1). This model is an interesting example to demonstrate that the accurate modelling of thermal inactivation dynamics is still far from evident due to, among others, the difficulty of including the shoulder and tailing effect. A model that attempted to include this effect was suggested by Juneja et al. (2014). They included a secondary model for the shape parameter p of the Weibull model. Given a concave curve the parameter p describes the shoulder effect and with a convex curve p describes the tailing effect. The model expressed the effect of the temperature, salt concentration, sodium pyrophosphate concentration, and sodium lactate concentration on the parameters δ and p:

$$\begin{aligned} ln\, \delta = {} & a_0 + a_1 \cdot (T - T^*) + a_2 \cdot (T - T^*)^2 + a_3 \cdot Cl \cdot (T - T^*) \\ & + a_4 \cdot P \cdot (T - T^*) + a_5 \cdot Cl \cdot L \end{aligned} \tag{15.30}$$

$$ln\, p = a_6 + a_7 \cdot (T - T^*)^2 + a_8 \cdot Cl + a_9 \cdot (T - T^*) \cdot P \tag{15.31}$$

where L is the sodium lactate concentration and T^* is the reference temperature, which was set equal to 60 °C by Juneja et al. (2014). From the modelling results, it was concluded that lactate only had an influence in the presence of salt. A similar model was developed by Santillana Farakos et al. (2013). However, this model only described the effect of temperature and water activity on the parameters of the Weibull model. The following simple equations were used for this:

$$ln\,\delta = a_0 + a_1 \cdot T + a_2 \cdot a_w \tag{15.32}$$

$$ln\,p = a_3 \cdot T \tag{15.33}$$

This model was applied to a wide range of temperatures (21–80 °C) for low-moisture food products ($0.19 \leq a_w \leq 0.54$) for *Salmonella* (Table 15.1). The authors found that water activity had a significant effect on the inactivation kinetics at different temperatures but that water mobility (i.e., the ability of water to move through the food product) had no independent effect from the water activity. In the same year, Villa-Rojas et al. (2013) constructed a similar model for the effect of temperature (56–80 °C) and water activity (0.601–0.946) on the inactivation kinetics of *Salmonella*. The model equations were however somewhat more complex (Table 15.1):

$$\sqrt{\delta} = a_0 + a_1 \cdot T + a_2 \cdot a_w + a_3 \cdot T^2 + a_4 \cdot T \cdot a_w \tag{15.34}$$

$$\frac{1}{p} = a_5 + a_6 \cdot T + a_7 \cdot a_w \tag{15.35}$$

The authors also made a comparison with a model based on a linear primary model, but found that the more complex nonlinear model and its two secondary models were required.

15.4 Concluding Remarks and Future Trends

The first mathematical models to describe the thermal inactivation of microorganisms were constructed at the beginning of the twentieth century. Research into this area has increased significantly since the 1980s. However, even today the relationship between environmental conditions and the thermal inactivation kinetics that can be applied to a wide range of processing conditions and food products remains difficult to be established. Consequently, recent studies are still reporting on the effect of different characteristics of food products on the thermal inactivation kinetics, highlighting the need for a more mechanistic understanding of these relationships (Jarvis et al. 2016). Given the current difficulties in modelling the full inactivation dynamics, these models are generally not advisable for industrial applications for which a fixed reduction of the microbial load is typically assessed or is known.

In the past years, some combinations of primary and secondary models have been proposed that allow the prediction of nonlinear inactivation kinetics over the full time domain. These models mostly rely on the Weibull model as a primary model in combination with polynomial secondary models. Both of these models are basically black box models. Consequently, the modelling of the full inactivation kinetics (i.e., including modelling of the shoulder and tailing effect) is still in its infancy. A move

towards grey box models, which include more mechanistic knowledge on the thermal inactivation of microorganisms, is required to make significant advances in this field.

The mechanistic information that is required to model the shoulder phase of microbial inactivation can be obtained from available genome-scale metabolic network (GNM) models (Van Impe et al. 2013). These models contain extensive information on the use of nutrients by microorganisms. As such, GNM models can quantitatively describe how microorganisms rely on limited resources to defend themselves from mild thermal treatments and how these resources run out. The shoulder phase of microbial inactivation is explained based on the heterogeneous nature of the cell population. Some studies have already been performed on the heterogeneous, stochastic nature of the thermal inactivation of microorganisms (Abe et al. 2018; Aspridou and Koutsoumanis 2015). Even though these are valuable additions to achieve more informative models, a systematic framework for modelling such heterogeneity is yet to be proposed. Consequently, future research in this field should focus on both the use of GNM models and modelling population heterogeneity to achieve improved and mechanistic dynamic models.

Also the implementation of microbial inactivation models should be made more detailed and realistic in the future to obtain higher model accuracy in real life applications. To this end, computational fluid dynamics can be applied to describe the complete processing variables in time and space during thermal processing (e.g., Knoerzer et al. 2007). Many applications would also benefit from constructing and using models that describe the microbial kinetics specifically for non-isothermal microbial inactivation. Such models have received increasing attention in the past years (e.g., Dolan et al. 2013).

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Chapter 16
Endpoints Method to Predict Microbial Survival, Nutrients Degradation, and Quality Loss at High and Ultra High Temperatures

Micha Peleg

16.1 Introduction

Thermal processing is still one of the most common methods to preserve foods. This is because exposure of microorganisms, spores, and enzymes in foods to high enough temperatures for a sufficiently long time always results in their permanent inactivation. Implementation of this principle in industrial food preservation is based on the identification of the most heat resistant potential target, creating time-temperature conditions that cause its destruction, frequently with an added safety factor, and protecting the processed food from recontamination by a suitable filling procedure and hermetically sealed package. The microbial target itself can be a heat-resistant spore such as the pathogenic *Clostridium botulinum*, or a surrogate, in the case of sterilization of low acid foods, or a vegetative bacterial cell, a heat resistant enzyme (e.g., pectinase in citrus juices), or an enzyme that serves as a maker (e.g., alkaline phosphatase) for refrigerated pasteurized milk. The assumption is that if the designated process assures the target's destruction, all forms of lesser heat resistant organisms or enzymes have been also destroyed.

Generally, heat preservation can be classified as sterilization or pasteurization. Sterilization is typically applied to low acid foods to inactivate the most heat resistant bacterial spores and hence will inactivate everything else. Pasteurization is typically applied to acidic foods such as fruit juices or pickles, where the low pH precludes the germination of bacterial spores in which case only vegetative cells and enzymatic activity are causes of safety and stability concerns. There is also a third diverse class of special cases, such as the heat preservation of tomato juice and products, or when an additional preservation method is involved as in the already mentioned

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refrigerated dairy products having a relatively short shelf life, but also canned cured meats where the presence of salts has a synergistic lethal effect. This class also includes intentionally slightly acidified products such as canned mushrooms and asparagus in jars, where the purpose is to protect texture. Pasteurized liquid eggs are also slightly acidified so that they can be thermally processed to eliminate pathogens without coagulating. Almost always, sterilization involves much higher temperatures than pasteurization. But in both cases, the choice of a proper time-temperature history and assessment of its effect on food product's safety and quality are based on the similar theoretical kinetic principles. Equally important are heat transfer considerations. In both the heating, holding, and cooling regimes of solid foods, the primary heat transfer mechanism is conduction while in liquids it is primarily convection. Heat transfer issues are only briefly mentioned at the end of this chapter whose main theme is the kinetics of microbial inactivation and chemical reactions during thermal processing of foods. The focus is on the possibility of exploiting theoretical principles and the availability of advanced mathematical software in order to: (a) Reduce the number of experimental microbial counts or concentration measurements and (b) Facilitate the experimental and calculation procedures, needed to determine kinetic parameters at high and very high temperatures in actual foods.

16.2 Microbial Inactivation Kinetics and Rate Constant-Temperature Dependence Models

16.2.1 The Traditional Microbial Survival Model

For many years, it has been assumed that inactivation of microbial cells, spores, and enzymes at any high enough constant temperature follows first order kinetics, i.e., that there exists a log-linear relationship between the momentary survival ratio and the exposure time whose slope is the logarithmic inactivation rate constant, k. It has also been assumed that the "D-value" (this rate constant's reciprocal) vs. temperature relationship is log linear too, characterized by the organism or spore's z-value. It has also been assumed that the rate constant's temperature-dependence follows the Arrhenius equation - see below. Despite growing evidence that microbial inactivation following the first order kinetics is the exception rather than the rule (van Boekel 2009; Peleg 2006), these two models are still treated as fundamental in almost every microbiology and food science textbook. They also continue to serve as the basis of microbial sterility calculations required by regulatory governmental agencies in the United Stated and around the world.

Mathematically, these models can be written in the forms:

$$Log_{10}S(t) = -k(T)t = -\frac{t}{D(t)} \tag{16.1}$$

where $S(t)$ is the momentary survival ratio, i.e. $N(t)/N_0$, $N(t)$ and N_0 being the momentary and initial count, respectively, $k(T)$ the temperature-dependent decay rate constant and $D(T)$ its reciprocal.

The temperature's effect on this model's single rate constant has been traditionally described by:

$$Log_{10}\left[\frac{D(T)}{D_{ref}}\right] = -\frac{T - T_{ref}}{z} \tag{16.2}$$

where T_{ref} is a chosen reference temperature in the pertinent temperature range, and the z-value the temperature increase in degrees Celsius or Fahrenheit which will reduce the D value tenfold, or vice versa.

The most common alternative to Eq. 16.2 as a temperature-dependence model of microbial inactivation is the ubiquitous Arrhenius equation, which can be written in the form:

$$k(T) = k_{Tref}Exp\left[\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] \tag{16.3}$$

where T is the absolute temperature in °K, k_{Tref} is the rate constant at a chosen reference temperature T_{ref} also in °K, E_{a} the "energy of activation" in kJ or kcal per mole and R the universal gas constant in commensurate units. Notice that according to this model, its popularity notwithstanding, a mole of bacterial spores has a mass on the order 100,000 metric tons (Peleg 2006), and of bacterial cells such as *E. coli* on the order 300,000 metric tons! [The common claim, or rather excuse, that E_{a} is the energy of activation of a "limiting chemical reaction" does not hold water. What's counted are the numbers of spores or cells, and there is no evidence that such a reaction really exists. Even if it existed, which is highly doubtful, there is no reason to believe that it must be the very same reaction at all lethal temperatures.]

16.2.2 Alternative Models

The most commonly encountered inactivation pattern is the Weibullian, which can be written in the form (Peleg and Cole 1998, Peleg 2006, van Boekel 2009):

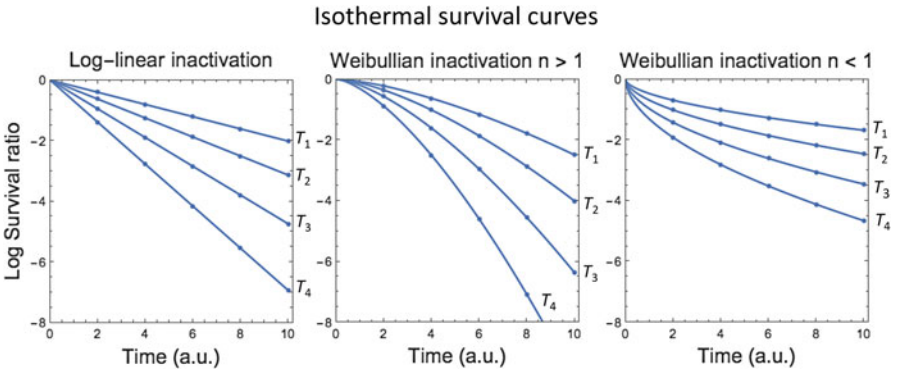

Fig. 16.1 Schematic view of the isothermal datasets used in the traditional methods to determine an organism or spore's inactivation kinetic model and extract its parameters ($T_4 > T_3 > T_2 > T_1$)

$$Log_{10}S(t) = -b(T)t^{n(T)} \tag{16.4}$$

where $b(T)$ is a temperature dependent rate parameter (or 'scale factor') and $n(T)$ a curvature measure (or 'shape factor'), usually a very weak function of temperature (van Boekel 2009) in which case it can be approximated by the expression:

$$Log_{10}S(t) = -b(T)t^{n} \tag{16.5}$$

The use of the term 'Weibullian' here and not 'Weibull' or 'Weibull distribution' as in many publications is intentional. Since the model's parameters are determined from the experimental $\text{Log}_{10}S(t)$ vs. t relationship and not $S(t)$ vs. t, which makes perfect sense for microbial inactivation, the calculated parameters values need not be the same as those of the original Weibull model' equation because of the different weights assigned to the high and low survival ratios.

According to the Weibullian model, concave downward semi-logarithmic survival curves are characterized by $n > 1$, and concave upward ("tailing") by $n < 1$, and from a purely formalistic viewpoint, the traditional first order kinetics is just a special case of the Weibullian model where $n = 1$ – see Fig. 16.1. Datasets similar to those shown in the figure are used to determine chemical degradation kinetics, except that the plot's ordinate (y -axis) need not be logarithmic, when it represents the residual concentration or concentration ratio instead of the count or survival ratio. In the case of synthesis, see below, the abscissa almost always represents the increasing concentration expressed in chosen concentration units. Notice the relatively large number of experimental counts (or concentration determinations) needed to generate such plots, especially when each data point represents two or more replicates.

Since microbial lethality or inactivation only starts at a certain temperature, one can replace the log-linear or Arrhenius equation (Eq. 16.3) by the log-exponential model (Peleg 2006; Peleg et al. 2008):

$$b(T) = Log_e\{1 + Exp[c(T - T_c)]\} \tag{16.6}$$

where T_c marks the lethal regime' onset of and k the slope of the $b(T)$ vs. T curve at temperatures well above T_c, i.e., where $T >> T_c$.

Under non-isothermal conditions ("dynamic inactivation"), i.e., where $T(t)$ is not a constant but varies with time, one can assume that the momentary inactivation rate, $dLog_{10}[S(t)]/dt$ is the isothermal rate at the momentary temperature, $T(t)$, at a time $t*(t)$ which corresponds to the momentary survival ratio, $S(t)$. This translates into the rate equation (Peleg 2006):

$$\frac{dLog_{10}[S(t)]}{dt} = -b[T(t)]n\left\{\frac{-Log_{10}[S(t)]}{b[T(t)]}\right\}^{\frac{n-1}{n}} \tag{16.7}$$

where $b[T(t)]$ is defined by Eq. 16.6 where T is replaced by $T(t)$. The boundary condition for this differential rate equation is $Log_{10}S\,(0) = 0$.

In this form the rate model has three kinetic parameters namely, c, T_c and n. The model can be expanded to situations where n is a function of temperature and hence of time, i.e., $n[T(t)]$. However, such a scenario is uncommon and hence will not concern us here.

Either way, Eq. 16.7 is an ordinary differential rate equation (ODE), which can be solved numerically for almost any temperature history. In other words, Eq. 16.7 can be solved when the temperature history, $T(t)$, is expressed algebraically, including when the expression contains 'If' statements, or when $T(t)$ is in the form of an Interpolating Function obtained from a digitized experimental time-temperature record. The numerical solution of Eq. 16.7, the sought dynamic survival curve $Log_{10}S(t)$ vs. t, is rendered in the form of an Interpolating Function which is treated by Mathematica® and other advanced mathematical programs as a regular function for mathematical operations such as derivation, integration and plotting, see below.

Similar considerations apply to alternative survival models and inactivation rate-temperature relationships. In first order kinetics, which is equivalent to the Weibullian model where $n = 1$, the momentary logarithmic decay rate is only a function of temperature but not of time. Therefore, the dynamic survival curve $S[T(t)]$ or $Log_{10}S[T(t)]$ vs. t can be calculated by direct numerical integration.

16.3 Kinetic Rate Temperature-Dependence Models for Chemical Degradation and Synthesis Reactions

16.3.1 Chemical Degradation Models

There is ample published evidence that many, perhaps most nutrients' degradation reactions, including vitamins and pigments loss, follow fixed order kinetics, i.e., obeying the rate equation (van Boekel 2009):

$$\frac{\mathrm{dC}(t)]}{dt} = -k[T(t)]C(t)^n \tag{16.8}$$

where $C(t)$ is the momentary concentration, $k(T)$ the temperature dependent rate constant, and n the reaction's kinetic order. The boundary condition here is $C(0) = C_0$, the initial concentration. If $C(t)$ is defined as the concentration ratio $C(t)/C_0$, then the boundary condition becomes $C(0) = 1$.

Under isothermal conditions, i.e., where $T(t) =$ constant $= T$, and $n = 1$, first order kinetics, integration of Eq. (16.8) renders the familiar exponential decay curve:

$$C(t) = Exp[-k(T)\, t] \tag{16.9}$$

For zero order kinetics, the analytical solution of Eq. 16.8 for isothermal degradation is:

$$C(t) = 1 - k(T)\, t \tag{16.10}$$

and for any n'th order (n $\neq$ 1):

$$C(t) = (1 + k(T)(n-1)t)^{1/(1-n)} \tag{16.11}$$

According to Eq. 16.10, when $t > 1 \,/\, k(T)$, $C(t)$ becomes *negative*, which has no physical meaning in our context, and according to Eq. 16.11, where $0 < n < 1$ and $t > (1/\, k(T))^{1\text{-}n}$, $C(t)$ becomes a *complex number*, which too has no physical meaning in our context. Such problematic situations can also emerge in non-isothermal degradation, but both can be avoided by adding an If statement to the model's equation, which sets the concentration to zero if and when the problem appears (Peleg et al. 2014).

The rate constant's temperature-dependence, $k(T)$, has been traditionally described and continues to be described by the Arrhenius equation (Eq. 16.3), in which case the mole in the energy of activation's definition refers to the degrading compound. The Arrhenius equation's widespread use in chemistry (and as already mentioned in quantitative microbiology) raises several theoretical and practical issues when applied to chemical reactions and biochemical processes taking place in foods (Peleg et al. 2012). Also, it has been shown that in a pertinent temperature range around T_{ref}, one can replace the Arrhenius equation with the simpler exponential model:

$$k(T) = k_{Tref} Exp\left[c\left(T - T_{ref}\right)\right] \tag{16.12}$$

without sacrificing the fit (Peleg et al. 2012, 2014). This simple exponential model eliminates the need to convert the temperature from °C to °K and the use of its reciprocal. Thus when Eq. 16.12 is plotted, $k(T)$ rises with temperature rather than falls with the temperature reciprocal as in the Arrhenius plot, which is counter

intuitive. The parameter c, in Eq. 16.12 together with the chosen reference temperature can be used to estimate the Arrhenius energy of activation, E_a, and vice versa, if and when applicable (ibid).

Since in Eq. 16.8 the momentary degradation rate is already expressed in terms of the momentary concentration ratio, $C(t)$, its application to dynamic conditions is straightforward and only requires that the momentary temperature, $T(t)$, be incorporated into the rate constant term, which yields the model:

$$\frac{\mathrm{dC}(t)]}{dt} = -k_{Tref} Exp\left[c\left(T(t) - T_{ref}\right)\right] C(t)^n \tag{16.13}$$

with the boundary conditions $C(0) = 1$ when $C(t)$ is defined in terms of the concentration ratio $C(t)/C_0$.

Eq. 16.13 has three adjustable parameters, namely, k_{Tref}, c and n. It is an ordinary differential equation (ODE) and can be solved numerically for a large variety of temperature histories, continuous or discontinuous.

16.3.2 Chemical Synthesis or Formation Models

Thermal processing of foods may not only cause the loss of nutrients, pigments, and other desirable compounds, but also the formation of undesirable ones. A case in point is the formation of hydroxymethylfurfural (HMF), a potential carcinogen in thermally preserved milk and other dairy products, and in other sugar containing foods.

In principle, the kinetics of a chemical compound's synthesis can be modeled in the same manner as that of a degradation reaction, except that the rate equation ought to reflect a concentration rise with time instead of fall. Theoretically, when a synthesis reaction follows fixed order kinetics, it can be described by rate model:

$$\frac{\mathrm{dC}(t)]}{dt} = k[T(t)]C(t)^n \tag{16.14}$$

where $C(t)$ is the momentary concentration in concentration units, n again is the kinetics order. The boundary condition here is $C(0) = C_0$, the actual initial concentration. If the synthesized compound is formed de novo, then $C_0 = 0$, and if it is already present in the food, $C_0 > 0$. At least in principle, the rate constant's temperature-dependence can be described in the same way as in degradation reactions, e.g., Eqs. 16.1, 16.3, 16.6, or 16.8. Therefore, if $k(T)$ follows the Arrhenius equation, and hence the exponential model (Eq. 16.12), and if the reaction's kinetic order n is temperature-independent, then the rate equation assumes the following form:

$$\frac{\mathrm{dC}(t)]}{dt} = k_{Tref} Exp\left[c\left(T(t) - T_{ref}\right)\right] C(t)^n \tag{16.15}$$

16.4 The Problem of Obtaining Meaningful Experimental Isothermal Temperature Profiles at High and Ultra Temperatures

Excluding storage and non-thermal food preservation, a most important purpose of microbial inactivation kinetics models, regardless of their particulars, is to assess the efficacy of existing heat treatments and/or predict those of contemplated ones. Similarly, the primary purpose of the kinetic chemical models, regardless of whether they describe the progress of degradation or synthesis reactions, is to assess and/or predict the nutritional and quality implications of such thermal preservation processes. Effective use of kinetic models to quantify and predict thermal processes' efficacy, and the amount of collateral damage that they cause to the food, has several requirements among them:

(a) That the chosen kinetic models faithfully describe the targeted organism or spore's inactivation mode (or that of a relevant surrogate) and the kinetics of the chemical reactions at the pertinent temperatures.
(b) That the chosen models' parameters have been correctly and accurately determined in the actual food or a relevant substitute.
(c) That the relevant temperature history is accurately determined. [In the case of microbial inactivation in solid foods this is the history of the coldest point, but when it comes to vitamins loss, for example, one might need to consider the entire package. For liquid foods, we will assume good mixing due to steam injection or turbulent flow – see below.]
(d) That the food's thermal properties and heat transfer mechanism are known or can be calculated with sufficient accuracy.

Since we are dealing with a principle here, statistical considerations associated with variability in the processing conditions and inevitable compositional non-uniformity within the processed food will only be mentioned, but not addressed in any detail.

For what follows, we assume that we already have an appropriate kinetic model, be it of microbial inactivation, chemical degradation or synthesis, and will explain how this model can be validated. We also assume that the pertinent temperature profile, $T(t)$, can be accurately measured, continuously or at sufficiently short time intervals. We will ignore heat transfer considerations and only comment on their implications at the end of this chapter. With these caveats, we are left with item (b) on the list, i.e., the chosen model's parameters determination, as a single issue for discussion.

Traditionally, the type of kinetic model, e.g., the kinetic order, log-linear or Weibullian, etc., has been determined from a set of isothermal microbial counts or survival ratios vs. time relationships. Or in the case of chemical reactions the compound of interest's concentration or concentration ratio vs. time relationships, recorded at various temperatures in the pertinent range as shown schematically in Fig. 16.1. For physical and technical considerations, achieving an ideal isothermal heat treatment starting with an instant temperature rise and ending with an instant temperature drop is unfeasible. But if the come-up and cooling times are very short relative to the holding time, and the changes in the microbial counts or monitored compound's concentration during the come-up and cooling times are negligible, one can consider the obtained data as *practically isothermal*. Unfortunately, this is rarely if ever the case in high temperature short time (HTST) thermal preservation, and is absolutely not the case in ultra high temperature (UHT) preservation. This is especially an issue when it comes to microbial inactivation, where the come-up time's lethality and destructive effect on nutrients cannot be ignored. Published reports on the kinetics of changes that occur at temperatures of 140–160 °C are frequently based on taking measurements after holding the food for tens of seconds (or even tens of minutes!) and then extrapolating the results to shorter times. One can question the relevance of such data because holding the food for such long times at such high temperatures almost certainly alter its physical and other properties. An attempt at extrapolation from lower temperatures would also be problematic because the underlying assumption that the kinetics at such high temperature remains unchanged is hard to confirm. In addition, there are technical difficulties in trying to accomplish very fast heating and cooling of solid foods, and even of liquids sealed in or flowing through a capillary, but these should not be discussed further.

All the above suggests that a way is needed to abandon the traditional reliance on isothermal data and come up with a method whereby the kinetic parameters, be they of microbial inactivation or a chemical reaction, can be extracted from the counts or concentrations determined after *the completion of a set of realistic non-isothermal* (*dynamic*) *treatments*, i.e., after the treated food has been cooled to ambient temperature and the process or reaction practically ceases.

16.5 The Endpoint Method

The endpoints method was developed at the Department of Food Science at the University of Massachusetts in 2008, motivated by the development of an extreme UHT milk processing at NIZO Food Research in the Netherland in which temperatures as high as 170 °C could be reached with a total processing time on the order of a third of a second. In the first published description of the endpoints calculation method, it was claimed, based on theoretical considerations, that the principle and methodology should be also applicable to chemical reactions at high temperatures (Peleg et al. 2008). But no effort had been made to substantiate the claim experimentally until 2014. Since then, under a NASA sponsored study of vitamins loss

kinetics in space-foods, it has been demonstrated with published data (Peleg et al. 2014, 2015) and experimental results that the method indeed works for thermal processing and storage. In the case of storage, the motivation of applying the method has been primarily logistic, i.e., to substantially reduce the needed number of samples taken for chemical analysis.

16.5.1 The Endpoints Method Application to Microbial Inactivation

Consider a hypothetical scenario of a particular food in which the targeted organism's or spore's inactivation follows the Weibullian model (Eq. 16.7) and its rate parameter temperature-dependence, $b(T)$, follows the log-exponential model (Eq. 16.6). If so, the resulting combined survival model has three parameters, namely, n, c and T_c, which we try to determine.

Suppose now that we perform three experiments in which we subject the targeted organism (or proper surrogate) in the actual food to three *different* carefully monitored dynamic lethal temperature histories, let's call them $T_1(t)$, $T_2(t)$, $T_3(t)$ as shown schematically in Fig. 16.2. After the heat treatment completion, i.e., after the product has been cooled an ambient temperature, samples are taken at times t_{final1}, t_{final2} and t_{final3} and the corresponding residual logarithmic survival ratios $\text{Log}_{10}S_{final1}$, $\text{Log}_{10}S_{final2}$, $\text{Log}_{10}S_{final3}$ determined as also shown in the figure. In the figure $t_{final1} = t_{final2} = t_{final3}$ for simplicity. However, this equality is not a prerequisite and as long as all three t_{final}'s are in the region where the inactivation has already ceased, their order and actual values are unimportant. Notice that each final survival ratio, $\text{Log}_{10}S_{final1}$, $\text{Log}_{10}S_{final2}$ and $\text{Log}_{10}S_{final3}$, must lie on its corresponding yet unknown survival curve $\text{Log}_{10}S[T_1(t)]$, $\text{Log}_{10}S[T_2(t)]$ or $\text{Log}_{10}S[T_3(t)]$, which is defined by Eq. 16.7 for each temperature profile $T_1(t)$, $T_2(t)$ or $T_3(t)$, respectively. This entails that the three experimental final survival ratios $\text{Log}_{10}S_{final1}$, $\text{Log}_{10}S_{final2}$ and $\text{Log}_{10}S_{final3}$ values create three simultaneous equations:

$$\text{Log}_{10}S[T_1(t_{final1}] = \text{Log}_{10}S_{final1} \tag{16.15a}$$

$$\text{Log}_{10}S[T_2(t_{final2}] = \text{Log}_{10}S_{final2} \tag{16.15b}$$

$$\text{Log}_{10}S[T_3(t_{final3}] = \text{Log}_{10}S_{final3} \tag{16.15c}$$

where the functions $\text{Log}_{10}S[T_1(t)]$, $\text{Log}_{10}S[T_2(t)]$ and $\text{Log}_{10}S[T_3(t)]$ are the numerical solutions of Eq. 16.7 for the three temperature profiles $T_1(t)$, $T_2(t)$ and $T_3(t)$. In these three equations, the three unknowns are n, c and T_c, the sought kinetic parameters of the targeted organism or spore's survival in the particular food.

With an advanced mathematical program such as Mathematica® (Wolfram Research, Champagne, IL, USA), one can express the numerical solution of a differential equation as an Interpolating Function which can be used for subsequent

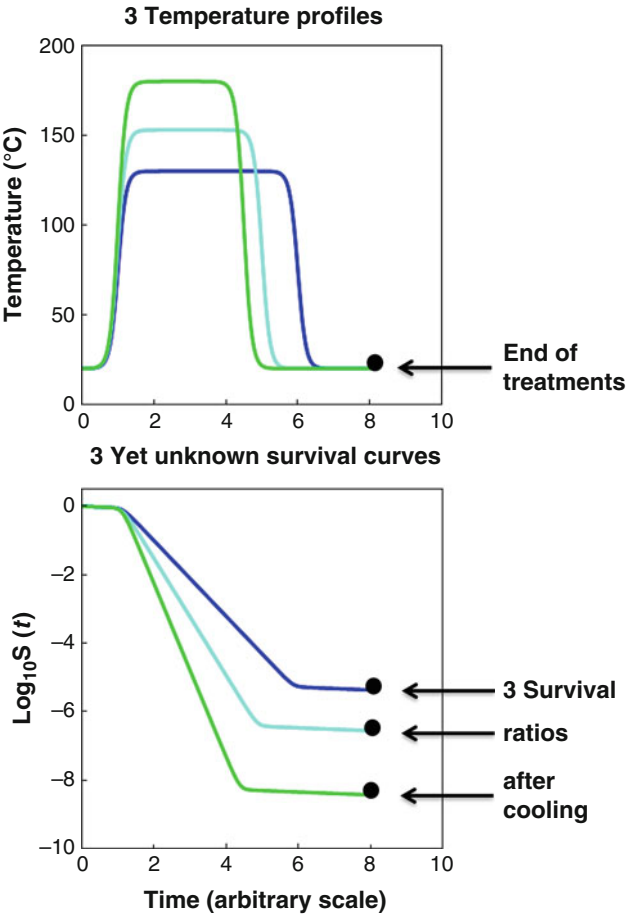

Fig. 16.2 Schematic view of the three endpoints method to estimate Weibullian microbial inactivation kinetic parameters from non-isothermal survival ratios obtained after three heat treatments completion. Notice that it also applies to isothermal survival curves wherever the come-up and cooling times' role is insignificant (Corradini et al. 2009)

mathematical operations and plotting. Mathematica® can also be used to solve these three simultaneous equations (Eqs. 16.15a, 16.15b, and 16.15c) numerically, for almost any conceivable temperature profiles to extract these three kinetic parameters using its FindRoot function.

To test the model and calculation method one should use the extracted n, c and T_c values to predict the final survival ratio at the end of a *fourth treatment* whose temperature profile, $T_4(t)$, *has not been used in their calculation*.

Here is an example taken from Peleg et al. (2008). It is based on NIZO's data on the inactivation of *Bacilus Sporothermodurans* spores in CASO growth broth, which are shown in a graphical form in Fig. 16.3.

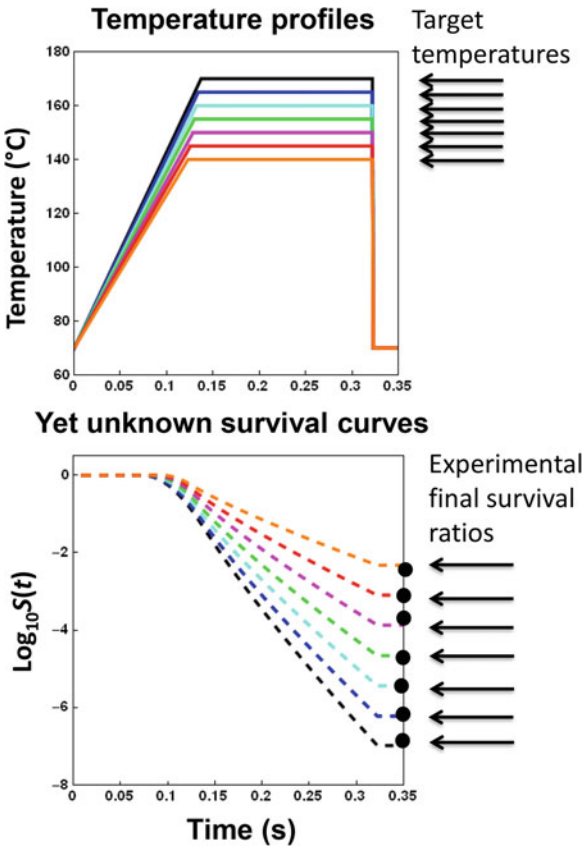

Fig. 16.3 Top: Temperature histories in the NIZO UHT process. Bottom: Corresponding experimental final survival ratios lying on yet unknown survival curves. Notice the very short process duration and the prominence of the come-up time

Table 16.1 Validation of the endpoint method to estimate a spore's inactivation parameters at ultra high temperatures

Endpoints profiles (°C)	4th validation profile (°C)	Predicted $\text{Log}_{10}S(t_{\text{final}})$	Observed $\text{Log}_{10}S(t_{\text{final}})$
145, 155 & 165	160	−5.7	−5.4
	150	−4.0	−4.25
	140	−2.4	−2.5
140, 150 &160	165	−6.2	−6.5
	155	−4.6	−4.7
	145	−3.1	−3.9

Table 16.1 shows that with one exception the predictions are all within 0.3 Log_{10} units or less, and that the exception is still within about 0.8 Log_{10} units only. Considering that a scatter on the order of ± 0.5 Log_{10} units in experimental microbial counts is quite common, these results can be viewed as validating the Weibullian survival model for the temperature range examined and confirming that the

calculation procedure has worked properly. Similar results have been obtained with other organisms under different temperature histories (Corradini et al. 2008).

The described endpoints method is not restricted to the Weibullian-log exponential model and it should work with any kinetic inactivation model that captures the organism or spore's isothermal survival pattern and how it is affected by temperature. Also, the endpoints method is not restricted to very high processing temperatures; it has been found equally applicable to heat treatments at relatively low processing temperatures, including isothermal treatments with negligible come-up and cooling times effects (Corradini et al. 2009). For low processing temperatures, the endpoints method's main advantage is primarily logistic; offering a simpler experimental protocol and a way to estimate the inactivation kinetic parameters from a considerably smaller number of microbial counts, in comparison with those required by the traditional methods.

The theoretical minimal number of experimental temperature profiles and their endpoints needed for the Weibullian model's kinetic parameters estimation is three. Nevertheless, at least four endpoints are always recommended: three for the survival parameters estimation and the fourth for the model and calculation procedure validation. But once the model has been validated by correctly predicting the fourth survival ratio, the four endpoints now available allow improvement of the parameters reliability and accuracy. One can now repeat the parameters calculation with different triplet combinations and averaging the results as follows: If we label the four different thermal treatments A, B, C and D, the number of triplet combinations is also three, i.e., A&B&C, A&B&D and B&C&D, in which case we can average three values of each parameter. However, with five different treatments, let's call them A, B, C, D and E, the number of possible triplet combinations rises to six i.e., A&B&C, A&B&D, A&B&E, B&C&D, B&C&E and C&D&E. In that case, the larger number of triplets not only increases the averaged parameters accuracy, but it also allows one to remove outliers identified by statistical criteria (Corradini et al. 2008). [For such criteria see https://en.wikipedia.org/wiki/Outlier or https://search.usa.gov/search?utf8=✓&affiliate=nist-search&sitelimit=www.itl.nist.gov&query=outlier&commit=Search.] Therefore the decision on the actual number of experimental treatments, as on the number of replicated counts, is primarily determined by logistic considerations rather than theoretical imperatives.

After the method has been evaluated and validated with one or more target organisms or spores, failure of the numerical solution to converge, or of the method to predict correctly new endpoints when applied to a new target, can be evidence that the inactivation of that new taget in the particular medium does not follow the assumed kinetic inactivation model and/or that there is a substantial error in one or more of the experimental survival ratios.

In principle, as already stated, one can apply the endpoints method with models other than the Weibullian-log exponential, but a word of caution is in order here. When one tries the method with a four-parameter model (instead of the three-parameter Weibullian-log exponential), it works perfectly well with simulated endpoints data having no or only very small introduced "experimental errors." But the method has failed miserably when the errors had been increased to levels that could

be still tolerated when the number of the sought parameters is only three. Whether future mathematical software will be able to overcome this predicament is unclear at this point, so for the time being the method is restricted to three parameters or two, see below.

The code of the Mathematica program to extract the Weibullian-log exponential model parameters by the endpoints method has been made publicly available in an Appendix to the Peleg et al. (2008) paper. To facilitate the calculations, they started with a very low or very high arbitrarily chosen initial n value, which was subsequently increased or decreased in small increments or decrements (of a magnitude also chosen by the user). For each iterative step, the program solves only two equations to extract c (called k in the code) and T_c, which together with the temporary value of n are used to calculate the third endpoint survival ratio. This calculated survival ratio is compared to the actual one and the iterations stop when the two agree within a user specified tolerance. In the future, the calculation method would be most probably simplified, and implemented with interactive software of the kind used for chemical reactions – see below.

The temperature profile, $T(t)$, for the kinetic parameters calculation, can be entered in two ways: as an explicit algebraic expression which has been derived by curve fitting the experimental time-temperature data, which can include 'If' statements to mark the heating, holding, and cooling regimes, or as an Interpolating Function determined directly from the digital record of the actual temperature history.

Despite being over ten years old by now and having obvious practical advantages over the traditional methods to calculate and predict heat inactivation patterns especially at high and ultra high temperatures, the endpoints method has received hardly any attention in the food microbiology community. It is highly doubtful that any research group engaged in microbial safety besides our own has ever tested the endpoint method performance and evaluated its potential utility. Hopefully, this state of affairs will change as a result NASA's interest in the methodology, which at the moment is only being applied to nutrients degradation in space-foods, the theme of the next section.

16.5.2 The Endpoints Method Application to Nutrients and Pigments Loss

The kinetics of vitamins, pigments, and other desirable compounds loss during thermal processing and storage has been amply studied during the last decades, and there is a large body of literature available on the subject. Recently, with NASA's plan to resume interplanetary human missions, interest in the topic has been revived, primarily in relation to maintaining the nutritious value of space-foods having a very long shelf life. And, since nutrients, especially heat labile vitamins, are

also lost during certain space foods heat preservation, interest in their degradation kinetics at high temperatures has been resurrected too.

The traditional methods to study chemical degradation kinetics at high temperatures have the same predicaments as those of microbial inactivation kinetics, and hence the motivation to replace them with the endpoints method. However, in contrast to microbial inactivation, which mostly follows nonlinear kinetics, chemical degradation, especially when monitored as the decay of a single compound, such as thiamin, or loss of a group of compounds of the same type, such as anthocyanins, usually follows first and occasionally other fixed order kinetics.

Fixed order degradation kinetics of order n follows Eq. 16.8 as a model. Thus if the rate constant's temperature-dependence, $k(T)$, follows the Arrhenius equation and thence the simpler exponential model (Eq. 16.12), the decay pattern is described by Eq. 16.13. In principle, if the kinetic order, n, of a particular heat labile compound in a particular food is known a priori, or can be assumed on the basis of published reports, then for any chosen T_{ref} in the pertinent temperature range, the model has only two adjustable parameters, namely k_{Tref} and c. These two parameters can be extracted from the compound's residual concentrations, C_{final}'s, determined after the completion of two different experimental thermal processes, i.e., after the food has been cooled to ambient temperature where on the pertinent time scale, the degradation can be assumed to have ceased. Thus, if the two temperature profiles were $T_1(t)$ and $T_2(t)$, and the corresponding experimentally determined concentration ratios are C_{final1} and C_{final2}, then we have two simultaneous equations:

$$C[T_1(t_{\text{final1}})] = C_{\text{final1}} \tag{16.16a}$$

$$C[T_2(t_{\text{final2}})] = C_{\text{final2}} \tag{16.16b}$$

where $C[T_1(t)]$ and $C[T_2(t)]$ are the numerical solutions of the rate equation (Eq. 16.13) for the corresponding temperature profiles $T_1(t)$ and $T_2(t)$, respectively. As before, the two temperature profiles can be entered either as algebraic expressions or digitized temperature records to be converted into Interpolating Functions, which Mathematica® recognizes. The two simultaneous equations (Eqs. 16.16a and 16.16b) have two unknowns, the sought k_{Tref} and c parameters, which can be extracted with Mathematica's using its FindRoot function. In other words, by having determined the residual concentration ratios C_{final1} and C_{final2} experimentally, monitoring their corresponding temperature histories, and knowing the degradation reaction's kinetic order, one can extract the kinetic parameters as the solution of two simultaneous equations.

Here too the assumed kinetic model and calculation procedure ought to be validated. And as before, this is done by using the extracted k_{Tref} and c parameter values to predict the final concentration ratio after completion of a third heat treatment, $T_3(t)$, whose temperature profile has not been used in their calculation. As in microbial inactivation, once the model has been validated by at least one

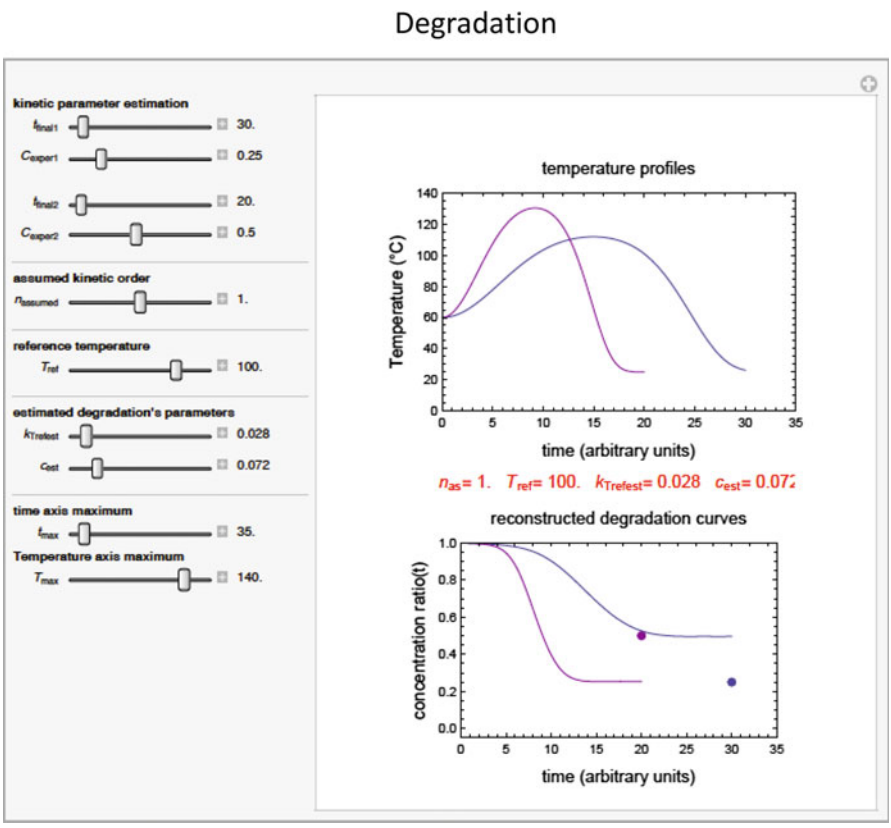

Fig. 16.4 Screen display of the interactive program to estimate kinetic degradation parameters by the two endpoints method, showing the entered temperature profiles, experimental endpoints, and chosen T_{ref} and n values. The two reconstructed degradation curves were generated with default $k_{Trefest}$ and c_{est} values using Eq. 16.15 as a model

additional new heat treatment, the k_{Tref} and c parameter values obtained from the three or more pair combinations (e.g., A&B, A&C, and B&C) can be averaged to improve their reliability and accuracy.

A freely downloadable Mathematica® program to estimate the degradation kinetic parameters by the two endpoints method, also available the Wolfram CDF Player and in a PDF format, has been made available on the Internet at: https://people.umass.edu/aew2000/nutrient_degradation/InterpolatedDegradation.html

Screen displays of the program for thermal processing are shown in Figs. 16.4 and 16.5. As can be seen in the figures, all the relevant model parameters, T_{ref}, n, etc., which are needed for the kinetic parameters calculation including the plot's range, can be entered either numerically or by moving their sliders on the screen. The two original temperature profiles, not shown in the figure, can be pasted as digitized time-temperature records in a variety of allowed formats.

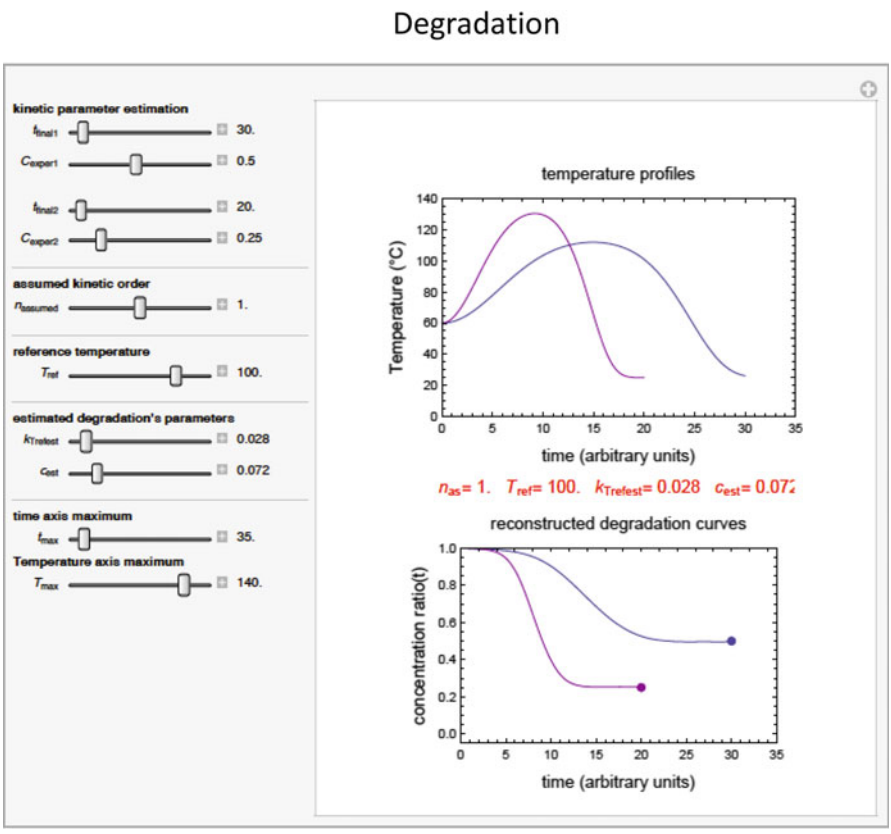

Fig. 16.5 The interactive program to estimate kinetic degradation parameters by the two endpoints method's screen display after matching the reconstructed curves with the corresponding endpoint concentration ratios, achieved by moving the k_{Trefest} and c_{est} sliders. When the endpoints and reconstructed curves match, these two sliders' positions are the sought k_{Tref} and c parameters' values

To estimate the k_{Tref} and c parameters, the user enters the two experimental endpoints and sets the kinetic order, n, and reference temperatures, T_{ref}. Once done, the user moves the k_{Tref} and c sliders until the two reconstructed degradation curves pass through their corresponding endpoints. When this is accomplished, the k_{Tref} and c sliders' positions mark these parameters' estimated values, which are also displayed on the screen in red. These estimates can be used as such to test the method or serve as the initial values for the FindRoot function in an attempt to improve their accuracy further, if and when deemed necessary. To test and validate the selected model and method, the user can replace one of the two original endpoints, it does not matter which, with that of the third experimental endpoint without moving the n, T_{ref}, k_{Tref} and c sliders. If the methodology is applicable, and none of the experimental endpoints has a substantial error, the new reconstructed curve will pass through or very close to the newly entered third point. The distance between the third

concentration ratio predicted with the estimated k_{Tref} and c parameters, as indicated by the sliders' positions, and the actual experimental values can then serve as a measure of the model and method's predictive ability in concentration ratio terms. Either way, it can and has been shown (Peleg et al. 2016, 2018) that small deviations from the assumed magnitude of n have little effect on the method's predictions at least for the examined compounds.

An example of the method's applicability tested with published degradation data on anthocyanins is given in Fig. 16.6. It shows that the predictions rendered by the endpoints method have been fairly close to the reported values at least when judged visually.

In principle, the method can be extended to degradation reactions of unknown kinetic order, in which case the minimal theoretical number of endpoints will be three. Thus, if the two endpoints method as described in Figs. 16.4 and 16.5 fails to produce two curves that pass through the entered experimental points regardless of the chosen k_{Tref} and c parameter combination, then the user can try adjusting the n slider to accomplish the task. Or alternatively, when the kinetic order is totally unknown, one should determine experimentally three endpoint concentrations and proceed to extract all three kinetic parameters (n, $k_{\mathrm{Tref,}}$ and c) by solving the following three simultaneous equations:

$$C[T_1(t_{\mathrm{final1}})] = C_{\mathrm{final1}} \tag{16.17a}$$

$$C[T_2(t_{\mathrm{final2}})] = C_{\mathrm{final2}} \tag{16.17b}$$

$$C[T_3(t_{\mathrm{final3}})] = C_{\mathrm{final3}} \tag{16.17c}$$

of which they are the three unknowns (Peleg et al. 2014). This can be done with Mathematica's FindRoot function, which is suitable for the purpose.

Nevertheless, one should always keep in mind that although failure to produce a satisfactory solution to the equations might indeed indicate a kinetic order substantially different from that assumed, or that the reaction follows the Weibullian model, for example (Peleg 2017), a more plausible explanation could be that there is a substantial error in at least one of the experimental concentrations. Therefore, one cannot overemphasize the need to determine at least four endpoints, when n is unknown a priori for the method to render reliable parameter values. Even with the additional experimental heat treatment or treatments, and the need to determine their final concentration ratio or ratios experimentally, the endpoints method still has a clear logistic advantage over the traditional methods based on a set of isothermal degradation curves, especially at when determined at high temperatures and having four or more points each.

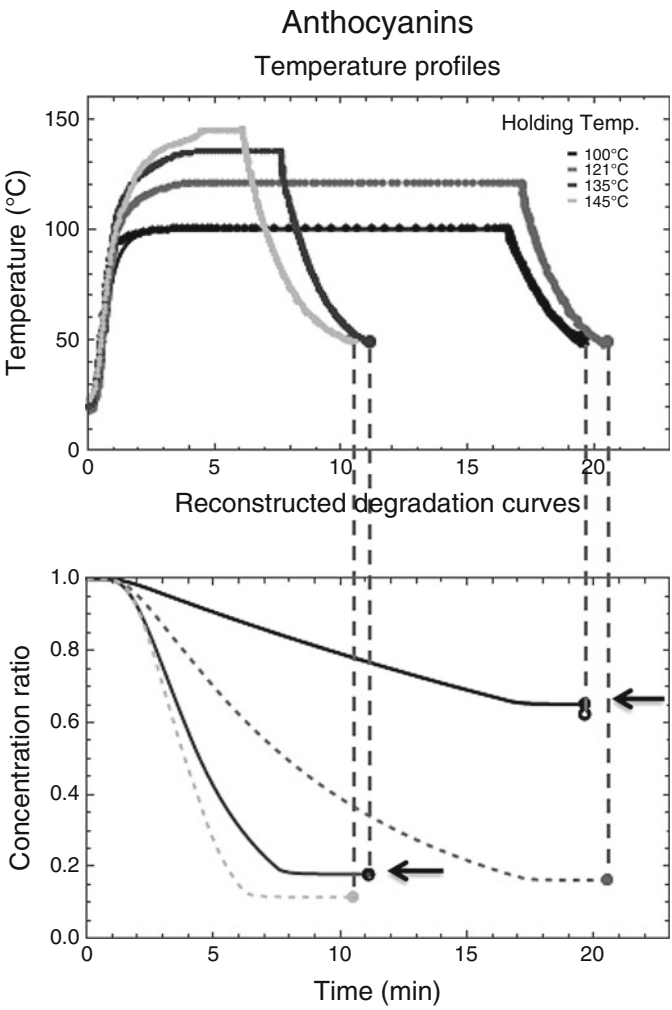

Fig. 16.6 The endpoints method applied to predict anthocyanins loss during thermal processing at sterilization temperatures. Top: The temperature profiles (gray and black). Bottom: The corresponding reconstructed concentration curves using Eq. 16.15 as a model (solid and dashed, respectively) on which the endpoints lie. Solid black circles – the predicted concentrations, empty black circles their reported values. (Adapted from Peleg et al. 2015. The original experimental data are from Sui and Zhou 2014)

16.5.3 Potential Application of the Endpoints Method to the Synthesis of Undesirable Compounds

From a purely formalistic viewpoint, the only differences between Eq. 16.15 and Eq. 16.13 are in the sign before the equation's right side, the boundary condition, and the parameters' magnitudes. Thus, at least in principle, all the arguments in favor of

the endpoints method for degradation reactions are equally appropriate for synthesis reactions (Peleg et al. 2016). But while the endpoints method's applicability to degradation reactions in foods at heat preservation (and storage) temperatures has been demonstrated with actual experimental and published data, to the best of the author's knowledge there are no parallel examples of its applicability to synthesis reactions in foods that occur at thermal processing temperatures. The closest reported application of the method has been to volatiles formation (TMA and TVBN) in stored fresh fish (Peleg 2016). Although chemically determined, they are actually used as markers of microbial activity and growth. Since noxious volatiles formation in fresh fish occurs at refrigeration or (at the most) ambient temperatures, and since their time scale is of hours or even days, it is difficult to see the relevance of its kinetics to heat preservation of foods. Therefore, what follows will only describe the endpoints method's *potential applicability* to the kinetics of synthesis reactions during thermal processing of foods. A modified program's layout based on synthesis instead of degradation kinetics is shown in Figs. 16.4 and 16.5. The two differ in that the parameters' ranges, especially those of the concentrations, have been adjusted to accommodate the *rising* synthesized compound's concentration (in contrast with the *falling* concentration in degradation reactions). Also, a new slider has been added to account for that the compound of interest, e.g., a Maillard reaction's product, might have been already present in the food prior to its thermal processing, in which case, the rate equation's boundary condition is $C(0) = C_0 > 0$. Two examples of the modified program's screen displays are given in Figs. 16.7 and 16.8. The first shows two temperature profiles where the higher peak temperature results in a higher concentration overall, and the second, where a considerably longer process at the lower processing temperature results in a higher concentration of the synthesized unwanted compound.

As with microbial inactivation and chemical degradation, the synthesis model and calculation method ought to be validated by comparing predicted endpoint concentrations after heat treatments that have not been used in the kinetic parameters calculation with the actual experimental ones. And here again, once the method has been validated in this manner, the calculated k_{Tref} and c parameters can be averaged to increase their reliability and accuracy. Also, if the reaction's order n is not known a priori and cannot be safely assumed, then a minimum of three endpoints would be needed to extract all three kinetic parameters simultaneously and a fourth one for the model and method validation.

16.6 Concluding Remarks and Future Trends

The endpoints method was originally developed for high and ultra high temperature processes where obtaining isothermal data is not a feasible option. But it also offers an economic way to estimate kinetics parameters at lower temperatures including from isothermal data on microbial inactivation, chemical degradation, and possibly the synthesis of undesirable or toxic compounds in processed foods. For the method

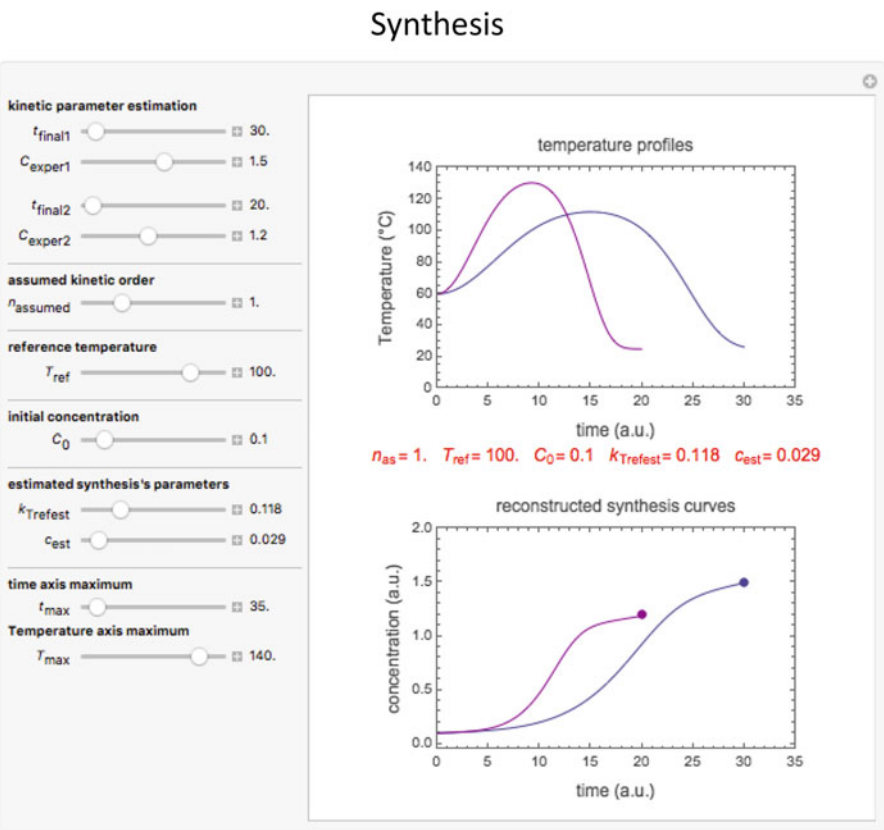

Fig. 16.7 The two endpoints method's synthesis version of the program showing matched reconstructed curves generated with Eq. 19.16 as a model. The k_{Trefest} and c_{est} sliders' positions are the sought values of the k_{Tref} and c parameters. Notice the relative levels of the final concentrations at the end of the shown processes having a comparable duration

to work properly and render correct and useful kinetic parameters, the temperature profiles have to be sufficiently distinct, i.e., as far away as possible from a repeated process. Also, the experimental final survival or concentration ratios (or absolute concentration in the synthesis case) ought to be determined from as many replicates deemed practical to minimize the effect of inevitable experimental errors. Failure of the reiterations in the numerical procedure to converge or if the rendered parameters are unrealistic or absurd, can be an indication that either the selected kinetic model has been inapplicable and/or that there has been a substantial error in one or more of the entered final survival or concentration ratios (or concentration in the synthesis case). Although not impossible, errors in one or more of the temperature records are less likely to be the reason especially if the instrumentation has been properly maintained and calibrated.

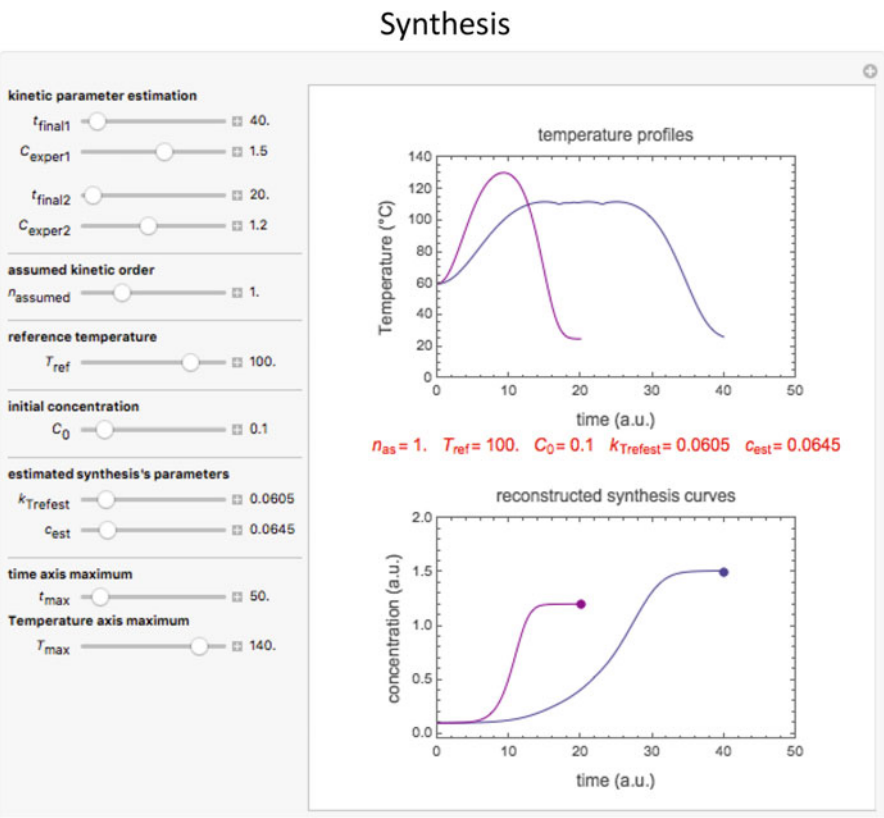

Fig. 16.8 The two endpoints method's synthesis version of the programs showing matched reconstructed curves generated with Eq. 19.16 as a model. The $k_{Trefest}$ and c_{est} sliders' positions are the sought values of the k_{Tref} and c parameters. Notice the inverted relative levels of the final concentrations as compare with Fig. 16.7 when the lower temperature heat treatment has been extended

The described software to do the kinetic calculations has been written in Mathematica® and made freely downloadable from the Internet. It includes the programs' codes, which the interested reader can use for writing his or her own version in another programming language. The method's performance is described in more details in the cited publications, which also have more examples of its successful applicability. Although microbial inactivation, degradation reactions, and synthesis are addressed separately in this chapter, they can be jointly monitored in the same set of experiments. In other words, at the end of the experimental heat treatments, the experimenter can determine not only the targeted organism or spore's survival ratio, but also the residual concentration or concentrations of a particular vitamin, pigment, or flavor marker and that of a non-enzymatic browning reaction, for example. Once the kinetic parameters have been obtained in this manner, they

can be added to the corresponding rate models and used to reconstruct the survival, degradations, and accumulation curves for the temperature histories of existing and/or contemplated thermal processes. Such curves, or even just their endpoints, can then be used for process optimization based on microbial safety, nutritional value, and/or other quality parameters determined simultaneously.

Since generating and analyzing a new survival, degradation, and/or synthesis curve only requires pasting a new logged time-temperature record, or a new $T(t)$ algebraic expression, and moving sliders on the screen, and since the calculation even for dense or elaborate temperature profiles rarely takes more than a minute and usually much less, one can examine numerous hypothetical and realistic scenarios in a very short time. In the case of vitamins degradation, such simulations can help in the decision on whether the product needs fortification prior to its heat preservation, for example. In the same manner, i.e., by just moving sliders on the screen, one can examine the potential effect of experimental errors and in the kinetic parameters themselves. Or alternatively, one can examine how variations in the kinetic parameters can affect the survival pattern and hence the process's safety or the product's nutritional quality.

This chapter has focused on the calculation method, but did not address heat transfer considerations. In well-mixed flowing liquids, or even space-foods packaged in narrow flexible pouches, the assumption of uniform, practically uniform or meaningful "representative" temperature $T(t)$ is not too far fetched, especially when judged against the inevitable scatter in microbial counts and concentration determinations. This is certainly not the case when it comes to canned meat, fish, and certain vegetables or fruits where the shortest distance to the center is on the order of two centimeters or more and a principal heat transfer mode is conduction. Although it has yet to be actually tried in the case of microbial inactivation, one can monitor the center temperature with an inserted thermocouple and after the completion of the thermal process, aseptically remove the product's around it and determine its microbial count. Repeating the process with different temperature profiles will provide the needed endpoints for the method application and the kinetic parameters. The results will refer to the product's coldest point whose temperature history determines the entire container's microbial safety.

This is not the case with nutrients loss and the accumulation of undesirable compounds, where the interest might be in their *total content* in the container or in a consumed portion of it. Here the endpoints method can only provide the sought reaction kinetic parameters in the actual food in a more economical way, and at temperatures where meaningful isothermal data are hard or impossible to obtain. But once these kinetic parameters have been obtained by the endpoints method, then at least in principle, they can be incorporated into a finite elements type program to calculate the concentration's spatial and temporal distributions based on heat transfer considerations and the container's geometry.

Acknowledgements The endpoints method for microbial inactivation was developed with support from the Massachusetts Agricultural Experiment Station at Amherst. Its adaptation to nutrients degradation in thermally processed and stored foods has been supported by NASA under Grant No. NNX14AP32G.

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Part V
Conventional and Novel Preventive Controls for Food Safety

Chapter 17
Conventional and Advanced Thermal Processing Technologies for Enhancing Food Safety

Jiby K. Kurian and G. S. Vijaya Raghavan

17.1 Introduction

Every year billions of people are at risk, and millions of people fall ill, and many die because of consuming unsafe food (WHO 2015). Though the detailed data on the economic cost of food-borne illnesses around the world is largely missing, the annual cost of food-borne illnesses in the United States alone is about $55.5 billion, estimated though a conservative economic approach (Scharff 2015). Thus, food-borne illnesses cause not only morbidity and mortality but also a significant impediment to socio-economic developments worldwide. Likewise, severe food-borne illnesses can cause reduced life expectancy and disabilities that affect the quality of life for the affected people (WHO 2015). The severity of risks associated with food contamination shows that food safety requires great concern in food production and processing. Food safety involves considering important factors such as microbial hazards, food chemistry, toxicology, processing capacity, process reactions, product and package interactions, and product stability over time. In addition to sufficient availability of foods, consumers expect the food to be produced in a sanitary manner and is safe to eat (Schoenfuss and Lillemo 2014). Thus, consumers require primarily safe foods in each mouthful consumed that are minimally processed and have freshness (Tapia et al. 2004). The government food regulatory bodies around the world are striving hard to ensure maximum food safety for its populace. Pasteurization and sterilization are the processes generally applied to destroy or inactivate microorganisms in foods to enhance food safety and storage life (Chandrasekaran et al. 2013).

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In addition to the microbial safety, food also needs to be safe from allergenic components. About 6–8% of children and 2–3% of adults around the world have food allergies, and allergic reactions to foods can be life-threatening (Umasunthar et al. 2013). Therefore, technologies are required to reduce the level of allergens in foods so as to ensure food safety for all.

Foods that are intended for long-term use must be processed using thermal or non-thermal technologies, or a combination of them, and safely stored. The objective of food processing is to produce a safe and nutritious product with acceptable quality attributes for the consumers. Since the thermal destruction of pathogens is the most effective method to ensure food safety (Dev et al. 2012), thermal treatment is one of the most traditionally and commonly applied methods for pasteurization and sterilization of foods. Thermal treatments not only destroy microorganisms and enzymes to prolong the shelf life of food, but also create acceptable taste, aroma, and appearance of food products (Kumar and Sandeep 2014).

For heat treatments, convection, conduction, and radiation are the three fundamental modes of heat transfer. Heating systems such as direct steam injection or steam infusion, retorts, heat exchangers, dielectric heating, and the combination of these systems are generally applied for blanching, pasteurization, hot filling, drying, evaporation, and sterilization of foods (Kumar and Sandeep 2014; Pereira and Vicente 2010). Thermal treatment of liquid foods is highly effective with conventional and advanced methods. However, thermal treatment of solid foods with low moisture content is challenging with both conventional and advanced methods due to increased resistance of microorganisms at lower water activity. Similarly, pasteurization and sterilization of packaged-foods using conventional methods are highly inefficient because heat has to transfer from the food surface to the interior of the foods. Also, the nutritional and sensory qualities of foods exposed to high-temperature treatment are significantly reduced. Currently, the demand for processed foods that retain their fresh taste and quality is increasing worldwide. Therefore, researchers and people in the food industry are working towards the development of advanced technologies for the processing of food (Neetoo and Chen 2014).

Investigators are developing new methods to improve the heat and mass transfer that involve conduction and convection mechanisms. Thermal processes which include the use of microwaves and radiofrequency, ohmic heating, and infrared irradiation are among the advanced technologies currently available for food processing. Compared to conventional technologies, the advanced technologies have reduced environmental footprint with high energy efficiency, reduced water consumption, and reduced emissions of greenhouse gases (Pereira and Vicente 2010). The increasing attention on the food safety and the efforts to develop new processing technologies to ensure food safety is evident from the increase in the number of research (journal articles, book chapters, conference paper, and review articles) articles published on these topics in recent years. Bibliometric analyses show that investigations on ‘food safety’ and ‘advanced thermal treatments’ have received increasing attention since the 1990s (Elsevier 2019). The trend in the number of research documents published every year since 2003 in the English language on the various aspects of “food safety” and the use of advanced thermal

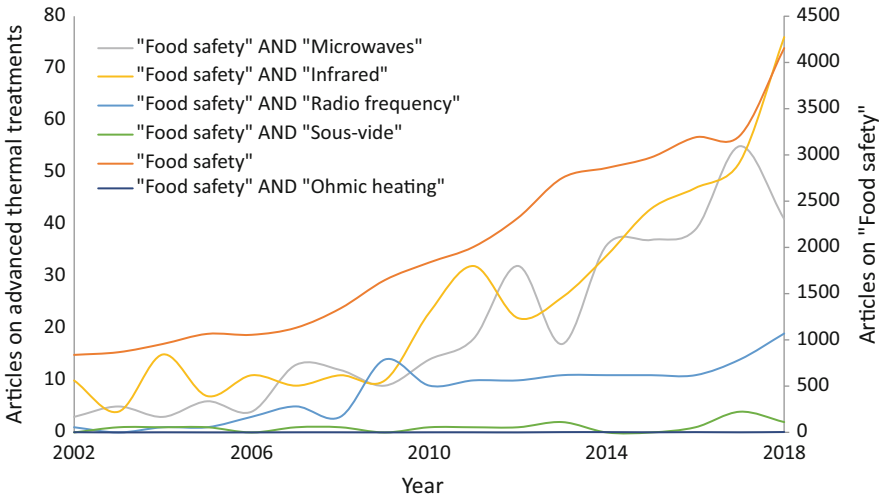

Fig. 17.1 Number of research articles published in English language on "Food Safety" and advanced thermal treatments during 2003–2018 (Elsevier 2019)

treatments to enhance food safety, is shown in Fig. 17.1. The research and development in the advanced thermal processing technologies for ensuring food safety are discussed in this chapter.

17.2 Enhancement of Heat Transfer in Conventional Heating Methods

Conventional technologies for the thermal processing of foods include retorting and heat exchangers. In these technologies, heat is transferred to the surface of food materials through conduction, convection and radiation mechanisms. Retorting technology requires the use of large amount of water and the heat transfer is inefficient in these methods (May 2001; Emond 2001). Therefore, investigations are being carried out to enhance the heat transfer in conventional heating methods. Particle to particle heat transfer is an advanced method of heat transfer involving the conduction and convection mechanisms. This method has great potential and is particularly useful for heat treatment of granular food materials such as grains and seeds (Sotocinal et al. 1997a). Because air has been used as a medium for heat transfer into the granular foods, the heat transfer is inefficient, especially when the hot-air becomes saturated with moisture from humid granular foods such as shelled corn (Sibley and Raghavan 1985). The use of the solid granular inert medium such as sand or salt for heat transfer can enhance the efficiency and the rate of food processing (Raghavan and Harper 1974). The use of molecular sieves has also been investigated for the enhanced heat transfer in the drying of grains such as

corn. Molecular sieves are materials with selective adsorption properties that can separate components of a mixture based on molecular size and shape differences. Examples of molecular sieves include zeolites and silicates (Szostak 1989). Studies have shown that the molecular sieves are better than salts for the drying of grains (Raghavan et al. 1988).

Several designs of particle-particle heat transfer have been developed by researchers (Sotocinal et al. 1997b) and the thermodynamics and the associated heat transfer mechanisms have been extensively studied (Raghavan and Pannu 1986; Raghavan et al. 1974). A combination of air and solid granular inert medium with continuous agitation and mixing of the media can further enhance the heat transfer to food materials. The agitation and mixing of media with the food material can be achieved through the rotation of the container or fluidization of the mixture. The media and food can be separated at the end of the operation and the media returned to the beginning of the process in a batch or continuous mode. This will ensure that the food is not contaminated with the heat transfer media. Complete immersion of food in the heat transfer media provide uniform heating of food and significantly enhances the rate of heat transfer that results in faster processing of food (Richard and Raghavan 1980).

This technique discovered and adapted decades ago is very useful at the current time for better efficiency in the processing steps and the use of this approach can contribute to reducing greenhouse gas emissions into the atmosphere.

Recently, solid nanoparticles such as Al_2O_3, Cu, CuO, SiO, TiO, etc. are being investigated for enhanced heat transfer in convective thermal processes. Incorporation of nanoparticles can significantly enhance the heat transfer capabilities of conventional heat transfer fluids such as oil and water. Further investigations in this area are required to understand the mechanisms of the movements and the behavior of the nanoparticles in the heating process (Kakaç and Pramuanjaroenkij 2009).

17.3 Dielectric Heating Technologies Using Radio Frequency (RF) and Microwaves (MW)

Conventional heating methods take a longer time to inactivate the pathogens at the cold spot(s) of a food product. The non-uniform heating and the long processing time result in undesirable changes in foods especially in solid foods with lower moisture content (<50%, wet basis). Dielectric heating is much faster than the conventional heating, and the heat is generated within the body of material being heated. The heat generation can be controlled more quickly in dielectric heating than in the conventional heating methods. The shorter treatment time and lower temperature can minimize the degradation of nutrients and desirable quality attributes of the food. Current demand for packaged foods that can ensure safety by reducing the post-processing handling of food, increases the need for advanced technologies that can process packaged foods. Processing of packaged foods requires volumetric

heating for increasing the internal temperature of foods to the required levels. However, the lack of suitable packaging materials makes the application of dielectric heating systems less successful for the processing of certain packed foods (Dev et al. 2012). These advantages and challenges have prompted significant research and development efforts in the dielectric heating technologies (Dev et al. 2012).

In general, the inactivation of microorganisms present in food occurs mainly due to the thermal effects of the dielectric heating treatments. Thermal treatments irreversibly denature enzymes, proteins, and nucleic acids that are essential for vegetative life and multiplication of microorganisms (Cebrián et al. 2017). If the processing is carried out in closed containers or packages, the steam generated inside will add to the lethality of the process and results in increased mortality of microorganisms (Dev et al. 2012).

Destruction or inactivation of microorganisms and enzymes by dielectric heating was explained by theories like selective heating, electroporation, cell membrane rupture, and magnetic field coupling (Kozempel et al. 1998). The selective heating mechanism involves the heating of microorganisms to a higher temperature than that of the foods and the surrounding medium. The electroporation mechanism involves the generation of high electrical potential across the cell membrane that causes the formation of pores and subsequent leakage of cellular materials. Cell membrane rupture occurs due to the high voltage and charges applied across the cell membrane. In magnetic field coupling, the electromagnetic energy will be coupled with the critical cell components such as DNA and proteins to disrupt the internal components of the cells (Kozempel et al. 1998; Chandrasekaran et al. 2013).

17.4 Microwave-Heating of Foods

Microwaves (MW) are electromagnetic radiations with a frequency range of 300 MHz to 300 GHz. However, domestic microwave appliances operate at 2.45 GHz frequency while industrial microwave systems operate at 915 MHz also. The penetration depth of 2.45 GHz microwaves is about 12 mm and that of 915 MHz microwaves is about 32 mm and it varies with the temperature of food. This limited penetration depth of microwaves causes heterogeneous heat distribution in foods, especially in the industrial applications (Herve et al. 1998).

The interaction of microwaves with dielectric materials results in the transformation of electrical energy into thermal energy within the dielectric materials. Microwave heating occurs through the dipolar rotation and ionic movement of molecules in foods. The dipolar molecules such as water rotates a million times per second as an effort to align with the oscillating electric field of microwaves. Similarly, ionic molecules present in foods do oscillatory migration due to the electromagnetic field of microwaves. These mechanisms cause the internal friction of molecules and thus heating of foods. In microwave heating, moisture content within the food may evaporate *in situ* and diffuses to the surface as vapor. When the temperature of the food reaches the boiling point of the solution in it, a positive pressure quickly

develops within the food that forces the vapor and liquid to the surface. The efficiency of heat generation and rate of processing are dependent on such factors as dimensions (size and shape), composition (moisture, minerals, lipids, etc.), dielectric properties of foods, phase (liquid, solid) of food components, agitation of foods, available microwave power, and processing time. The dielectric properties of foods are influenced by temperature, moisture content, and the concentration of components such as salt and sugar. These properties change substantially during the heating of foods. In addition to dielectric properties, heat and mass transfer properties, microstructure, heat capacity, and heat of vaporization of food are also affected, and they significantly influence the outcome of the microwave heating process. These complex changes make it difficult to predict the outcome of the microwave heating (Scaman et al. 2014; Raaholt et al. 2014). In general, the knowledge of the dielectric properties of the materials involved can be used to obtain the appropriate conditions for microwave heating and desired lethality of the process (Chandrasekaran et al. 2013).

The microwave oven has become a common household appliance and large-scale microwave units have been increasingly used in the food processing industry as well. Microwave energy can be used for pasteurization, sterilization, tempering, dehydration, blanching, baking, coagulation, coating, gelatinization, puffing, roasting, and cooking of foods. Industrial processing of meats, fish, potatoes, and acidified vegetables are also done with the use of microwaves. Microwave heating results in no or minimally change in sensory qualities of the foods. Also, many studies have shown that MW processed foods have a better retention of nutrients than that in foods processed with conventional thermal processes.

Examples for industrial application of microwave heating include the pasteurization of pouch-packed meals and yogurts. Also, the pasteurization of packaged bread, cakes, and confectionary has been achieved using microwaves. Pasteurization using microwaves can be applied when the use of chemicals is not permitted for the inhibition of molds or when the application of chemicals significantly affects the volume and aroma of the products. Similarly, sterilization of packaged and pre-cooked foods has been achieved with the use of microwaves. Microwave pasteurization and sterilization of fluids and semi-fluids have been developed for industrial applications. Continuous processing is achieved by heating the pumpable foods during transportation through the tube. Foods such as milk, soups, sauces, and purees have been pasteurized or sterilized using microwaves (Raaholt et al. 2014).

Microwave thawing of frozen foods such as meat, fish, and butter has been applied in food industries worldwide to reduce the space and time required and control the growth of microorganisms in foods. By using microwaves, especially at 915 MHz frequency, the space requirement can be reduced by six times and the time required can be reduced to minutes or hours that can prevent the growth of microorganisms (Raaholt et al. 2014).

Drying is the most energy-intensive operation in the food industry, and the efficiency of drying is largely dependent on the effective transfer of energy into the foods for in-depth heat generation and moisture transfer. Microwave drying technology is efficient in providing in-depth heating that can lead to increased drying

rates, shorter drying time, and removal of pathogens. It can reduce the space required for the processing of foods and improve the quality of food products. Foods dried with microwaves have less shrinkage, better color and rehydration properties, and high nutritional qualities than products dried using conventional methods (Raaholt et al. 2014). Additionally, the dehydration of partly-dried and porous solid materials is very challenging in conventional methods, and microwave heating is highly advantageous in the drying of these materials. Microwave heating has been used in the commercial drying of sugar cubes, potato slices, pasta, and vegetables. It has also been used in puffing of snacks, baking of half-baked thin foods such as biscuits, and drying of grains (Raaholt et al. 2014).

Microwaves have been used for pre-cooking of poultry, meat patties, and bacon to improve yield, improve product appearance, reduce nitrosamine formation, improve product stability, and increase the quality of rendered fat. Baking of foods using microwaves requires less time and space, and the color and structure of the products are more uniform than in the case of conventional baking. Microwave frying of doughnuts has led to shorter frying time and lower fat uptake than conventional frying methods (Raaholt et al. 2014).

Similarly, high-temperature-short-time sterilization using MW produces foods that are superior in quality than the foods produced through conventional sterilization processes (Add reference). The decontamination of powdered black pepper at different moisture levels was achieved using MW in continuous and intermittent mode applications and about 90% reduction in microbial load was achieved with 82% of volatile compounds retained in the process. Investigations have shown that MW-treated spices such as black pepper, oregano, red chili, rosemary, and sage have microbial loads within the limits set by the International Commission on Microbiological Specifications for foods (****). . Also, the control of *Aspergillus parasiticus* which produces aflatoxin in hazelnuts was achieved by MW heating. The MW processing did not affect the sensory qualities of in-shell hazelnuts. Similar results were observed in the processing of walnuts and almonds (Dev et al. 2012).

Microwaves are increasingly used in the post-harvest processing of agricultural products. Stored cashew kernels infested with adult *Tribolium castaneum* were treated with microwaves for the removal of pests. More than 90% of the pests were killed after exposure to 80 °C for 180 minutes (McBratney et al. 2000). Similarly, barley seeds infected with the loose smut pathogen *Ustilago nuda* were treated with microwaves for the inactivation of the pathogen. The treatments were effective for the inactivation of the pathogen without significantly affecting the germination of the seeds (Stephenson et al. 1996). Researchers have used microwaves for the eradication of seed-borne pathogen *Diaporthe phaseolorum* in soybean seeds, and the microwave treatment did not significantly affect the viability of seed and the vigor of seedlings (Reddy et al. 1995). Also, the effect of microwaves on the degree of inactivation of *Fusarium graminearum* on wheat seeds, seed germination, and seedling vigor was investigated. The results have shown that the pathogen eradication increased with the increase in microwave power applied, but the seed viability and seedling vigor were adversely affected (Reddy et al. 1998).

Researchers have investigated the continuous pasteurization of water, milk, and cream using microwaves and found that milk was heated more rapidly than water because of the protein contents in milk. Among the components of milk, proteins heat up faster than fat and lactose (Kudra et al. 1991). Most of the commercial applications of MW sterilization are for the processing of liquid foods such as milk and juices and are applied by only a few industries. The non-uniform heating of solid foods and the lack of reliable methods to ensure the achievement of food safety standards results in slow adaptation of MW- sterilization process by the industries. Excessive heating of the corners and edge of foods occurs due to the localized concentration of the MW field in these areas. Use of 915 MHz, instead of 2450 MHz, for sterilization can result in a more uniform heating of foods due to the deeper penetration of MW into the foods. Researchers at Washington State University have been developing MW system for continuous sterilization of solid and semisolid foods using 915 MHz frequency (Tang et al. 2008). Among the applications of 915 MHz, MW sterilization of vacuum-packaged sliced beef in gravy and whey protein samples was investigated. The studies have shown that the 915 MHz single-mode system can be used for the sterilization of heterogenous foods such as fish in gravy in pouches and chicken meat in gravy in trays (Tang et al. 2008; Dev et al. 2012).

Development of commercial MW systems for continuous sterilization of pumpable foods such as vegetable purees have been investigated. Patents were issued for cylindrical applicator MW systems operating at 915 MHz. Similarly, pasteurization of in-shell eggs using 2450 MHz frequency with rotation of eggs, was investigated to achieve the different levels of pasteurization temperature required for the egg yolk (61.1 °C) and egg white (57.5 °C), as well as to overcome the challenges of pressure build-up and explosion of in-shell eggs during their processing with MW (Dev et al. 2012).

Microwave heating has also been used for the inactivation of allergens and protein inhibitors in foods such as soybeans (Vagadia et al. 2018). Investigations have shown that allergens in fruits, vegetables, and nuts can be inactivated by using microwaves. For example, allergens in celery, kiwi fruits, hazelnuts, cashew nuts, walnuts, and almonds were inactivated by treatment with microwaves (Vanga et al. 2017). The reactivity of potentially allergenic wheat gliadin was significantly increased after exposure to microwaves at 40 kJ. However, exposure to higher intensity of microwaves (80 kJ and 150 kJ) did not increase the reactivity of allergens. Therefore, further investigations are needed to develop optimized processing methods to reduce the allergenicity of foods. Altering the conformation of allergens through thermal and nonthermal processing can be explored to reduce the food allergy (Shriver and Yang 2011).

Many applications of microwaves have been claimed successful in laboratory and pilot scales, and the number of commercial implementations of such successful applications is slowly increasing. Microwave application is highly used for the sterilization of food packaging materials such as glass, plastic, and paper (Fito et al. 2004). However, some of the industrial applications of microwave heating were not successful due to the lack of conformity with the needs and specifications of

the food processing plants. One of the main limitations of microwave processing for the sterilization of packed foods is the strict requirement of the absence of metallic content in foods. The non-uniform heating adversely affects the level of microbial inactivation (Fito et al. 2004). Moreover, the microwave equipment manufacturers have to design individual systems according to the requirements of industries that process different food materials. Also, the personnel in the food industry must be trained in handling the microwave equipment. However, the recent advances in modeling and simulation capabilities help in scaling up of successful applications of microwave processing methods. (Raaholt et al. 2014).

17.5 Radiofrequency Heating of Foods

The radiofrequency (RF) heating technology uses the electromagnetic radiation in the frequency range of 300 kHz to 300 MHz. The RF heating systems consist of the RF generator and electrodes. The RF generator creates an alternating electric field between the electrodes where food is placed for processing. The electric field alternates millions of times per second, which causes the dielectric molecules in the food to alternate in orientation while trying to align themselves with the alternating electric field. The rotation of molecules and the corresponding friction between the molecules and the space charge displacement cause heat generation within the food. The factors influencing the heat generation include the frequency and voltage applied, and the dielectric loss factor and dimensions of the food product (Tang et al. 2004).

RF can be applied to process large quantities of food products with high ionic conductivity. However, depending on the food characteristics and volume, each RF processing system requires specific design and tuning. To avoid interference with the telecommunications, only certain bands of radio frequency are legally allowed for industrial and scientific food processing applications. For example, 13.56 MHz, 27.12 MHz, and 40.68 MHz are allowed in North America for industrial RF processing of food. These frequencies have corresponding central wavelengths of 22 m, 11 m, and 7.3 m, respectively (Orsat and Raghavan 2014).

Early developments in RF pasteurization and sterilization were affected by the difficulty in measuring the temperature and pressure in electromagnetic fields. The development of fiber optic sensors for online measurement of temperature and pressure, infrared thermal imaging systems, dielectric properties measurement systems, chemical marker techniques, and computer simulation of electromagnetic fields have enabled the rapid development of RF technologies for the pasteurization and sterilization of foods (Tang et al. 2004).

Radiofrequency has been applied in the food processing industry for a long time for blanching, thawing, drying, baking, pasteurization, and sterilization. RF baking and post-baking processing of biscuits, crackers, and snack foods are widely used. Drying of grains and moisture leveling in finished products are also done with the help of RF. Moisture leveling is achieved in finished products because RF will

produce more heat in wet regions than in the drier regions of the foods. This moisture leveling helps in improving the quality and consistency of the finished products (Neetoo and Chen 2014).

In one of the earliest investigations on the use of RF as a germicidal agent, the destructive effect of RF on *E. coli* was demonstrated and the use of electrolytes was suggested for enhanced bactericidal effects (Fleming 1944). The destruction of microbial cells is possible if heat is generated much faster in the cells than in the surrounding medium. Since most of the microbial cells bear a negative charge, the cells can be oscillating rapidly in an alternating electric field, and when the elastic limits of the cell structure exceeded, the cells will be ruptured to cause the death of the microorganisms (Orsat and Raghavan 2014).

Investigations have shown that the RF heating of apples and cherries kills pests but does not affect the sensory qualities of the fruits. Similarly, oranges treated for the control of Mediterranean fruit flies and persimmons fruits treated for the control of Mexican fruit fly larvae have shown no significant changes in qualities such as firmness, weight, total soluble solids, acidity, and volatiles. Vacuum-packed carrots treated with RF had higher qualities and extended shelf life than the carrots treated with conventional methods such as dipping in chlorinated or hot water (Orsat et al. 2001). The control of contamination by pathogenic bacteria such as *Salmonella, Escherichia coli*, and *Listeria monocytogenes* on alfalfa seeds, without affecting their germination, was achieved by using short-term RF treatments. Almonds were treated with RF to reduce contamination with *Salmonella* Enteritidis without affecting the nutritional qualities (Marra et al. 2015; Tang et al. 2004).

Pasteurization of sliced bread loaves using RF was reported by Cathcart et al. as early as 1947. RF pasteurization prevented mold growth and staling in bread loaves stored for 10 days at room temperature. It controls the growth of *Aspergillus* and *Penicillium* in bread loaves (Tang et al. 2004). Pasteurization of meat emulsion samples using RF can reduce the processing time by 79% when compared to steam pasteurization (Tang et al. 2004). RF heating of comminuted meats for the reduction of vegetative cells and spores of *Bacillus cereus* and *Clostridium perfringens* was also found as effective (Marra et al. 2015). RF heating was investigated to reduce microbial counts on fresh meats and chum salmon (*Oncorhynchus keta*) eggs. RF heating has also been used in control of *Clostridium botulinum* spores in foods. However, in some cases, the non-uniform heating of the surfaces of foods leads to an insufficient reduction in microbial counts. Increasing the salt content and the highest processing-temperature of the foods can increase the inactivation of microorganisms in processed foods (Tang et al. 2004; Orsat and Raghavan 2014).

Compared to conventional methods, RF treatment can produce juices (e. g., orange, peach, and quince juices) with better bacteriological and sensory qualities (Demeczky 1974). Studies have shown that RF heating can be successfully used for the inactivation of *Escherichia coli* and *Listeria* in milk under continuous laminar flow conditions (Awuah et al. 2005). Similarly, RF heating was used for sterilizing 6-pound trays of macaroni and cheese, and the treatment effect was compared with the conventional 90 minutes long retort process. RF treatment achieved the target

sterilization within 30 minutes and it did not induce significant changes in color and flavor (Wang et al. 2003; Marra et al. 2015).

Mathematical modeling and computer simulation have been used for rapid development of RF systems for food processing. Finite element modeling (FEM) of RF pasteurization of in-shell eggs was carried out to determine the hot and cold spots generated in the material when the eggs were under different strengths of the electric field and in different orientations. The simulation results have shown that the in-shell eggs should be rotated inside the RF field to obtain uniform heating and proper pasteurization (Dev et al. 2012). Additionally, the continuous processing of foods with RF requires the adaptability of the system for different geometry of the foods. The change in geometry of the foods changes the RF coupling power. Therefore, continuous monitoring of the geometry of foods and the corresponding adaptation and moving of RF electrodes is required for continuous RF-based processing of foods (Dev et al. 2012). Computer-aided development of RF systems improved the heating uniformity and coupling of power into the applicators. Commercial and in-house developed software programs are available for modeling and simulation purposes. The correlation between simulated and experimental data is still a challenge because of the complexity of the underlying process and product behavior (Tang et al. 2004).

In addition to the application of RF to process high moisture containing foods, researchers have been investigating the control of pathogens in foods with low moisture content. RF pasteurization of almonds and peanut butter cracker sandwiches has been studied (Jiao et al. 2018). About 2–4 minutes of RF treatment could produce 5-log reduction of *Salmonella* in almonds (Gao et al. 2011). Similarly, about 1.5 minutes of RF treatment of creamy peanut butter resulted in log reduction of 4.29 log CFU/g for *S. Typhimurium* and 4.39 log CFU/g for *E. coli* O157:H7. Also, there was no significant effect of the RF treatment on the sensory qualities of the peanut butter and crackers (Ha et al. 2013). Treatment of wheat flour with RF resulted in 5–7 log reduction in *Salmonella* after 8.5–9 minutes of treatment (Villa-Rojas et al. 2017; Jiao et al. 2018).

17.6 Ohmic Heating of Foods

Ohmic heating is also known as Joule heating, electrical resistance heating, electroconductive heating, and electro-heating. This process involves the passing of low-frequency (50–60 Hz) or high-frequency (up to 25–30 kHz) alternating electric currents through foods to generate heat. The application of low-frequency electricity requires the use of specially designed graphite electrodes to avoid electrolysis and metal dissolution into food. Stainless steel electrodes can be used if high-frequency electricity is used for Ohmic heating (Chen 2015). Ohmic heating was practiced in the 1930s for milk pasteurization, but due to the high cost of the process and the requirement of suitable inert electrode materials, Ohmic heating was not highly pursued until 1980s. Developments in areas such as power electronics and the

availability of low-cost ohmic heaters have greatly advanced the improvement and refinement of ohmic heating technology. These developments have helped in the control of electrolytic reactions taking place at the electrodes during ohmic heating (Sastry 2004). Currently, it has been investigated for blanching, pre-heating, sterilization, and thawing of food materials. High-frequency power systems and online process control technology helps to incorporate ohmic heating into high-temperature short-time (HTST) processing of liquids and liquid foods containing particulates. Rapid heating, high-quality products, less fouling, and greater energy efficiency are some of the benefits of ohmic heating technology (Chen 2015).

Ohmic heating can be applied for the continuous processing of viscous foods and foods containing particulates. Large heating tubes with lower shear rates are used for the heating of fragile particles. The main parameter that influences the rate of ohmic heating is the electrical conductivity of foods which depends on temperature, ionic constituents of foods, the structure of foods, and electric field strength. Addition of salts and liquids increases the ionic concentration and thereby the electrical conductivity of solid foods. Thus, the foods can be soaked in saline solutions before ohmic heating for rapid processing (Goullieux and Pain 2014).

Ohmic heating has been applied to a wide variety of foods such as juices, sauces, meats, soups, liquid egg products, fruits, vegetables, and seafood. It can be used to sterilize foods and to produce high-quality shelf-stable processed foods. Foods processed with ohmic heating retained their texture and had high nutrient content, color, and flavor than the traditionally processed foods (Neetoo and Chen 2014). However, ohmic heating is suitable only for materials containing ions and is not suitable for food materials like oils. The difference in electric property between ingredients makes the control of the process very difficult. Also, the direct temperature measurement of the multiphase particles in a food product is challenging (Chen 2015).

Several investigations were carried out on the lethality of ohmic heating for microorganisms. The heat generated within food is largely responsible for the inactivation of microorganisms. Since ohmic heating is faster than conventional heating to increase the food temperature, foods can be sterilized in a short time with ohmic heating. The extent of microbial inactivation is dependent on the strength of electric field applied, treatment time, microorganism targeted, and food type (Goullieux and Pain 2014). Higher electric field strengths and longer treatment time results in greater reduction of microbial loads. The non-thermal effects of ohmic heating on microbial inactivation is caused by the electric current at low frequency (50- 60 Hz), which can lead to the accumulation of charges and forming pores on the cell wall of microorganisms. Investigations have found enlarged periplasmic space and uneven cell wall structure in *E. coli* cells after ohmic heating. The electroporation phenomenon was observed when yeast (such as *Saccharomyces cerevisiae*) cells were treated. The leakage of cellular contents was increased significantly with an increase in electric field strength (10–20 V/cm), and the electroporation of the cell membrane was irreversible (Goullieux and Pain 2014).

Inactivation of *E. coli* and *Bacillus subtilis* spores in saline water and orange juice was investigated with ohmic heating at a high alternating current electric field

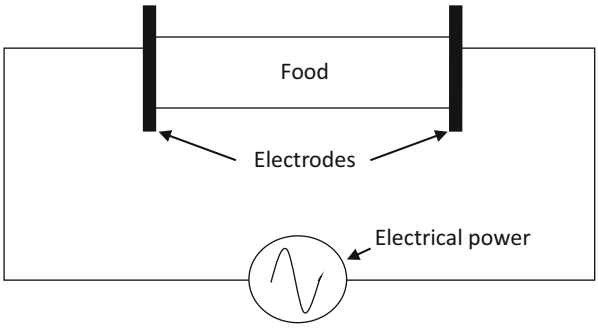

Fig. 17.2 Diagram of Ohmic heating system for food processing. (Adapted from Chen 2015)

(20 kHz, 7–17 kV/cm). About 5-log reduction of *E. coli* was observed when 0.1% saline water was heated to 74 °C using 20 kHz, 14 kV/cm electrical system (Uemura and Isobe 2002). Similarly, about 4-log reduction of *B. subtilis* spores was observed when orange juice was heated to 121 °C using 20 kHz, 16.3 kV/cm electrical system (Uemura and Isobe 2003). It has been postulated that the ohmic heating induces the leakage of ionic compounds such as calcium dipicolinic acid from the core, and denatures the enzymes on the coat, of bacterial spores. The leaked ionic compounds further increase the electrical conductivity of the spores to increase the destructive effect of ohmic heating (Goullieux and Pain 2014; Uemura and Isobe 2002; Uemura and Isobe 2003).

The nutritional quality of infant formula sterilized with ohmic heating was found as not significantly different from the infant formula sterilized with conventional methods such as ultra-high temperature for a short time (130 °C for 6 s). After the ohmic heating, the concentrations of Maillard reaction compounds such as furosine, carboxymethyl lysine, lactulosyl-lysine, fructosyl-lysine, pyrraline in infant formula were comparable with that of the infant formula sterilized with conventional methods. Also, the level of vitamin C was better preserved in infant formula processed using Ohmic heating (Roux et al. 2016). Therefore, ohmic heating can be applied to produce safe foods with high nutrients and vitamin levels (Goullieux and Pain 2014).

The configuration of the ohmic heating system (Fig. 17.2) needs to be adjusted depending on the type of foods to be processed. The flow behavior and electrical conductivities of the foods and particles need to be understood for better control of heating and to achieve homogenous temperature distributions in the foods. Modeling and simulation of the process help in designing ohmic heating systems that can provide maximum possible efficiency in sterilization and nutrient retention in foods. However, more work is needed to fully understand electrical conductivity, rheological properties, and particle sizes of food during ohmic heating. The changes in flow behaviors and electrical properties of solid-liquid mixtures, especially with large sized particles at high concentration, need to be understood for commercial applications of ohmic heating (Goullieux and Pain 2014).

17.7 Infrared Heating of Foods

Infrared (IR) is the electromagnetic radiation emitted due to the vibrational and rotational movements of molecules in a hot source. It is predominantly responsible as radiant energy for the heating effect of sunlight. The heating effect of infrared has been used traditionally for the thermal processing of foods to ensure safety and shelf life. Thus, the application of IR heat is one of the oldest methods of food processing. The IR radiation spectrum is categorized mainly into three bands, viz. near infrared (NIR) radiation with wavelength in the range of 0.75–3 μm, mid-infrared (MIR) radiation with wavelength in the range of 3–25 μm, and far-infrared (FIR) radiation with wavelength in the range of 25–1000 μm. The IR radiation band of 2.5–200 μm is generally used in the advanced methods for food processing (Das and Das 2015).

The commercially used IR heating equipment consists of a radiator which radiates IR in all directions and a reflector (waveguide) which directs the IR to a target such as food (Fig. 17.3). Foods absorb, transmit, and reflect the IR radiation falling on it. The extent of IR absorption depends on the composition and the radiation properties such as absorptivity, reflectivity, and transmissivity of foods. Water and the various organic molecules such as proteins, lipids, and starch in food absorb IR radiations at different wavelengths. Proteins absorb IR in the range of 3–4 μm and 6–9 μm, lipids absorb IR in the range of 3–4 μm, 6 μm, and 9–10 μm, and sugars absorb IR in the range of 3 μm and 7–10 μm. The molecules that absorbed IR radiation will generate heat through stretching vibrations (Das and Das 2015).

IR processing can be applied to provide a high amount of heat directly into the food within a short time, without heating the surrounding air. Compared to conventional methods, the heating efficiency of IR heating is very high and changes in the quality of the foods are minimal (Pan et al. 2014). Compared to microwave and radiofrequency technologies, IR heating can provide more uniform heating of the surface and core of foods. The depth of penetration of IR into foods is in the range of

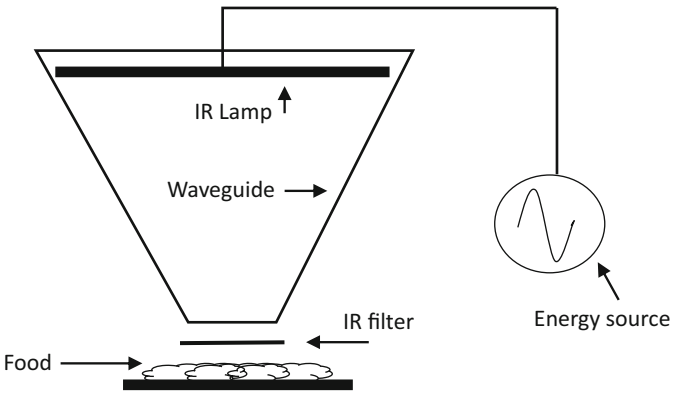

Fig. 17.3 Schematic of IR heating system for food processing. (Adapted from Jun and Irudayaraj (2003)))

1–18 mm which significantly influences the temperature and moisture level of the final products. Shorter penetrating IR significantly increases the surface temperature of foods. Generally, IR processing is considered a safer and cleaner method than many of the conventional methods (Neetoo and Chen 2014; Das and Das 2015).

IR heating has been applied for baking, drying, and cooking of foods with smooth surfaces and modest thickness. It has also been applied for the surface pasteurization of bakery products, decontamination of packaging materials, and thawing of frozen foods. The IR heating was successfully used for drying of shrimps, barley, and oysters. Similarly, it has been investigated for the cooking of in-shell eggs and bread baking. The rehydration capacity of foods dried through IR heating was found to be higher than the foods dried using microwave heating and hot air methods. As in the case of other processing methods, IR heating also requires strict process controls because exposure of foods to IR for longer duration results in discoloration of the food surfaces (Neetoo and Chen 2014).

IR heating has been used for pasteurization and sterilization of foods, and the process has high thermal efficiency and fast heating rate than the conventional heating process using steam. IR radiation is absorbed by the water molecules in microorganisms that lead to the rapid increase in cell temperature and consequent inactivation and death of all types of microorganisms. IR heating damages DNA, RNA, ribosome, cell envelope, and proteins in microorganisms. IR treatments can destroy all vegetative cells and spores of bacteria, yeast, and molds in solid and liquid foods based on the treatment conditions. The efficacy of IR sterilization and pasteurization is dependent on the IR power level applied, temperature of the food, wavelength and bandwidth of the IR emitter, size and type of foods, nature and concentration of microorganisms present in food, and moisture content of foods (Das and Das 2015; Pan et al. 2014).

The efficacy of IR heating for enhancing food safety has been investigated for pathogen inactivation, sterilization of milk, decontamination of fruit surfaces, pasteurization of nuts, and disinfestation of grains (Pan et al. 2014). Packed solid dairy products such as cottage cheese in a plastic container were pasteurized with IR at 71 °C for 5 minutes to reduce the count of yeast and molds on the surface and about 1 cm deep. It improved the shelf-life of the product by 3–4 weeks when stored at 4 °C. Investigations have shown that, at a given temperature, IR heating is more effective than conductive heat against *E. coli* and therefore, a given pasteurization target can be achieved faster with the use of IR. The IR heating method has been successfully applied to decontaminate *Bacillus subtilis* from wheat, *Rhodotorula mucilaginosa* from fig fruits, *Staphylococcus aureus* from milk, *Listeria monocytogenes* from hot dogs, *Salmonella* Enteritidis from almonds, *Aspergillus niger* and *Fusarium proliferatum* from corn meal, and *Bacillus cereus* spores from paprika powder. Among the different methods investigated for the pasteurization and decontamination of *Salmonella enterica* serovar Enteritidis, IR heating effectively decontaminated and preserved the quality of raw almond kernels (Das and Das 2015; Pan et al. 2014).

The IR processing conditions recommended for the pasteurization of raw almonds were heating of almonds to 100–120 °C and holding at 90–100 °C for

5–10 minutes. In addition to the inactivation of *Salmonella* species, these conditions provide over 5.5 log reductions of *Pediococcus* bacteria also. The IR heating methods can replace the chemical methods of using methyl bromide for disinfesting freshly harvested and stored rice. Also, these treatments reduce the moisture content which helps in the milling of rice. The recommended temperature for killing all moths in freshly harvested rice is 60 °C, and for stored rough rice it is 50 °C, for 1 minute (Pan et al. 2014).

In addition to the pasteurization of foods, IR heating was also used to decontaminate the food-contact surfaces to eliminate microorganisms and thereby improving the shelf-life of foods. For example, baking trays are sterilized using IR before the dough was put on them. Additionally, the selective heating of fungal spores was investigated by applying IR in the range of wavelengths suitable for the denaturation of proteins. Selective IR heating had a higher degree of lethality than the nonselective irradiation process. Moreover, the systems equipped with emitters that can release IR in the absorption band of water in food can be more effective in food processing. However, successful commercial applications of IR heating are still a challenge due to the small cost recovery, productivity issues, and nonuniform quality of IR-processed products (Das and Das 2015).

17.8 Sous-Vide Processing of Foods

Sous-vide processing involves heating of vacuum-packaged food under low-temperatures (65–95 °C) for 7–8 hours and storing the processed food in the refrigerator at (0–3 °C). This technology is particularly applied to process meat, fish, ready-to-eat meals, etc., in the food service industry (Park et al. 2014). It is considered as one of the delicate and healthy methods of food processing. Most of the nutritional contents and flavors of the food are preserved in this processing method through the control of heat, oxygen level, and moisture content. The reduced oxidation helps in maintaining the qualities of essential polyunsaturated fatty acids in foods. Sous-vide cooked foods have desirable organoleptic properties such as fresh-like texture, good flavors, and wholesomeness that are appealing to consumers (Neetoo and Chen 2014).

Sous-vide processing was reported to reduce the counts of pathogenic bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, and *Listeria monocytogenes* to below detectable levels on fish samples and extend the shelf-life of the processed fish to more than 45 days when stored at 2 °C. Compared to conventionally cooked fish cakes, the sous-vide cooked fish cakes had improved microbial safety and an eightfold increase in shelf-life when stored at 3 °C. Similarly, the addition of salts such as calcium lactate and sodium lactate had completely inhibited the growth of *Bacillus cereus* on sous-vide processed beef goulash samples

(Neetoo and Chen 2014). However, the low-temperature applied in sous-vide processing does not always inactivate harmful bacterial spores, and the vacuum conditions may support the survival of anaerobic bacteria such as *Clostridium botulinum* (Park et al. 2014).

Though the sous-vide processing of foods is appealing to consumers, the industry must use high-quality ingredients to start with and the environment should be properly sterilized to prevent the initial contamination of the foods. Also, the strict monitoring and control of the process temperature and time required to inactivate the many possible pathogens and the need for proper storage conditions to keep the processed foods for long-term use limit the applicability of in sous-vide processing in the food industry and home kitchens (Neetoo and Chen 2014).

17.9 Combination of Thermal Methods for Food Safety

A summary of the different thermal technologies and their applications for ensuring food safety discussed in this chapter is given in Table 17.1. No single technology is applicable to all food types and process requirements. Ensuring food safety throughout the supply chain requires effective and efficient strategies that involve combining multiple technologies to inactivate pathogens in foods and increase product stability. Though many dielectric technologies have been developed for the processing of food, the commercial implementation of these techniques is very limited. Physicochemical damages to the foods such as fresh fruits subjected to thermal treatments, nonuniform heating of solid foods, and the post-processing loss of quality of foods lead to investigations on combining different methods of food safety to compensate for the disadvantages and make use of the advantages of the individual methods (Dev et al. 2012). Some of the combined methods investigated for enhanced food safety are discussed in this section.

Radiofrequency heating combined with hot-air treatment was investigated to reduce mold growth in packaged bread loaves. Vacuum-packed ham slices were pasteurized by Orsat et al. (2004) using RF with 600 W at 27.12 MHz and found that the storability of vacuum-packed hams was improved by decreasing the bacterial load and moisture loss. The sensory qualities and product acceptance of the ham slices were not significantly changed after the RF treatments (Marra et al. 2015; Orsat et al. 2004). Advances in the IR-based thermal imaging technology have been helped in the development of RF-based systems for food quality assurance and safety assessment. Applications of thermal imaging include temperature validation, detection of the bruise and foreign bodies, and evaluation of product quality in food processing (Gowen et al. 2010).

Table 17.1 Summary of advanced thermal treatments investigated for food safety

Process	Process parameters	Advantages	Disadvantages	Examples of food safety applications
Dielectric heating (microwave and Radiofrequency)	Composition, dielectric properties, dimensions, size, and mixing and agitation of food and media Power level, and frequency of MW or RF applied Processing time	Volumetric and faster heating Quick control of heating is possible Can be applied to packed foods High retention of nutrients in food	Non-uniform heating of heterogeneous solid foods Unpredictable process outcome Non-metallic food-containers required High capital investment required	Pasteurization and sterilization of milk (*E. coli* and *Listeria*) (Awuah et al. 2005), bread (*Penicillium*) (Liu et al. 2011), scrambled egg (*Clostridium sporogenes*) (Luechapattanaporn et al. 2005), almonds (*Salmonella enteritidis*) (Gao et al. 2011), alfalfa seeds (*Salmonella, E. coli*, and *Listeria monocytogenes*) (Nelson et al. 2003). Pest removal from cashew nut (*Tribolium castaneum*) (McBratney et al. 2000), walnut (orangeworm (*Amyelois transitella*)) (Wang et al. 2007), etc.
Infrared heating	Wavelength of IR radiation Composition, size, and radiation properties of food Processing time	Uniform surface heating Processing of packed-foods High retention of nutrients in food Good for the sterilization of food contact surfaces	High capital cost required Non-uniform product quality	Decontamination of *Bacillus subtilis* from wheat (Daisuke et al. 2001), *Rhodotorula mucilaginosa* from fig fruits (Hamanaka et al. 2011), *Staphylococcus aureus* from milk (Krishnamurthy et al. 2008), *Listeria monocytogenes* from hot dogs (Huang and Sites 2008), *Enterococcus faecium* from almonds (Yang et al. 2010), *Aspergillus niger* from corn

(continued)

Table 17.1 (continued)

Process	Process parameters	Advantages	Disadvantages	Examples of food safety applications
				meal (Jun and Irudayaraj 2004), *Bacillus cereus* spores from paprika powder (Staack et al. 2008), etc.
Ohmic heating	Electrical conductivity, ionic concentration, and structure of food Electric field strength Temperature Processing time Configuration of the system	Heating of particulate foods Retention of nutrients and sensory qualities of food	Not suitable for oils Non-uniform heating of heterogeneous solid foods containing ingredients with different electrical properties	Inactivation of *E. coli in saline water* (Uemura and Isobe 2002) and *Bacillus subtilis* in orange juice (Uemura and Isobe 2003).
Sous-vide heating	Vacuum packing of food Temperature Processing time	High retention of nutrients Reduced oxidation Desirable organoleptic properties and wholesomeness of processed foods	Low temperature insufficient for inactivation of all pathogens (e.g., *Clostridium botulinum*)	Control of *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, and *Listeria monocytogenes on rainbow trout* (González-Fandos et al. 2004)

17.10 Summary and Conclusions

The application of advanced thermal technologies holds the potential for producing high-quality and safe food products. A summary of the different thermal technologies and their applications for ensuring food safety discussed in this chapter is given in Table 17.1. It can be seen that, each of the technologies available for food processing is applicable to particular food types and process requirements. Also, ensuring food safety throughout the supply chain requires effective and efficient strategies that involve combining multiple technologies to inactivate pathogens in foods and increase product stability. Despite the many advantages and successful application of advanced thermal technologies at laboratory scale to ensure food safety, the industrial applications of these technologies are limited due to the relatively high capital cost required and the nonuniform heating obtained in solid foods (Dev et al. 2012). Physicochemical damages to the foods such as fresh fruits subjected to thermal treatments, nonuniform heating of solid foods, and the post-processing loss of quality of foods lead to investigations on combining different

methods of food safety to compensate for the disadvantages and make use of the advantages of the individual methods (Dev et al. 2012).

Utilization of the advantages of each technology while minimizing and eliminating the disadvantages of them is an important engineering challenge for the researchers and people in the food industry. Advance in computer-aided modeling and simulation of the thermal pasteurization and sterilization of different food products will help in developing highly efficient technologies to meet the food safety standards in the food industry. Methods should be developed for inducing appropriate conformational changes to the allergenic components of the foods to ensure food safety for all.

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Chapter 18
Gamma Ray, Electron Beam, and X-ray Irradiation

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18.1 Introduction

Ionizing irradiation has been studied for over a century. The first patents were issued for use of ionizing radiation to kill bacteria in foods in the early twentieth century (Farkas and Mohácsi-Farkas 2011; Josephson 1983). Irradiation research and its applications on food progressed significantly after World War II. Food irradiation was one of the nuclear technologies that originated from President Dwight Eisenhower's "Atoms for Peace Program" in the 1950s (Pilat et al. 2018). Consequently, the U.S. Army began a series of experiments on various foods to establish the safety of irradiated foods including toxicological studies, testing for wholesomeness, and effectiveness of the irradiation process in preserving foods. As a result of the U.S. Army program and other research, food irradiation was considered to have the potential to preserve food for military troops in the field. In 1958, U.S. Congress extended Food and Drug Administration (FDA)'s authority to regulate food irradiation processes under the 1958 Food Additives Amendment to the Food, Drug, and Cosmetic Act (Morehouse and Komolprasert 2004). Since then, the FDA has approved food irradiation processes for many foods, including wheat, spices, meat, poultry, fruits, and vegetables (Table 18.1). Irradiated beef, pork, smoked turkey, and corned beef have been consumed by U.S. astronauts. In the U.S., research on applications of irradiation for food safety purposes have mostly been conducted by a handful of universities and federal institutions. The early food irradiation program with the U.S. Army was transferred

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Table 18.1 Foods permitted to be irradiated under FDA's regulations (21 CFR 179.26)

Type of food	Purpose	Maximum dose (kGy)
Fresh, non-heated processed pork	Control of *Trichinella spiralis*	1.0 kGy
Fresh produce	Growth and maturation inhibition	1.0 kGy
Fresh produce	Arthropod disinfection	1.0 kGy
Dry or dehydrated enzyme preparations	Microbial disinfection	10 kGy
Dry or dehydrated spices/seasonings	Microbial disinfection	30 kGy
Fresh or frozen, uncooked poultry products	Pathogen control	3.0 kGy
Frozen packaged meats (solely NASA)	Sterilization	44 kGy
Refrigerated, uncooked meat products	Pathogen control	4.5 kGy
Frozen uncooked meat products	Pathogen control	7.0 kGy
Fresh shell eggs	Control of *Salmonella*	3.0 kGy
Seeds for sprouting	Control of microbial pathogens	8.0 kGy
Fresh or frozen molluscan shellfish	Control of *Vibrio* species and other foodborne pathogens	5.5 kGy
Iceberg lettuce and spinach	Control of foodborne pathogens and extension of shelf-life	4.0 kGy
Unrefrigerated (as well as refrigerated) uncooked meat, meat byproducts, and certain meat food products	Control of foodborne pathogens and extension of shelf-life	4.5 kGy
Crustaceans	Control of foodborne pathogens and extension of shelf-life	6.0 kGy

https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=179.26

to the USDA, Agricultural Research Service (ARS), Eastern Regional Research Center (ERRC) in 1980 (Shieh et al. 1985). Research on irradiation has been conducted by ARS scientists to improve microbial safety while maintaining quality of various foods, though the research activities at ERRC has been downsized over the last few years. There have been a number of recent publications on food irradiation including many book chapters (Nam et al. 2016; Ahn et al. 2017; Pillai and Shayanfar 2017), and books (Fan and Sommers 2013; Ferreira et al. 2017). This current chapter provides an overall review of present status on various food irradiation technologies. Topics includes are types and benefits of ionizing radiation, pathogen inactivation, irradiation-induced changes in food quality, regulatory approval, labeling of irradiated foods, and consumer acceptance.

18.2 Definition and Type of Irradiation

The term radiation refers to energy in the electromagnetic spectrum (Fig. 18.1). We are surrounded by radiation, such as visible light, UV light, infrared, and radio frequency, virtually all of which are non-ionizing radiations. Gamma-ray, X-ray, and electron beam are called ionizing radiations because they are capable of producing ions (i.e. electronically charged atoms or molecules), by knocking electrons out of the normal orbits of atoms or molecules. Gamma and X-rays have much shorter wavelengths, higher frequency and higher energy than other types of radiation on the electron magnetic spectrum (Fig. 18.1). In order to produce ionization, a certain minimum energy level of radiation is needed. When electrons orbiting around the nucleus absorb energy, the atoms enter an electronically "excited" state from the ground state. If the electron absorbs sufficient energy, it leaves the atoms and becomes free from the control of the nucleus, which is called ionization. Depending on the atom, energy (ionization potential) required to free electrons from atoms is between 4 and 20 eV. X-rays and gamma rays have energy greater than the required 4–20 eV (Urbain 1986). When the amount of energy derived from the radiation is less than that needed for ionization, most of the excitation energy in molecules is converted to heat, which is the basis for microwave and radio frequency applications for food preservation. Conventionally, there are three types of ionizing radiation technologies that can be used for food applications: namely, gamma ray, X-ray, and electron beam.

Gamma ray and X-ray are photons, which are type of elementary particles traveling at the speed of light with zero mass and zero charge. Upon photons encountering matter, only part of the photon's energy is transferred to a particular atom. After interaction, the incident photons continue to pass through the absorber (food), though in a changed direction and with less than its original energy. The transferred energy is used to free an orbital electron from the atom. Gamma rays are generated from radioisotope sources. Gamma radiation sources, which are permitted for food processing are cobalt–60 with emission energy levels of 1.17 and 1.33 MeV and cesium-137 with emission energy of 0.66 MeV (Fig. 18.2). Due to its better penetrating power and availability, cobalt-60 is the main source of gamma

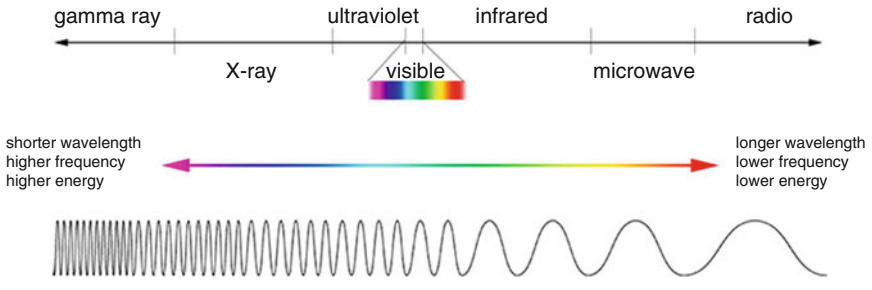

Fig. 18.1 Electromagnetic spectrum. (From: https://imagine.gsfc.nasa.gov/Images/science/EM_spectrum_compare_level1_lg.jpg)

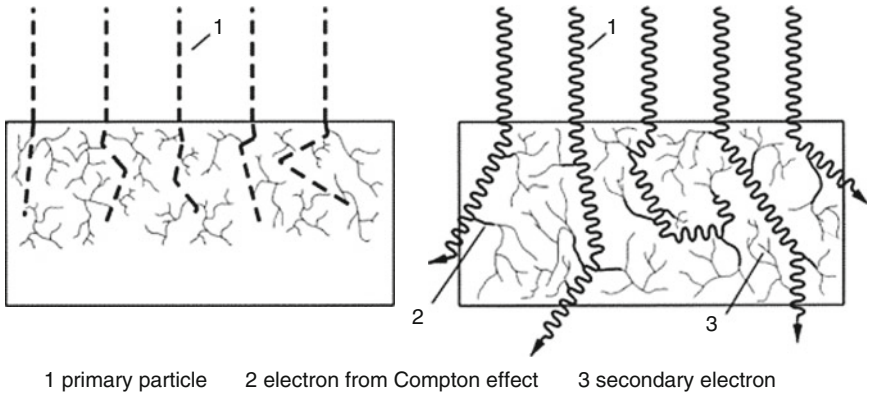

Fig. 18.2 Principal mechanisms for electron and photon interaction. (Modified from Mittendorfer 2016)

irradiation for commercial applications. Because of higher energy level, gamma rays produced from cobalt-60 have greater penetration power than that from cesium-137. However, cesium-137 has a longer half-life of 30.17 years, compared to that of cobalt-60 at only 5.27 years (Lagunas-Solar and Matthews 1985). Therefore, cobalt-60 needs to be replenished more frequently to maintain similar strength of radiation. Gamma rays (and x-rays) have considerable penetrating ability, because they have no mass and carry no charge (Hansen and Shaffer 2001; Lambert 2004).

Electron beams are high energy electrons with a maximum energy level of 10 MeV being allowed for food application by U.S. FDA. High energy electron beams are produced by a particle accelerator (Cleland 2013). A major advantage of such a system is that the machine can be turned on and off at will, does not require replenishment of the source and there is no radioactive waste. However, production of high energy electron beam requires high electric power consumption and potentially high maintenance cost. In addition, the electron beams have lower penetration ability than gamma- and X-rays, with a practical penetration of only 3.9 cm for 10 MeV electrons in high moisture foods (Commonwealth of Australia 2014). Therefore, it is suitable only for foods with small dimensions or low density.

As an electron beam penetrates into foods, there is a continuing stepwise loss of energy. Some electrons are scattered in directions different from beam direction near the surface. At some point below the surface, the range of the scattered electrons, both primary and secondary electrons, is inadequate to enable escape from foods, which creates a region of maximum energy transfer at the sub-surface of foods (Fig. 18.3) (Urbain 1986). At depths greater than this, attenuation of the beam intensity reduces the amount of energy available for transfer. The reduction of available energy continues with increasing depth until none remains and the limit of penetration is reached. The result is that maximum energy transfer occurs below the surface of foods. A similar effect occurs with X-ray and gamma ray except that it is less pronounced.

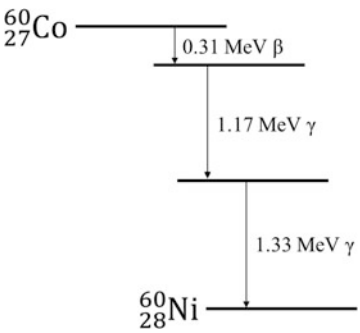

Fig. 18.3 Decay of Cobalt-60 and emission of gamma rays

Table 18.2 Ionizing radiation sources for food use

	Electron beam	X-rays	Gamma rays
Power source	Electricity	Electricity	Radioactive isotope (mainly Co-60)
Power activity	Electrical on/off	Electrical on/off	5.27 years half-life (Co-60)
Property	Electrons mass $= 9.1 \times 10^{-31}$ kg	Photons $\lambda = 4.1 \times 10^{-3}$ nm	Photons (1.25 MeV) $\lambda = 1.0 \times 10^{-3}$ nm
Charge	1.6×10^{-19} C	None	None
Emission	Undirectional (can be scanned and bent by magnets)	Forward peaked	Isotropic
Penetration	Finite range	Exponential attenuation	Exponential attenuation
Dose rate	360,000 kGy/h	100 kGy/h	10 kGy/h

Source: Adopted from International Irradiation Association (2011)

As a comparison, X-rays are produced by bombarding a heavy metal target (such as tantalum or gold) with fast electrons produced by an accelerator. Similar to gamma rays, X-rays are photons with no mass and no charge and have considerable penetrating ability. X-ray ionizing radiation requires less shielding than gamma rays, and the X-ray tube can also be turned off when it is not in use. Although X-rays can be an effective sterilization method for large volumes of high density product, their use has been limited partially because the conversion of electrons to X-rays is very inefficient (normally in the range of 8–12%) and has a high electricity consumption, which can be expensive.

As mentioned earlier, accelerated electrons have low penetrability in high moisture (water equivalent) food (Commonwealth of Australia 2014; Farkas 2006). Compared with electron beam, gamma- and X-rays have higher penetrability facilitating treatment of products even in pallet-size containers. Except for different penetration, the effect of electromagnetic ionizing radiations and electrons are equivalent in food irradiation (Farkas 2006). Tables 18.2 and 18.3 summarizes some characteristics of the three types of ionizing radiation and their advantages

Table 18.3 Advantages and disadvantages of gamma ray, electron beam and x-ray for food application

Gamma-ray	Electron beam	X-ray
Low operation/maintenance cost	High operation/maintenance cost	Highest operation/maintenance cost
High penetration	Low penetration	High penetration
Isotopes, disposal of radioactive material	Machine can be turned off	Machine can be turned off
Require heavy shielding	Require less shielding than x-rays	Require shielding not as much as gamma ray
Require regular replenishment of radioisotopes	High energy cost	High energy cost
Good dose uniformity	Poor dose uniformity	Good dose uniformity

and disadvantages for food application. There was no difference in bacterial inactivation effectiveness of X-ray, gamma-ray, and electron beam (Song et al. 2016; Tallentire and Miller 2015).

18.3 Dose and Dosimetry

Measurement in radiation dose is based on the amount of energy deposited in food being treated and is referred to as absorbed dose. The international unit for the absorbed dose is the Gray (Gy). One Gray is defined as one joule of energy absorbed by one kilogram of water. Since 1 joule equals 0.24 calories, temperature of water due to ionizing radiation only increases by 0.24 °C every kiloGray (kGy). Ionizing irradiation is regarded as one of the non-thermal processing technologies for food applications. An older unit of absorbed dose is known as the rad. The conversion between gray and rad is as follows: 1 krad = 10 Gy, 1 Gy = 100 rad = 0.1 krad, and 1 kGy = 1000 Gy = 100 krad. The dose rate is the quantity of radiation absorbed per unit time. The shorter the time required to deliver a dose, the higher the dose rate. A higher dose rate increases throughput.

Dosimetry is the measurement and calculation of absorbed doses that foods receive from ionizing radiation. The dosimetry system should be calibrated in accordance with appropriate international or national standards such as the ISO/ASTM 51261 Guide for Selection and Calibration of Dosimetry Systems for Radiation Processing (McLaughlin and Desrosiers 1995). Factors affecting dose mapping commonly include density and composition of the foods, variations in shape and size, variations in orientation of the product, stacking, volume and packaging. There are several types of dosimeters including those that measure free radicals with an EPR spectrometer (such as alanine) and those that measure the changes in optical density in radiosensitive dyes such as polymethyl methacrylate and radiochromic films (Hansen and Shaffer 2001; Kuntz and Strasser 2017).

Table 18.4 Comparison of D_{10} values (kGy) of foodborne pathogens between meats/beef and fresh produce

Pathogens	Meat/beef	Fresh produce
E. coli O157:H7	0.25	0.11–0.47
L. monocytogenes	0.51–0.59	0.16–0.39
Salmonella spp.	0.48–0.70	0.16–0.46

Adopted from Ahn et al. (2017), Fan (2012)

In general, low doses (0.2–1.0 kGy) are used for disinfestation of fruits and vegetables by sterilizing insects such as the fruit fly, moderate doses (1–5 kGy) can be used to inactivate vegetative bacteria for food safety purposes, while higher doses (>10 kGy) are used for sterilization of some dry foods such as spices, herbs, and health care products. Bacterial spores and viruses are considerably more resistant to irradiation than vegetative bacteria, probably due to the variation in sizes of DNA/RNA. Radiation sensitivity of a microorganism is inversely related to the size and complexity of the organism. A small-sized microorganism is more resistant to ionizing radiation compared to a large one (Urbain 1986). Viruses are smaller in size (typically 10–100 nm) than bacteria (1–3 μm) with corresponding smaller genomes, and consequently have higher resistance to irradiation. Therefore, inactivation of viruses and bacterial spores requires a higher dose of irradiation for inactivation.

The D_{10} value, sometimes also referred to as the D value, is used to define the irradiation dose required to achieve a one log cycle reduction in the population of a target organism (i.e. 90% reductions in survival population). The D values are an indication of a microorganism's sensitivity to irradiation. D values are often calculated as the reciprocal of the radiation dose vs reduction or populations of microorganisms. There are great variations in D values of common pathogens in the literature, probably due to use of different preparation and inoculation methods, irradiation temperature, food composition, water activity, etc. Overall, it seems that D values of pathogenic bacteria on fresh and fresh-cut produce are lower than those on meat products (Table 18.4). On cut fruits and vegetables, water activity is higher than on the surface of meat products, making it easier to inactivate microorganisms. In addition, for determining D values in meat products, pathogens are sometimes inoculated inside meats (e.g. mixed into ground beef). Food components in meat products may protect pathogens from irradiation.

18.4 Mechanisms of Ionizing Irradiation

Pathogen inactivation or chemical changes in food induced by irradiation can be a result of either the so-called direct effect or of an indirect effect (Fan 2013). The direct action occurs when gamma or X-rays or an electron beam directly damages a target within food, such as DNA or vitamin molecules. DNA is the most critical and direct target of irradiation. A dose of 0.1 kGy irradiation can cause damage to 2.8%

DNA, 0.14% of the enzymes, and 0.005% of amino acids in bacterial cells (Diehl 1995). The bases of DNA molecules are highly susceptible to ionizing radiation which result in the cleavage of phosphodiester bonds of DNA. Then, DNA damages would cause bacterial cells to lose their ability of replication, and eventfully lead to the death of bacterial cells.

In the indirect effect, ions, free radicals and other reactive species produced from radiolysis of water are involved in the inactivation of microorganisms or chemical degradation. Upon irradiation at 25 °C, the following species are formed (Simic 1983):

$$H_2O \rightarrow e_{-aq}(2.8) + \cdot OH\ (2.8) + H\ (0.5) + H_3O^+(2.8) + H_2(0.4) + H_2O_2(0.8)$$

where ·OH is hydroxyl radical, e_{-aq} is hydrated electron, ·H is hydrogen atom (ion), H_2 is hydrogen, H_2O_2 is hydrogen peroxide, and H_3O^+ is hydrated proton. The numbers in the parenthesis are relative amounts produced and expressed as G-value (number of species produced per 100 eV absorbed). Free radicals, such as hydroxyl radicals, hydrogen ions, and hydrated electrons, can attack DNA, cell membranes and other components of food and microorganisms. In most common foods such as fresh produce, meat and meat products, water is the major reactive component. Consequently, radicals from radiolysis of water play an important role, and most changes caused by irradiation in many foods are through indirect effects. Inactivation of microorganisms by irradiation in high moisture foods are primarily due to free radicals such as hydroxyl radicals formed within their cells. The radicals react with the base and sugar moieties of DNA, such as purine and pyrimidine bases and deoxyribose sugar, resulting in breakage of sugar-phosphate bonds and loss of the replication function. Oxygen has a great influence on the course of water radiolysis and the subsequent reactions of the primary radicals from water. Oxygen oxidizes free radicals and leads to formation of peroxides. Foods with high fat content are particularly affected by oxygen during irradiation, resulting in development of off-odors. Ozone, a strong oxidant, can also be formed from oxygen during irradiation.

18.5 Factors Affecting Irradiation for Microbial Inactivation

Irradiation is an effective method to inactivate enteric pathogens associated with foodborne outbreaks. The populations of most common enteric pathogens such as *Escherichia coli* O157:H7, *Salmonella* serotypes, and *Listeria monocytogenes* can be significantly decreased or eliminated by low-dose irradiation (< 3.0 kGy). However, enteric viruses and endospores of the genera *Clostridium* and *Bacillus* are highly resistant to ionizing radiation (Thayer 1995). While radiation is a broadly effective antimicrobial intervention, there are a number of factors, which can affect

the sensitivity of the target organism. These factors can broadly be grouped into intrinsic characteristics of the organism, the suspending or supporting menstruum (i.e. the commodity being irradiated), and the conditions of irradiation. They are connected to the two primary modes of action for lethality of ionizing radiation: (1) intracellular ionization of water molecules and other cellular components, and (2) direct strand breakage of DNA and RNA.

Species vary in their response to irradiation. Viruses and fungi tend to be more resistant to irradiation than bacteria, and bacterial spores being more resistant than vegetative bacteria (Sommers et al. 2002). In part, this range of responses is related to the nature and configuration of DNA within the various organisms. In viruses and fungal cells, and in eukaryotes in general, DNA is wound tightly in compact clusters. Where the DNA strands present less of a cross-sectional target for the energetic electrons (electron beam) or photons (X rays or gamma rays), there is a reduced probability for direct DNA/RNA strand breakage. Also, DNA, which is tightly wound, is less available for interaction with the oxygen and hydroxyl radicals generated during ionization of water within the cells. This minimizes the oxidative/reductive DNA damage from irradiation. In both cases, a higher radiation dose is required to enact the same level of kill that would be obtained in a more sensitive organism. A comparable effect is achieved for bacteria living in protective biofilm communities, which have entered the biofilm physiological state. Such cells can be twice as resistant to irradiation as the same bacteria under planktonic conditions (Niemira and Cooke 2010).

Within bacteria, there is a wide range of radiation sensitivity, with one of the most resistant bacteria, *Deinococcus radiodurans*, having D_{10} values comparable to the quiescent viruses (Makarova et al. 2001). Even within the same genus or species, strain variation in radiation sensitivity is a well-known phenomenon (Sommers et al. 2004; Byrne et al. 2014). While the physiological details of this strain variation are complex, they generally relate to cellular mechanisms, which either help to prevent the cells to quickly repair damage done by irradiation before the destabilizing effects become lethal, or help the cell from being damaged in the first place. In some species, trans-membrane proteins are more effective at stabilizing osmotic shock and membrane depolarization resulting from damaged cell membranes, which have been partially or wholly perforated by irradiation-engendered chemical radical species. Gram-positive species are typically more radiation resistant than Gram-negative, with the cell wall serving as a support system that allows time for membrane repair (Sommers and Niemira 2007). Species (or strains within a species) with more efficient DNA repair systems can respond more rapidly to overcome the damage done by irradiation, whether enacted by direct strand breakage or by radical species intermediates. This strain variation can occur naturally or can be induced by the selection for antibiotic-resistant strains where the mechanism of antibiotic resistance is related to DNA repair, membrane stabilization, or some other mode of action consanguineous with irradiation (Niemira and Lonczynski 2006; Byrne et al. 2014).

The supporting medium can influence the effective radiation sensitivity of target organisms by interfering with the antimicrobial modes of action of the irradiation. For irradiation systems using e-beam, exceptionally dense products (e.g. bone-in

meats) can serve to limit depth of penetration of the electrons. For X-ray or gamma systems, with much higher penetration of photons, any direct blockage or shielding is not typically an issue with food commodities. Therefore, interference of the suspending menstruum comes in the form of chemical components which can tie up and neutralize oxygen and hydroxyl radicals in tissues before they have the chance to damage the contaminating pathogen (Alvarez et al. 2006). Antioxidants are a well-known class of radical scavengers, and studies showing the radiation-protective effect of antioxidant solutions are well-represented in the literature (Sharma et al. 2017). In addition to antioxidants that are suspended in solution or are free-floating in tissue fluids, cell-wall-bound antioxidants can play a role in limiting the antimicrobial efficacy of irradiation. These bound antioxidants can continue to contribute alterations to the effective radiation sensitivity of suspected pathogens, even if they are bound to the cell membrane or the cell wall fragments, rather than to intact tissue structures.

Finally, the conditions of irradiation can limit the lethality of the process, leading to an effective increase in radiation resistance in suspended, contaminating bacteria. When dissolved oxygen is limited, as in anoxic modified atmosphere packaging, the generation of oxygen radicals is reduced, and the antimicrobial impact of irradiation is blunted (Niemira et al. 2005). This, therefore, requires a higher radiation dose to achieve the same level of kill. For products with abundant free water, high-energy electrons or photons ionize water molecules, the resultant oxygen and hydroxyl radicals migrate and interact with their surroundings. This means that tissues and contaminating pathogens undergo chemical reactivity. Where there is limited free water, the radicals are less able to migrate away from the point of creation. Statistically, there is a greater likelihood of their simply recombining, rather than enacting damage to adjacent tissues or cells. In practice, this means that foods with a low water activity, or in frozen state, do not support the migration of radical species, and irradiation is less effective at killing associated organisms. It should be noted that, in a positive sense, this reduced migration of oxygen and hydroxyl radicals also means that dry and/or frozen foods can tolerate much higher doses of irradiation before suffering sensory or organoleptic impacts (Niemira and Lonczynski 2006).

18.6 Impact on Food Quality and Nutrients

Studies on irradiation of meats have been conducted for several decades. In general, irradiation at doses to enhance microbial safety did not affect the sensory property of meats such as ground beef (Fan et al. 2004). Increased redness may be a problem in irradiated white (light) meats and gray discoloration may be an issue in irradiated raw red meat under aerobic conditions and high doses (Nam and Ahn 2003a; Ahn et al. 2017). Irradiation of meat under vacuum conditions or addition of ascorbic acid to aerobically packaged meat can prevent color development in ground beef. Ascorbate also significantly slowed down the development of lipid oxidation in ground beef during storage, when combined with double-packaging (aerobically packaged

then vacuum packaged). Therefore, double-packaging in combination with ascorbate can be a good strategy to prevent overall quality changes in meats (Nam and Ahn 2003c).

Irradiation at low doses does not affect the flavor of meats. However, irradiation of meats at high doses may induce a development of an off-odor. It appears that volatile sulfur compounds (VSC) such as dimethyldisulfide and dimethlsulfide (Fan et al. 2002) are most responsible for the off-odor due to irradiation (Ahn and Lee 2002; Fan et al. 2004; Fan et al. 2011). The VSCs are mainly formed from sulfur-containing amino acid such as methionine, cysteine, peptides (glutathione and cystine), proteins, or others (thiamine, coenzyme A). To prevent or minimize VSCs and off-odor production of irradiated foods, various additives and packaging types have been investigated (Nam and Ahn 2003b).

Water-soluble vitamins, such as the vitamin B family in meats are more sensitive to irradiation than fat-soluble vitamins. The loss of vitamins due to irradiation depends on the nature and composition of the food. In addition, the content of many vitamins often decreases during storage, and vitamins often degrade during thermal processing or cooking. Furthermore, many environmental factors affect the stability of vitamins. For example, oxygen and temperature during irradiation and post-irradiation storage must be considered when studying the degradation of vitamins.

Fresh produce items are relatively more sensitive to irradiation compared with meats, as all fresh fruits and vegetables are living organisms which undergo physiological and biochemical changes. Alternation and impact on physiological and biochemical changes of fresh produce ultimately lead to the changes in quality during post-treatment storage. The major quality parameters of fresh produce are appearance, texture, nutrients and flavor. Studies have demonstrated that most fresh and fresh-cut fruits and vegetables can tolerate up to 1 kGy of radiation without noticeable deterioration in quality (Fan and Sokorai 2008a; Fan 2012).

It appears that irradiation may result in softening and sogginess of some fruits and vegetables. Some studies have suggested that the softening may be due to irradiation-induced changes in enzyme activities involved in the synthesis of cell wall components (Melo et al. 2018). However, the softening of fresh produce due to irradiation is observed immediately after irradiation, suggesting the changes in texture is probably a result of direct effects on cell wall or membrane, leading to loss of cell turgor pressure.

Among the common vitamins in fresh produce, ascorbic acid is most sensitive to irradiation. Many fresh fruits and vegetables are good sources of vitamin C. Upon irradiation, ascorbic acid is converted to dehydroascorbic acid. With increasing radiation dose, ascorbic acid content decreases while the amount of dehydroascorbic content increases.

Cut fruits and vegetables tend to be more tolerant to irradiation than their whole counterparts (Fan 2012). There are several possible reasons for the differences in radio-sensitivity between whole and cut produce. Processing of some cut produce items, such as cut fruits, often involves removal of skins, which eliminates possible radiation-induced skin disorders. In addition, cut fruits and vegetables have a shorter

shelf-life than their whole counterparts, therefore cut fresh produce may reach their limit of shelf-life before any irradiation-induced quality deterioration occurs. Furthermore, fresh-cut fruits and vegetables are stored in refrigeration temperatures and often in modified atmosphere packaging, which slow down or prevent the occurrence of irradiation-induced damages.

Although irradiation has been proven to be effective in inactivating human pathogens, preserving quality, reducing spoilage, and providing other benefits, irradiation may cause changes in quality of some foods, such as fresh fruits and vegetables. Therefore, for some applications, it is desirable to combine irradiation with other technologies and treatments to minimize the negative effects of irradiation on product quality. When irradiation treatment is combined with other treatments, the dose necessary to eliminate the pathogen may be reduced due to increased bacterial sensitivity and synergistic/additive effects. The combined treatments may have a number of benefits such as reduced cost as lower doses are used, and maintained organoleptic and nutritional quality. The combinations can be other non-thermal processing such as high pressure processing, modified atmosphere packaging, antimicrobial films and coating, natural antimicrobials, and antioxidants such as essential oils (Fan 2012; Maherani et al. 2018; Mukhopadhyay et al. 2013).

18.7 Labeling and Consumer Acceptance

As regulated by U.S. FDA, irradiated foods should carry the Radura label (Fig. 18.4), and a statement that the food has been "treated with radiation" or "treated by irradiation". Labeling requirements apply only to bulk foods that have been irradiated. Foods containing irradiated ingredients such as spices are not required

Fig. 18.4 The international food irradiation symbol, Radura

to bear a label. In Europe, the use of Radura symbol is optional. Labeling of irradiated food gives the consumer a choice for irradiated products. Additional labels are permitted to inform the consumer reasons for applying irradiation, and how to store the food product. The radiation disclosure statement is not required to be more prominent than the declaration of ingredients. In terms of commercial application on food, irradiation is most commonly used to disinfect spices and dried vegetables (Kume et al. 2009). In recent years, it has been increasingly used to treat fresh fruits for the phytosanitary purpose although the total amount of fresh produce treated by irradiation is still low.

No evidence of genotoxic or teratogenic effects has been found in animals that were fed with irradiation-sterilized foods (World Health Organization 1994). In recent years, two groups of compounds have drawn attention: 2-alkyl cyclobutanones (2-ACBs) and furan (Fan 2013; Sommers et al. 2013). It is believed that 2-ACBs are unique radiolytic products and produced from fat upon irradiation. These compounds are good markers of irradiation treatment and have been used to detect irradiated foods (Driffield et al. 2014). The levels of 2-ACBs are in sub-ppm levels in meat products irradiated for the purpose of enhancing microbial safety. Most fresh fruits and vegetables have low fat content, and therefore the formation of 2-ACBs is not a concern. However, furan, a possible carcinogen, has been shown being induced by irradiation from a number of food compounds such as sugars, ascorbic acid and fatty acids (Fan 2005, 2015). Studies demonstrated that irradiation at a dose of 5 kGy induced less than 1 ppb levels of furan in most fresh fruits and vegetables tested, levels which are much smaller than those in many thermally processed foods (Crews and Castle 2007; Fan and Sokorai 2008b). In addition, there is evidence that furan is naturally occurring in some foods with thermal processing steps. For example, mesquite pod flour, a high sugar-containing food, contains up to 13.0 ng/g of furan (Fan et al. 2015). Furthermore, other non-thermal treatments such as UVC can also induce furan formation (Fan 2015).

There have been slow advancements in the commercial application of food irradiation. Consumer acceptance is a major challenge in the widespread application of irradiation. Other factors that influence commercialization of irradiation include limited number of foods that are approved by regulatory agencies, cost, and the public uncertainness of this technology. Studies on marketing and purchase intent of irradiated foods have demonstrated that educated consumers are willing to buy irradiated foods. Typically, at least 50% of consumers in the US will buy the irradiated food, if given a choice between irradiated product and the same non-irradiated products (Eustice and Bruhn 2013). If consumers are first educated about what irradiation is and benefits of irradiation, increased number of consumers (approximately 80%) would buy the product in these marketing tests (Eustice and Bruhn 2013). Therefore, educating consumers would increase consumer acceptance of irradiated foods and advance commercialization of the technology.

18.8 Irradiation Facilities

18.8.1 Gamma Rays

There are about 180 gamma ray facilities worldwide used for various industrial applications, mainly for sterilizing medical devices and for food irradiation (Dethier 2016). Gamma rays from cobalt-60 and cesium-137 are allowed for use by the U.S. FDA (FDA 2018). However, cesium-137 is seldom used because large cesium-137 sources are not readily available. As mentioned earlier, cobalt-60 is produced by placing metallic slugs of stable cobalt-59 in a nuclear power reactor in which cobalt-59 absorbs neutrons. There are about 40 nuclear reactors in eight counties producing cobalt-60 (Dethier 2016). Canada, Russia, and UK produced most of the cobalt-60, accounting for more than 80% of the global cobalt-60 supply. The activated metal (cobalt-60) is doubly encapsulated as rods or discs in stainless steel casings before being released to radiation facilities. Most cobalt-60 sources are often in the form of a "pencil", 45.2 cm in length and 1.11 cm in diameter (Cleland 2013). The pencils are doubly encapsulated in stainless steel tubes, which are loaded into flat and vertical racks (Fig. 18.5).

For most gamma irradiation facilities, the cobalt-60 in racks is stored into water-filled pools when not in use, where water serves as a shield. To irradiate products, the racks are raised above the pool water where products pass by the source rack on a conveyor. The treatment room is surrounded by thick concrete walls, which protects operating personnel from gamma radiation when the source rack is in the raised position (Cleland 2013). The absorbed dose is determined by the time the products are in the irradiation field and the distance from the source rack. To capture as many gamma rays (photons) as possible from the cobalt-60 source, products are positioned in close proximity to the source. The portion of energy that is absorbed by the product may range from 15% to 40% (Dethier 2016).

The gamma irradiator designed by Gray Star (Mt. Arlington, NJ) is different from many other gamma ray facilities. The Gray∗Star Genesis system, a self-contained irradiator, irradiates products under water (Fig. 18.6), which serves as built-in shielding. The benefit of the design is that no above ground concrete shielding is required as the source is always in water. For irradiating, products are placed into a container which is closed at the top but open at the bottom. Water is kept out of the containers by injecting air with increasing pressure as they are lowered into the pool.

18.8.2 Electron Beam

Accelerated electrons with energies up to 10 MeV are allowed by the U.S. FDA to treat food (FDA 2018). The energy limit is set to avoid inducing radioactive nuclides in the food as described earlier. The penetration of an electron beam increases in proportion to the electron energy, so it is advantageous to use energies of at least

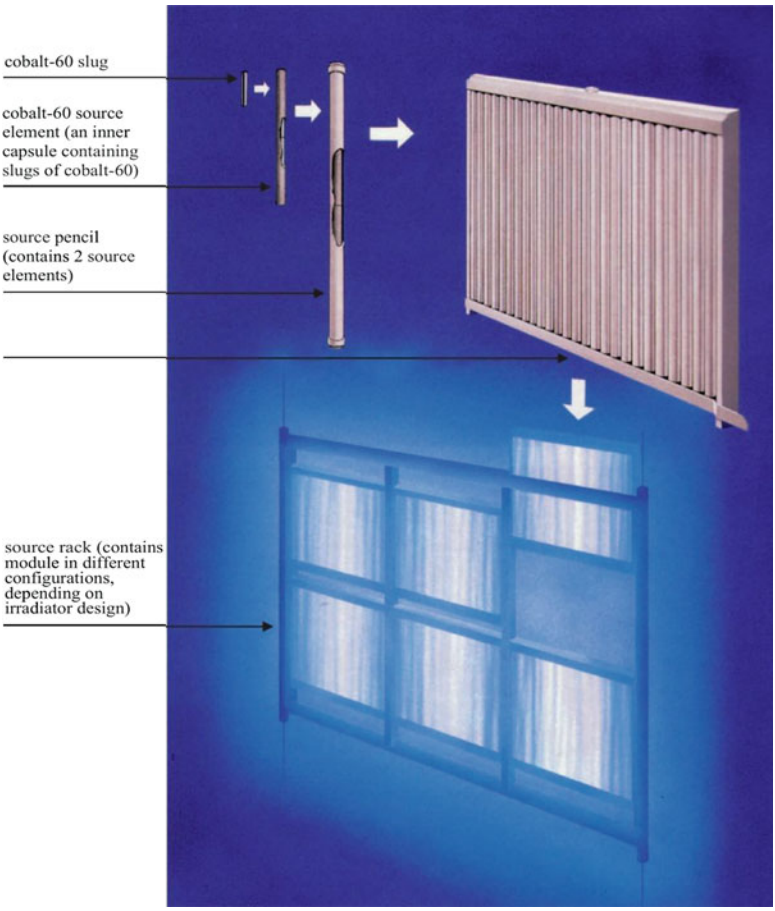

Fig. 18.5 Illustration of a typical cobalt source rack built from slugs, pencils and modules. (Nordion, Ontario, Canada)

3 MeV for packages of foods. Lower energy electrons can be used for irradiating small sized foods such as grains and for surface pasteurization of food. Due to the limited penetration of electron beams, they are effective for the sterilization of low density or small, uniformly packaged product but have limited application for other types of products. Electron beams can be applied on two sides of packages to increase dose uniformity (Pillai and Shayanfar 2017).

More than 1400 industrial electron beam accelerators are used around the world for mainly non-food purposes, such as for sterilizing single-use medical devices (Cleland 2013). Only a few of these machines are being used for food irradiation. Several different methods are used to produce high-energy, high-power electron beams. These include constant-potential, direct-current systems, microwave linear accelerators (linacs), and radio-frequency, resonant cavity systems (International

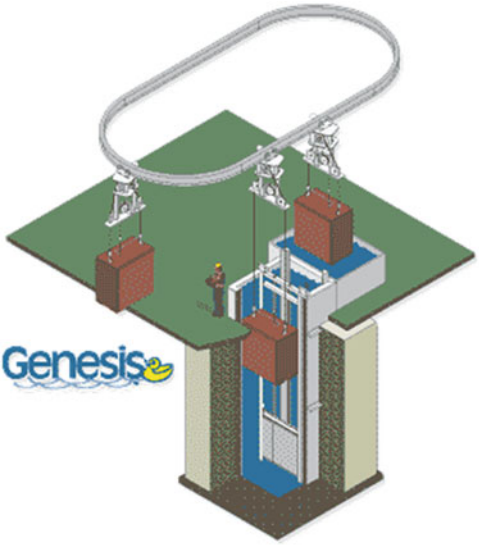

Fig. 18.6 Genesis, a gamma ray irradiator designed by Gray∗Star. http://www.graystarinc.com/images/genesis.gif. (Gray∗Star 2005)

Irradiation Association 2011). The choice of the type of accelerator for a particular application is usually dependent on the process requirements for electron energy and beam power.

18.8.3 X-rays

X-rays with energies up to 7.5 MeV are allowed by the U.S. FDA for food applications (FDA 2018). The first U.S. commercial X-ray facility started operation in Hawaii in 2000 for phytosanitary treatment of fresh fruits and vegetables shipped to mainland U.S. (Follett 2014; Hallman and Loaharanu 2016). X-rays are generated by bombarding electrons to a target material, which converts the beam of electrons into a ray of photos. The efficiency for converting electron beam power to emitted X-ray power increases with increasing atomic number of the target material and the electron energy. Therefore, metals with high atomic numbers are used to convert electron beams to X-rays. Increasing the energy also improves the X-ray penetration and allows the treatment of thicker packages or heavier products, such as fresh foods.

Dual technologies, i.e. electron beam systems with an option to produce X-rays, have been installed in several countries in recent years (Dethier 2016). One example of such a system is IBA's Rhodotron® DUO (IBA Industrial, Edgewood, NY) which is a 10 MeV E-beam system with 7 or 5 MeV X-ray capabilities. The system has benefits of both X-ray (such as penetration) and E-beam (dose rate), reducing capital and operation cost compared with two separate systems.

18.9 Dose Mapping and Process Optimization

As mentioned earlier, irradiation has limitations in penetration ability, especially for electron beam which can penetrate to a depth of only 3.8 cm in meat (Nam et al. 2016). In addition, maximum doses are not observed on the surface of food due to the Compton effect. Instead, maximum doses occur at some distance from the surface of food if irradiation is applied on one side (Fig. 18.7a). The location of the maximum dose for single side irradiation depends on the density and composition of the food and energy levels of electron beams. The occurrence of maximum dose in the sub-surface of food is due to the fact that secondary electrons are knocked out by the primary electrons. There will be more secondary electrons in the sub-surfaces, so that the total number of ionized particles is increased in the sub-surface (known as "Compton scattering"). To overcome the penetration limitation, two electron beams positioned opposite may be simultaneously applied. When irradiation is applied on both sides of the food, the total absorbed dose will be the sum of two single side irradiation treatments (Fig. 18.7b, c). As a result, maximum doses may not be the sub-surface of food. In the particular example shown in Fig. 18.4b, the maximum dose is in the center of food where the minimum dose is on the surface. It is important to minimize the ratio between maximum and minimum doses to ensure the irradiated food receives a relatively uniformed dose throughout the package. If some parts of the food receive too high of a dose, the quality of the portion of the food may be compromised. If there is a cold spot (low absorbed dose), the intended purpose, such as a targeted 5 log reduction of pathogens, may not be achieved for the particular area. To increase the energy efficacy, the thickness of the food may be adjusted so that the minimum doses occur at the center and surface of the food (Fig. 18.7c). There will always be dose variations within the same package

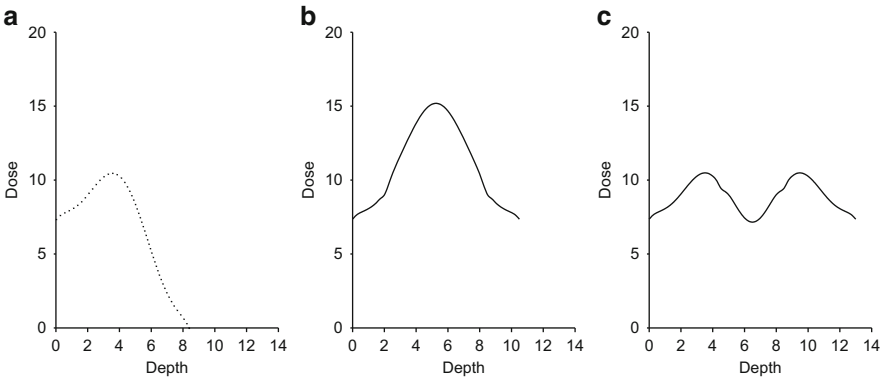

Fig. 18.7 Theoretical depth-dose curves in food irradiated by e-beam on one side (**a**) and both sides of food with two different thickness (**b**, **c**)

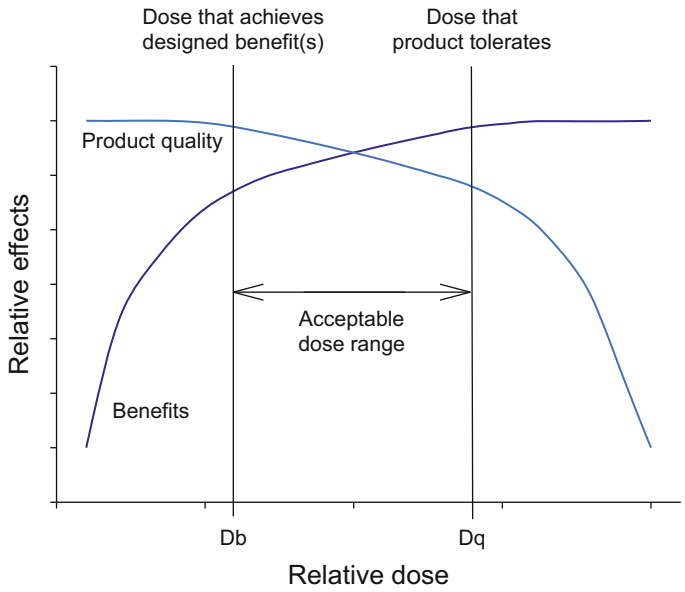

Fig. 18.8 Process optimization of ionizing irradiation: Balancing the benefits and quality retention

of foods due to penetration limitations and Compton effect. In order to deliver the required dose for a particular purpose, some parts of the product will absorb higher doses. The ratio between maximum dose and minimum dose is called Dose Uniformity Ratio (DUR).

To obtain more uniform dose distribution when applying electron beam irradiation to materials with an irregular surface, an electron scatter chamber (Maxim Chamber) was developed (Maxim et al. 2014). Basically, a stainless steel mesh was placed around a cylindrical area where the target sample was placed. Upon contacting with the mesh, electrons scatter and are directed onto the target from multiple angles, eliminating the electron beam linearity and resulting in a relatively uniform dose distribution over the target surface. The effect of irradiation in the Maxim Chamber on dose distribution was tested on rabbit carcasses, and results indicated that the dose uniformity ratio (DUR) on the rabbit carcasses was 1.8.

It is important to balancing the benefits of irradiation with irradiation-induced quality deterioration. As absorbed dose is increased, the extent of beneficial effects of irradiation is increasingly realized (Fig. 18.8). The beneficial effects could be pathogen reduction, shelf-life extension, or insect disinfestation. On the other hand, as irradiation dose increases, product quality deteriorates even though there may be no change (or beneficial changes) in the low dose range. For example, too high of a dose may make fruit too soft to be accepted by consumers. Therefore, to achieve a desired benefit, there is a prescribed dose (Db) required to achieve the desired benefits. However, each food product has its own tolerance to irradiation in terms of product quality at which doses significant changes in quality parameters occur.

Therefore, there is an upper limit dose (Dq) at which the products can tolerate without deterioration in food quality. The doses between Db and Dq are the acceptable dose range for a particular product. The range (window) can be small or large depending on the targeted benefits and the type of food. The doses applied to any particular food for a specific purpose must fall within the acceptable range.

18.10 Conclusions and Future Trends

In summary, ionizing irradiation has been studied for over a century, and has been used for various purposes, such as insect disinfestation, shelf-life extension, and inactivation of spoilage and pathogen microorganisms, although commercial application is still very limited in terms of both quantity and types of food being treated with radiation. There are three types of irradiation: gamma ray, X-rays, and electron beam, each having its own advantages and disadvantages for food applications. Irradiation facilities are available to enhance microbial safety of food by effectively inactivating human pathogens on foods. Inactivation of microorganisms involves two types of mechanisms: direct effect in which radiation interacts directly with molecules of food, and indirect effect which involves radicals generated from radiolysis of water. For application to improve microbial safety, many factors should be considered to maximize its benefits and minimize possible damage to food quality.

Consumer acceptance remains a major challenge for wide commercial use of this effective technology. Understanding consumer perceptions and attributes are keys to the acceptance of irradiation and other novel technologies (Rollin et al. 2011). Studies have shown that an increasing percentage of consumers is willing to purchase irradiated food if they are properly informed about the benefits of irradiation on food (Eustice and Bruhn 2013). Therefore consumer education and proper communication are important to increase the awareness and eventually acceptance of the technology. Many factors may affect success of educating consumers including trust in the source of information, interaction with the public and cultural variation (Rollin et al. 2011). In recent years, consumers pay more attention to naturalness of food, clean labeling, and environmental impact. The acceptance of the technology remains a formidable task, because irradiation is associated with inducing formation of unique radiolytic compounds, disposing radioactive waste, and labeling requirement of irradiated foods. Advancements in research and adaptation of e-beam and X-ray may help alter the consumer's image of the process. In terms of research directions, there may be a need to develop programs to educate consumers on food irradiation using social media. Combinations of irradiation with other technologies and treatments may be explored to reduce the doses needed to achieve targeted benefits using synergistic mechanisms. As with any other technologies, irradiation should be applied and incorporated as part of an overall good manufacturing practice.

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Chapter 19
Microbial Decontamination of Food by Light-Based Technologies: Ultraviolet (UV) Light, Pulsed UV Light (PUV), and UV Light-Emitting Diodes (UV-LED)

Joshua R. Cassar, Beining Ouyang, Kathiravan Krishnamurthy, and Ali Demirci

19.1 Introduction

A vast number of technologies currently exist that act as effective microbial reduction interventions for the food industry. Thermal methods of food microbiological decontamination often lead to unfavorable organoleptic or nutritional changes in the product (Bolder 1997; Huffman 2000; Elmnasser et al. 2007). Especially, temperature sensitive products can deteriorate rapidly when treated by thermal processing methods (Demirci and Ngadi 2012). Fortunately, nonthermal methods in the form of chemical and physical treatments can provide better alternatives. However, the use of chemicals as microbial reduction interventions creates concerns of toxicity or development of undesirable residues (Demirci and Ngadi 2012). On the other hand, physical interventions such as high hydrostatic pressure (HHP) processing, pulsed-electric field (PEF), ultraviolet (UV) light, and pulsed UV light (PUV), and UV light-emitting diode (UV-LED) are gaining popularity as alternatives to thermal and chemical decontamination methods (Demirci and Ngadi 2012).

Among the emerging technologies, this chapter will evaluate the application of UV light, PUV light, and UV-LED, which are low energy input and low-cost alternatives that are gaining attention as effective food microbial reduction

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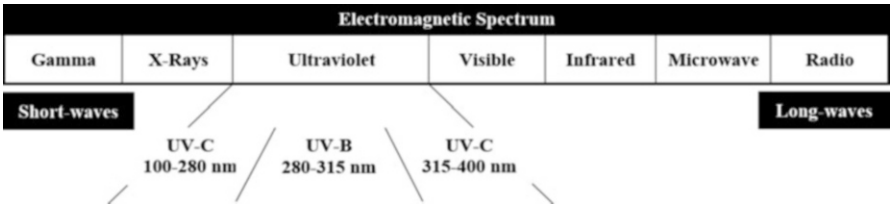

Fig. 19.1 Electromagnetic spectrum of light

technologies (Guerrero-Beltran and Barbosa-Canovas 2004; Demirci and Ngadi 2012; Xiong and Hu 2013). These light-based technologies are known to reduce the pathogenic and spoilage microorganisms on the surfaces of food products and have demonstrated microbial reduction capabilities in transparent and some translucent liquids (Guerrero-Beltran and Barbosa-Canovas 2004; Shur and Gaska 2010).

Ultraviolet (UV) light originates artificially from mercury lamps that emit an electromagnetic radiation spectrum with wavelengths ranging from 100 to 400 nm (Fig. 19.1). The complete UV spectrum can be further broken down into four specific regions that have differing characteristics. The four regions are as follows: Vacuum UV (100–200 nm), UV-C (200–280 nm), UV-B (280–315 nm), and UV-A (315–400 nm) (Krishnamurthy et al. 2010; Demirci and Ngadi 2012).

Within the total UV spectrum, wavelengths between 220 and 300 nm are considered to have germicidal effects against a variety of microorganisms including bacteria, viruses, protozoa, molds, yeast, and algae. Between 250 and 270 nm, the germicidal effect of UV light is the highest. Therefore, low-power mercury lamps having a continuous emittance of 254 nm are designed to work in this wavelength range. UV light is a low-cost, easy to handle and environmentally friendly nonthermal, non-chemical treatment with germicidal effects. Historically, UV light has been used to decontaminate drinking water and wastewater (Demirci and Ngadi 2012). Recently, UV light has also been applied for disinfection of surfaces in pathogen prone facilities such as hospitals and food processing operations (Demirci and Ngadi 2012). However, its low energy output prevents its use, especially in the food industry, which processes significant amounts of material in a short time. Currently, the majority of UV light disinfection systems use low or medium pressure mercury lamps, which have raised concerns of possibility of mercury leakage (Song et al. 2016). Furthermore, these mercury lamps have a short lifetime of about 10,000 hours and low energy dissipation (Xiong and Hu 2013; Shin et al. 2016).

PUV light (also known as pulsed light) is a more recent technology, which delivers much higher instantaneous power outputs compared to conventional UV light lamps. Like UV light, PUV is also a non-chemical technology and during short exposure time, nonthermal. PUV light is generated as short duration, high intensity pulses when high voltage is applied to an inert gas (e.g. xenon). The gas molecules enter an excited state and release photons in the wavelength range of 100–1100 nm (Krishnamurthy et al. 2007; Demirci and Ngadi 2012). The power of PUV light is amplified by building and storing the energy in a capacitor and releasing it over short

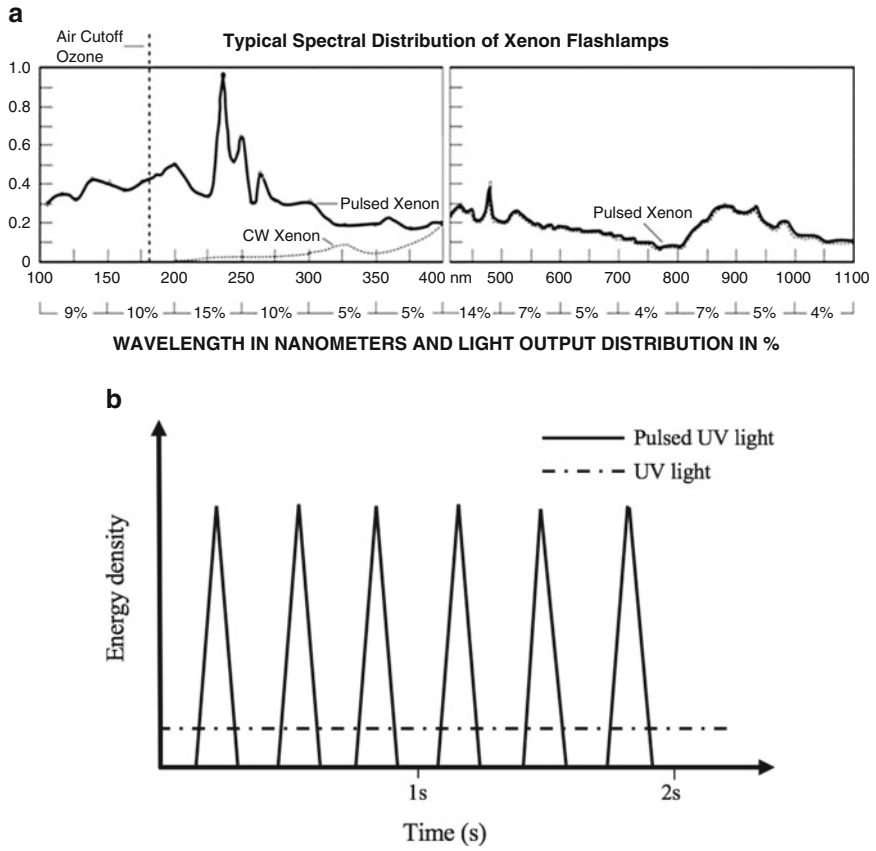

Fig. 19.2 (**a**). Spectral output of a Xenon flashlamp (Sonenshein 2003). (**b**) Comparison of energy density of PUV light and UV light (not to scale)

bursts (Gomez-Lopez et al. 2007). The intense energy pulses produced from electromagnetic radiation between 100 to 1100 nm include portions of UV, visible and infrared spectra (Fig. 19.1). The term PUV light is specific to Xenon flashlamps, accredited to the understanding that 54% of the energy is derived from the UV region (Fig. 19.2a). The total percentage of UV energy depends on the gas type, lamp pressure, input voltage, and other factors. The effectiveness of microbial reductions, the penetration ability, and lamp safety are advantages of PUV light compared to continuous UV light (Demirci and Ngadi 2012). Figure 19.2b compares the spectral distribution of PUV light and UV light.

A third UV light technology is UV light-emitting diodes (UV-LED), which emits radiation from 210 to 400 nm (Xiong and Hu 2013; Song et al. 2016). UV-LEDs possess unique properties including diversity in wavelengths, small form-factor, adjustable design (Rattanakul and Oguma 2017), low radiant heat emissions (D'Souza et al. 2015), faster start-up time, mechanical robustness, and ease of

operation. It can efficiently be turned on and off at high frequencies (Xiong and Hu 2013). In addition, UV-LED lamps have a longer lifespan, roughly ten times more than mercury lamps and are more efficient in converting electric energy into light energy (Xiong and Hu 2013). The UV-LED can also generate pulsed emittance at specific frequencies. Compared to pulsed UV lamps, UV-LED has a lower power requirement for operation and its pulse pattern is more controllable (Song et al. 2018). Most of the studies on UV-LED inactivation of pathogens is focused on drinking water and wastewater treatment.

The next sections of this chapter will provide a more in-depth understanding of UV, PUV, and UV-LED pertaining to food decontamination. The fundamental characteristics of microbial reduction and effects on food quality are discussed as well. Current trends, applications, and limitations are also provided.

19.2 Fundamentals of UV, PUV, and UV-LED

UV light, PUV, and UV-LED can be characterized by the interactions between light and matter. A photochemical interaction is initiated when light photons are received by matter. Atoms that absorb the photon energy are in a state of excitement allowing for the building and breaking of molecular bonds creating different chemical structures. In order for these reactions to occur, it is necessary for photons to be absorbed by the matter and there are adequate conditions for molecular bonds to be reestablished (Blatchley and Peel 2001). Max Planck, a German physicist defined light photon energy as:

$$E = h \cdot v$$

where E is defined by photon energy (eV or kJ/Einstein), h is Planck's constant ($\approx 6.63 \times 10^{-34}$ J·s), and v is frequency (s^{-1}) defined by:

$$v = c/\lambda$$

where c is the speed of light ($\approx 3.00 \times 10^{8}$ m/s) and λ is the wavelength (m).

According to the equation, photon energy is correlated to the frequency of the photons. This equation implies that shorter wavelengths in the electromagnetic spectrum produce higher photon energy (Demirci and Ngadi 2012)

19.2.1 Ultraviolet Light

UV light is the portion of the electromagnetic spectrum between 100 and 400 nm (Fig. 19.1). UV light consists of roughly 10% of the total radiation produced by the earth's sun (Demirci and Ngadi 2012). Each sub-portion of the UV spectra namely,

Table 19.1 UV type and characteristics

Type	Wavelength	Range (nm)	Characteristics
UV-A	Long	320–400	Changes in human skin (tanning)
UV-B	Medium	280–320	Skin burning (cancer)
UV-C	Short	200–280	Germicidal effects (DNA)
UV-V	Very short	100–200	Vacuum UV range

Modified from Guerrero-Beltr et al. (2004)

vacuum-UV, UV-C, UV-B, and UV-A have the following photon energy ranges (eV): 12.4–120, 12.4–4.43, 4.43–3.94, and 3.94–3.10, respectively. Photon energy derived from each sub-portion of the UV region correlates to differing bimolecular reactions, as described in Table 19.1.

Photochemical interactions can either be beneficial or adverse for living organisms. Wavelengths in UV-A portion of the spectra are correlated to skin tanning and can enhance the synthesis of vitamin D. Conversely, UV-B wavelengths in the UV spectra can cause skin damage which can lead to cancer. UV-C wavelengths produce germicidal effects through the formation of pyrimidine dimers (especially, thymine dimers) in the DNA of living cells. These altercations in the DNA lead to microbial inactivation. The germicidal effects of UV light were first acknowledged in 1877 by Downes and Blunt, two English scientists, who discovered the ability of the sun to prevent microbial growth (Downes and Blunt 1877). The germicidal effect of UV light was later correlated to dosage (intensity $\times$ time), wavelength of radiation, and sensitivity of the specific microorganism. In 1930, Gates published the first analytical bactericidal experiment that presented the peak effectiveness at 265 nm (Gates 1934). Throughout the rest of the twentieth century, research investigating the germicidal effect of UV light was limited. In recent decades, increasing interest has sparked the application of UV light for microbial reduction in foods and medical devices. With the renewed interest in the bactericidal effects of UV light, varying technologies have been developed to produce UV light.

Today, there are various lamps that emit either monochromatic or polychromatic UV light wavelengths. Evaluating the efficacy and cost effectiveness of each process is critical in determining the most appropriate UV light source. The following sections summarize various types of UV lamps. A schematic diagram of a flow-through UV disinfection system is presented in Fig. 19.3.

19.2.1.1 Low Pressure Mercury (LPM) Lamps

Low pressure mercury lamps emit a monochromatic wavelength with 85–90% of cumulative energy at 253.7 nm (Reed 2010). This monochromatic wavelength yields relatively low light irradiance and is relatively close to the peak germicidal wavelength recognized by Gates (1934). LPM produces germicidal wavelengths (200–300 nm) with a germicidal efficiency of 35–40%. The lamps operate at a mercury vapor pressure of 0.1–10 Pa and an operating temperature of 30–50 °C.

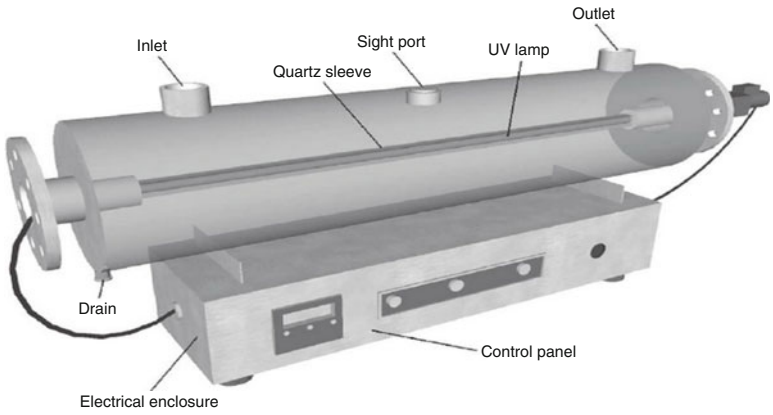

Fig. 19.3 Schematic diagram of a flow-through microwave UV disinfection system (Demirci and Ngadi 2012)

High output LPM lamps operate at increased temperatures (60–100 °C) and result in less germicidal effectiveness (30–35%). The lifespan of LPM lamps ranges from 18 to 24 months at an intensity of 0.1 W/cm^2 (Demirci and Ngadi 2012).

19.2.1.2 Medium Pressure Mercury (MPM) Lamps

Medium pressure mercury lamps produce a much broader range (185–600 nm) of polychromatic light. Compared to the LPM lamps, MPM lamps have a higher vapor pressure (50–300 kPa) and operating temperature (600–900 °C). The MPM lamps have a much greater intensity (~12 W/cm^2) compared to the LPM lamps, but only result in a 15–20% germicidal efficiency. Gas mixture and operating temperature determine the spectrum of light produced. The life span of the MPM lamps with an intensity of ~12 W/cm^2 is a couple of weeks. Both LPM and MPM lamps produce continuous UV light (Demirci and Ngadi 2012).

19.2.1.3 Excimer Lamps

Excimer lamps produce monochromatic radiation when electric potential is applied to rare gases across a dielectric barrier. Wavelengths produced by excimer lamps are determined by the mixture of gas (Ar, Kr, Xe, ArCl, KrCl, and XeCl) in the lamp, which allows for manipulation of output wavelengths (Kogelschatz 2004; Demirci and Ngadi 2012). The lamps have an electrical to optical energy conversion efficiency of 10–35%.

19.2.1.4 Low Pressure Amalgam (LPA) Lamps

Low pressure amalgam lamps were produced as an alternative to LPM lamps. LPA lamps emit from 254 to 285 nm and are 35% efficient at 254 nm. Operating temperature of LPA lamps ranges from 90 to 120 °C and does not affect the UV intensity. Negligible heat generation, high efficiency, low operating costs, and long lamp lifespan are advantages to the LPA lamps (Koutchma 2009).

19.2.1.5 Microwave UV Lamps

Microwave UV lamps produce UV wavelengths without any electrodes. Microwave energy is used to excite the mercury atoms which in turn release radiation. Pressures and temperatures produced by microwave UV are similar to LPM lamps. Though LPM lamps have a lamp life three-fold greater (Koutchma 2009).

19.2.2 Pulsed UV Light

PUV light or pulsed light (PL) is a novel UV technology that amplifies the germicidal mechanism produced by continuous UV light. PUV light is generated as short duration, high intensity pulses when high voltage is applied to an inert gas (e.g. Xe, Ar, Kr) in a flash lamp. This produces a broad spectrum of electromagnetic radiation ranging from 100 to 1100 nm (UV, visible, and infrared wavelengths). Typically, a majority of the energy comes from the UV light spectrum (eg. a typical xenon pulsed light system produces approximately 54% of the energy in the UV spectrum), justifying the term pulsed "UV" light. The frequency can range from 1 to 20 pulses per second and the duration of each pulse can be reduced to a few hundredth of microseconds. The high energy density (J/cm^2) of each pulse is produced by storing the electrical energy in a large storage capacitor. The stored energy is released to excite the gas molecules within the flash lamp resulting in high intensity light pulses. The system delivers energy that is 20,000 times more intense than that of the sun on the earth's outer surface (Elmnasser et al. 2007). PUV light provides increased germicidal effectiveness compared to continuous UV light. Flash lamps and surface discharge (SD) lamps are used for generating pulsed light. Typical flash lamps are activated by releasing energy between two electrodes confined to a small envelope of inert gas. SD lamps produce plasma by discharging high power electricity along the surface of a dielectric substrate (e.g. fused silica tube) inside an envelope containing xenon gas (Koutchma 2009; Demirci and Ngadi 2012). Figure 19.4 depicts of the SteriPulse-XL 3000 Pulsed Light System (Fig. 19.4a) and a cross-section of the longitudinal axis of the xenon flashlamp with reflector (Fig. 19.4b).

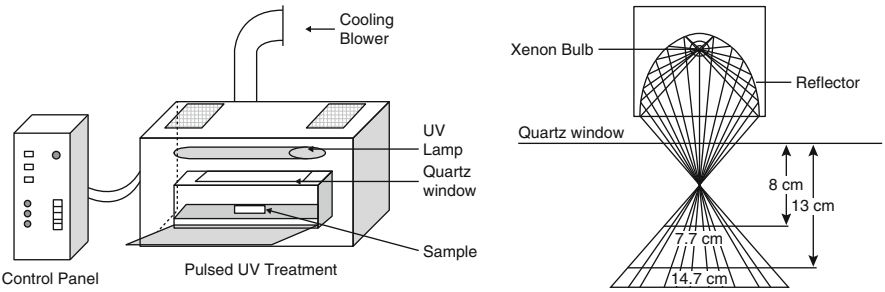

Fig. 19.4 (**a**) Depiction of the SteriPulse-XL 3000 Pulsed Light System (Bialka et al. 2008) and (**b**) Distribution of light in a cross-section of the longitudinal axis of the xenon flashlamp with reflector (Xenon 2006)

19.2.3 UV-LED Light

The first practical visible LED was developed in 1962. Since then, LED technologies have been enhanced significantly and applied extensively, especially in the lighting industry, because of the increasingly higher efficiency and lower cost (Chen et al. 2017). LED is a semiconductor device that utilizes semiconducting materials to convert direct current (DC) into light of varying emission wavelength by a p-n junction (hole and electron) The radiation emits at the junction when the electrons and holes recombine (Song et al. 2016). The different wavelength of the radiation generated is based on the type of semiconducting materials (Song et al. 2016).

By using different semiconducting materials, LED can emit various wavelengths (Song et al. 2016), such as red (GaAsP semiconductor materials with 620–750 nm emission) and blue (GaN; 450–400 nm) (Chen et al. 2017). The common materials for UV range (200–400 nm) are III-nitride, including gallium nitride (GaN), aluminum gallium nitride (AlGaN), and aluminum nitride (AlN). GaN-based UV-LEDs can reach a wavelength as low as 365 nm (Chen et al. 2017). AlGaN based UV-LEDs which consist of AIN and GaN in appropriate proportions have wavelength from 210 to 365 nm, covering from deep UV to near visible regions (Song et al. 2016). The shortest wavelength among semiconductors is reported as 210 nm (deep UV) by AIN UV-LEDs (Song et al. 2016). LEDs which emit radiation at wavelengths below 300 nm exhibit germicidal action (Chen et al. 2017).

19.3 Mechanisms of Microbial Inactivation

The germicidal effects associated with UV, PUV, and UV-LED lights are a result of predominantly photochemical changes that disrupt cellular DNA structures. It is known that photons with wavelengths in the range of 240–280 nm are effectively

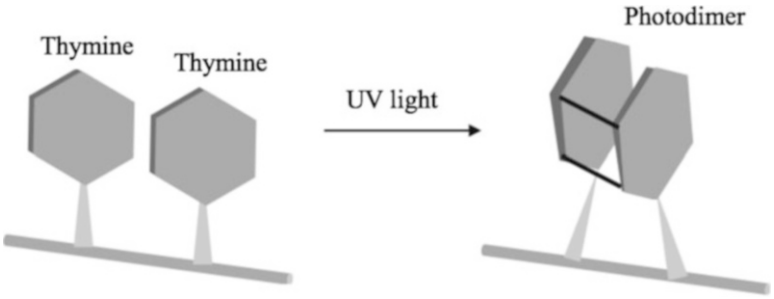

Fig. 19.5 Depiction of Thymine dimer formation after exposure to UV light

absorbed by DNA in living cells (Chen et al. 2017). After absorbing the UV light, DNA is damaged, ultimately impeding replication and causing cell death (Chen et al. 2017). Additional mechanisms such as photothermal and photophysical changes are hypothesized to contribute to microbial reduction by PUV light (Krishnamurthy et al. 2010). Ultimately, it has been confirmed that broad spectrum (e.g. PUV light) has no germicidal effect when wavelengths lower than 320 are filtered out (Elmnasser et al. 2007).

19.3.1 Ultraviolet Light

When UV wavelengths penetrate a cell wall, the germicidal UV-C wavelengths create disruptions in cellular DNA, which inhibits a microorganism's ability to reproduce. UV light results in the formation of photo-dimerization of pyrimidine bases and photo-hydration of cytosine (Fig. 19.5). The bonds formed by these disruptions make the DNA unable to unzip for replication effectively inhibiting cellular reproduction. If a cell lacks the ability to repair such DNA disruptions, cell death will occur due to mutations, impaired replication, and DNA transcription (Miller et al. 1999; Elmnasser et al. 2007; Krishnamurthy et al. 2010). However, UV damage on cellular DNA can be repaired under certain conditions by the cell's repair mechanisms (Elmnasser et al. 2007; Demirci and Ngadi 2012).

19.3.2 Pulsed Ultraviolet Light (PUV)

In addition to DNA damage, PUV light has photothermal and photophysical effects on microorganisms (Elmnasser et al. 2007; Krishnamurthy et al. 2010).

19.3.2.1 Photothermal Mechanism

PUV light emits a broad spectrum that includes visible and infrared wavelengths. High power pulses create an opportunity for localized heating leading to cell wall drying and cellular destruction. The water in microorganism becomes vaporized, which generates a steam flow at the cell membrane evacuating intercellular content. At high dosage and prolonged treatment times, temperature of the product significantly increases. It is reasonable to infer that at low energy levels where temperature is not significantly increased at the surface of a product that temperature increase only minimally contributes to the microbial reduction.

19.3.2.2 Photophysical Mechanism

Similar to photothermal effects, physical altercation of a microorganism is another phenomenon of PUV light. Studies have reported that microscopic examination revealed the physical damages of microorganisms caused by PUV light. Other studies have reported complete cell lyses or destruction beyond the DNA interactions. It is hypothesized that the broad-spectrum wavelength emitted in short powerful bursts could contribute to intense micro-vibrations on the cell membranes that lead to distortion and lyses (Fig. 19.6).

PUV light damages DNA too severely to be repaired by enzymes and also destructs the cellular structures. It is hypothesized that the intensity of the energy delivery damages the DNA repair enzymes, effectively inactivating their function (Elmnasser et al. 2007; Demirci and Ngadi 2012).

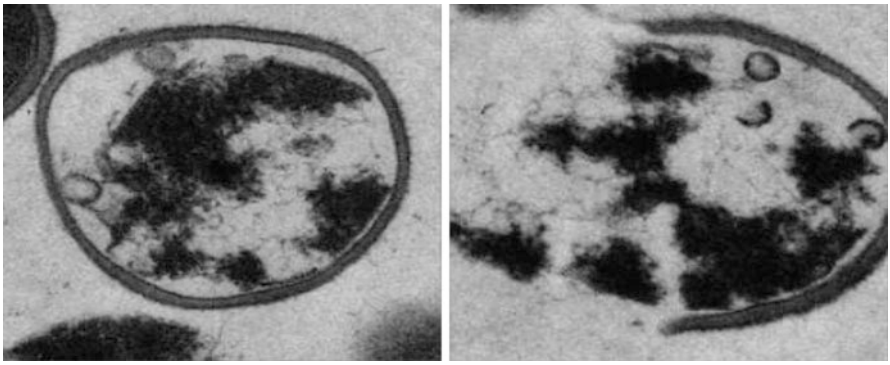

Fig. 19.6 Example of a cell membrane collapse after exposure to PUV light (Krishnamurthy et al. 2010)

19.3.3 UV-LED

As an ultraviolet light source, UV-LED have the same mechanisms of microbial inactivation as the traditional mercury-based UV lamps (Song et al. 2016). Besides, the UV-LED can be designed to emit various wavelengths based on the composition of the semiconductor material and different peak emissions can be combined into a single device that emits a spectrum most suitable to a specific microorganism (Chen et al. 2017). For example, it has been reported that the combined wavelengths of 280/365 nm, or 280/405 nm, could synergistically increase the inactivation efficiency of pure cultured strains of *E. coli* and *Enterococcus faecalis*, as well as indigenous fecal indicator bacteria in treated wastewater (Chen et al. 2017).

UV-LED can also be pulsed offering high flexibility for pulse pattern with various frequencies and duty cycles. However, very limited research is available on the efficacy of pulsed UV-LEDs on food and other applications. Thus, the mechanisms of pulsed UV-LEDs were barely explored (Song et al. 2018).

19.4 Applications of UV, PUV, and UV-LED for Food Decontamination

Studies have demonstrated that UV, PUV, and UV-LED have a germicidal effect on microorganisms in water and foods. UV, PUV, and UV-LED disinfection has been effective in reducing or eliminating bacteria, fungi, viruses, and protozoa (Wright and Cairns 1998; Demirci and Ngadi 2012; Song et al. 2016). Predominantly, UV light has been used to disinfect water (Wright and Cairns 1998). UV light has also been used to disinfect food-contact surfaces, packaging containers, and packaging films. Overall application of UV light is limited in the industry due to the low throughput of commercial-scale systems for treating food. Regardless of its limited use in commercial food decontamination, research continues to investigate the effectiveness of the technology.

PUV light offers a similar germicidal effectiveness as UV light, but the technology is more efficient, safer, and faster in its ability to decontaminate the surface of a food product. Especially for surface decontamination of food or food contact surfaces, PUV light is effective.

UV-LEDs cover the ultraviolet (UV) range down to 210 nm and enable new applications for air, water, and surface sterilization and decontamination (Shur and Gaska 2010). A substantial number of studies have demonstrated the usefulness of LEDs for water treatment. However, research on the efficacy of UV-LEDs for decontamination of food is very limited. Many studies about UV-LEDs disinfection are still restricted to batch system and water treatment. Thus, some selected studies on water disinfection are summarized and discussed below.

19.4.1 UV Light

Chun et al. (2010) tested the efficacy of 5 kJ/m^2 UV-C light on inactivation of *Campylobacter jejuni, Listeria monocytogenes,* and *Salmonella* Typhimurium inoculated on the surface of chicken breasts. The populations of *C. jejuni, L. monocytogenes, and S.* Typhimurium were reduced by 1.26, 1.29, and 1.19 $\log_{10}$ CFU/g, respectively, at 5 kJ/m^2 (Chun et al. 2010). Wallner-Pendleton et al. (1994) inoculated broiler carcasses with *S.* Typhimurium and exposed them to UV-C light at a wavelength of 253.7 nm for 5 min. This exposure resulted in a 61% reduction of *S.* Typhimurium. UV has been used to significantly reduce bacteria from the surface of shell eggs (Kuo et al. 1997). Coufal et al. (2003) developed a conveyor system for continuously treating eggs with UV light. After a 4 min treatment at 4–14 mW/cm^2, aerobic plate counts, *S.* Typhimurium, and *E. coli* were reduced by 1.3, 4.0 and 4.5 $\log_{10}$, respectively. Wright et al. (2000) investigated the reduction of *E. coli* O157:H7 in apple cider by ultraviolet light treatment. The cider contained a cocktail of multiple strains of acid-resistant *E. coli* O157:H7 (6.3 log CFU/ml) and was treated using a thin-film UV disinfection unit. The UV dosage ranged from 9402 to 61,005 μW/cm^2/s resulting in a mean reduction of 3.81 $\log_{10}$ CFU/ml for *E. coli* O157:H7. Tables 19.2 and 19.3 provide a more complete representation of the microbial reduction capabilities of UV light for solid and liquid foods, respectively. The data represented in the following tables focuses on a small portion of published data to provide a general range of microbial reduction by food type and microorganism.

19.4.2 Pulsed UV Light

Keklik et al. (2010a) studied the effect of PUV light on reduction of *S.* Typhimurium on the surface of boneless, skinless chicken breast. They reported that reduction in *Salmonella* population ranged from 1.2 to 2.4 $\log_{10}$ CFU/cm^2. Chun et al. (2010) tested the efficacy of UV-C for reduction of *C. jejuni*, *L. monocytogenes*, and *S.* Typhimurium. UV-C (254 nm) treatment at 5 kJ/m^2 reduced the populations of *C. jejuni*, *L. monocytogenes*, and *S.* Typhimurium by 1.26, 1.29, and 1.19 $\log_{10}$ CFU/g, respectively.

In another study, McLeod et al. (2017) subjected boneless, skinless chicken breast fillets inoculated with pathogenic bacteria to PUV light with fluences ranging from 1.25 to 18 J/cm^2 resulted in average reductions from 0.9 to 3.0 $\log_{10}$ CFU/cm^2 of *S.* Enteritidis, *L. monocytogenes*, *Staphylococcus aureus*, *E. coli* EHEC, *E. coli* ESBL, *Pseudomonas* spp., *Brochothrix thermospacta,* and *Carnobacterium divergens*.

Can et al. (2014) reported that a PUV light treatment reduced *Penicillium roqueforti* and *L. monocytogenes* on both packaged and unpackaged hard cheese. After 40 seconds of exposure at 5.6 J/cm^2/pulse, the PUV light treatment produced a

Table 19.2 Summary of published data for microbial inactivation of solid food by UV light

Food type	Microorganism	Contamination method	Wavelength (nm)	Dosage	Initial population (Log_{10} CFU/cm^2)	Reduction (Log_{10} CFU/cm^2)	Reference
Chicken meat	*Campylobacter jejuni*	Natural	254.0	9.4–32.9 W/s	~7.00	0.6–0.8	Isohanni and Lyhs (2009)
Frankfurters (beef/pork)	*Listeria monocytogenes*	Artificial	254.0	1–4 J/cm^2	~8.00	1.31–1.93	Sommers et al. (2009)
Lettuce	Hepatitis A virus	Artificial	253.7	40–240 mW/cm^2/s	7.00–9.00	4.29–4.62	Fino and Kniel (2008)
Onions (green)	Hepatitis A virus	Artificial	253.7	40–240 mW/cm^2/s	7.00–9.00	4.16–5.58	Fino and Kniel (2008)
Peaches	*Debaryomyces hansenii*	Artificial	254.0	7.5 mW/cm^2	~8.00	33–48%	Stevens et al. (1997)
Shelled eggs	Aerobic plate counts	Natural	254.0	4–14 mW/cm^2	3.75–4.61	1.83–1.93	Coufal et al. (2003)
	Salmonella typhimurium	Artificial	254.0	4–14 mW/cm^2	4.67–6.33	3.44–4.63	
	Escherichia coli	Artificial	254.0	4–14 mW/cm^2	7.17–7.73	4.58–4.91	
Strawberries	Hepatitis A virus	Artificial	253.7	40–240 mW/cm^2/s	7.00–9.00	1.28–2.60	Fino and Kniel (2008)
Watermelon	Aerobic plate counts	Natural	254.0	4.1 kJ/m^2	–	>1.00	Fonseca and Rushing (2006)

Table 19.3 Summary of published data for microbial inactivation of liquid foods by UV light

Food type	Microorganism	Contamination method	Wavelength (nm)	Dosage	Initial population (Log_{10} CFU/mL)	Reduction (Log_{10} CFU/mL)	Reference
Apple juice	*Listeria innocua*	Artificial	200–280	0.177 W/cm^2/s	~5.00	>5.00	Caminiti et al. (2012)
	Escherichia coli	Artificial	200–280	0.177 W/cm^2/s	~5.00	>5.00	Caminiti et al. (2012)
Egg (liquid)	*Escherichia coli* K12	Artificial	254.0	3.96–26.44 mJ/cm^2	~8.00	0.270–0.896	Unluturk et al. (2010)
	Escherichia coli O157:H7	Artificial	254.0	3.96–26.44 mJ/cm^2	~8.00	0.219–1.403	
	Listeria innocua	Artificial	254.0	3.96–26.44 mJ/cm^2	~8.00	0.215–0.960	
Grape (white) juice	*Escherichia coli* K12	Artificial	–	75.04 mJ/cm^2	5.73	5.71	Hakgüder (2009)
Milk (goat)	*Listeria monocytogenes*	Artificial	254.0	15.8 mJ/cm^2	7.30–7.44	>5.00	Matak et al. (2005)
Orange juice	Aerobic plate counts	Natural	–	144.36 mJ/cm^2	6.04	1.76	Hakgüder (2009)

reduction of 1.32 $\log_{10}$ CFU/cm^2 on unpackaged cheese and 1.24 $\log_{10}$ CFU/cm^2 on packaged cheese.

PUV light has also proven to be effective for treatment of liquid products. Krishnamurthy et al. (2007) investigated the inactivation of *Staphylococcus aureus* in milk using a flow-through PUV light treatment system. Milk was treated at 5, 8, or 11 cm from the xenon flash lamp at flow rates of 20, 30, or 40 mL/min resulting in reductions ranging from 0.55 to 7.26 $\log_{10}$ CFU/mL.

Tables 19.4 and 19.5 further depict the germicidal response of PUV light on the surface of solid foods and in liquids, respectively. The data represented in the following tables focuses on a small portion of published data to provide a general range of microbial reduction by food type and microorganism.

19.4.3 UV-LED

Oguma et al. (2013) applied UV-LED with peak emissions at 265, 280, and 310 nm to batch and flow-through water decontamination systems. At fluences of 10.8 and 13.8 mJ/cm^2, over 4 $\log_{10}$ CFU/mL inactivation of *E. coli* was obtained in the batch reactor with UV-LED at 265 and 280 nm, respectively, while 0.6 $\log_{10}$ CFU/mL inactivation was observed at 310 nm of UV-LED at a fluence of 56.9 mJ/cm^2. Rattanakul and Oguma (2017) reported that UV-LED is effective for inactivating *Pseudomonas aeruginosa* and *Legionella pneumophila*, and surrogate species, including *Escherichia coli*, *Bacillus subtilis* spores, and *bacteriophage* Qβ in water. The study demonstrated that among UV-LED with nominal peak emissions at 265, 280, and 300 nm, the 280 nm UV-LED required the lowest energy consumption for achieving a 3 $\log_{10}$ inactivation in all microbial species tested.

Kim et al. (2017) used UV-LED treatment at 4 different peak wavelengths (266–279 nm) for inactivating four major foodborne pathogens in media including *E. coli* O157:H7, *Salmonella* spp., *L. monocytogenes*, *S. aureus*, and spoilage yeasts including *Saccharomyces pastorianus* and *Pichia membranaefaciens*. The results showed that UV-C-susceptibility followed the sequence of Gram-negative bacteria > Gram-positive bacteria > yeasts. The reduction of *E. coli* O157:H7 and *Salmonella* spp. was over 5 $\log_{10}$ CFU/mL after 0.4 and 0.6 mJ/cm^2 of irradiance, respectively. Gram-positive bacteria were reduced by 3–5 $\log_{10}$ CFU/mL after 0.6 mJ/cm^2 of irradiance. The inactivation of yeasts varied from 1 to 4 $\log_{10}$ CFU/mL after 0.6 mJ/cm^2 treatment depending on the species.

Similarly, Shin et al. (2016) examined the effect of UV-LED for inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on solid media and in water. The results showed that 1.67 mJ/cm^2 on solid media was sufficient to obtain over 6 $\log_{10}$ CFU/mL reduction of *E. coli* O157:H7 and *S.* Typhimurium and over 5 $\log_{10}$ CFU/mL reduction of *L. monocytogenes*. In addition, the results of this study showed that *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was reduced by 6.38, 5.81, and 3.47 $\log_{10}$ CFU/mL, respectively, in water with a continuous flow system at 0.5 L/min of flow rate and 200 mW output power of UV-LED.

Table 19.4 Summary of published data for microbial inactivation of solid foods by pulsed UV light

Food type	Microorganism	Contamination method	Energy/pulse	Total pulses	Total energy (J/cm^2)	Initial population (Log_{10} CFU/cm^2)	Reduction (Log_{10} CFU/cm^2)	Reference
Alfalfa seeds	*Escherichia coli*	Artificial	5.6 J/cm^2	135	–	~8	0.94–1.82	Sharma and Demirci (2003)
Black pepper	*Saccharomyces cerevisae*	Artificial	1.95 J/cm^2	64	–	~9	2.93	Fine and Gervais (2004)
Blueberries	*Salmonella*	Artificial	5.6 J/cm^2	15–180	–	~7	1.3–3.8	Bialka and Demirci (2007)
	Escherichia coli O157:H7	Artificial	5.6 J/cm^2	15–180	–	~7	1.3–4.9	Bialka and Demirci (2007)
Carrots	Aerobic Mesophiles	Artificial	7 J	675–2700	–	~8	1.64–1.67	Gómez-López et al. (2005)
Chicken carcass	*Escherichia coli* K12	Artificial	5.6 J/cm^2	90–540	–	~5–6	0.87–1.43	Keklik et al. (2011)
Chicken frankfurters	*Listeria monocytogenes*	Artificial	5.6 J/cm^2	15–180	2.9–67.0	~5–6	0.3–1.9	Keklik et al. (2009)
Chicken breast	*Salmonella* typhimurium	Artificial	5.6 J/cm^2	15–180	2.9–67.0	~5–6	1.2–2.4	Keklik et al. (2010a)
	Campylobacter spp.	Artificial	1.18 J/cm^2	–	106.2	~4	0.96	Haughton et al. (2011)
	Escherichia coli	Artificial	1.18 J/cm^2	–	106.2	~3	1.13	Haughton et al. (2011)
	Salmonella Enteritidis	Artificial	1.18 J/cm^2	–	106.2	~3	1.35	Haughton et al. (2011)
Corn meal	*Aspergillus Niger*	Artificial	5.6 J/cm^2	300	–	~5	1.35–4.95	Jun et al. (2003)
Eggs (shelled)	*Salmonella* Enteritidis	Artificial	5.6 J/cm^2	3–90	–	~8	2.0–7.7	Keklik et al. (2010b)

Lettuce	Aerobic Mesophiles	Artificial	7 J	675–2700	–	~8	1.24–1.97	Gómez-López et al. (2005)
Salmon fillets skin	*Listeria monocytogenes*	Artificial	5.6 J/cm^2	45–180	–	8.7	0.88–1.02	Ozer and Demirci (2006)
	Escherichia coli O157:H7	Artificial	5.6 J/cm^2	45–180	–	8.7	0.52–1.09	Ozer and Demirci (2006)
Soybean sprouts	Aerobic Mesophiles	Artificial	7 J	675–2700	–	~8	0.65	Gómez-López et al. (2005)
Strawberries	*Botrytis cinerea*	Artificial	7 J	3750	–	~5	>1.00	Marquenie et al. (2003)
	Salmonella	Artificial	5.6 J/cm^2	15–180	–	~8	0.3–3.4	Bialka and Demirci (2007)
	Escherichia coli O157:H7	Artificial	5.6 J/cm^2	15–180	–	~8	0.4–3.9	Bialka and Demirci (2007)
Wheat flour	*Saccharomyces cerevisae*	Artificial	1.95 J/cm^2	64	–	~9	0.7	Fine and Gervais (2004)
White cabbage	Aerobic Mesophiles	Natural	7 J	675–2700	–	~8	0.64–0.84	Gómez-López et al. (2005)

Table 19.5 Summary of published data for microbial inactivation of liquid foods by pulsed UV light

Food type	Microorganism	Contamination method	Energy/pulse	Total energy (J/cm^2)	No. of pulses	Intial population (Log_{10} CFU/mL)	Reduction (Log_{10} CFU/mL)	Reference
Apple cider	*Escherichia coli*	Artificial	5.6 J/cm^2	13.1	3–36	~9.00	5.76	Sauer and Moraru (2009)
	Escherichia coli	Artificial	5.6 J/cm^2	13.1	3–36	~9.00	7.15	
Honey	*Clostridium*	Artificial	5.6 J/cm^2	–	135	6.32	0.89–5.46	Hillegas and Demirci (2003)
Milk (bulk tank)	Aerobic Mesophiles	Natural	0.5–2.0 mJ/cm^2	25 J/cm^2	110	~3.00	>2	Smith et al. (2002)
	Serratia marcescens	Artificial	0.5–2.0 mJ/cm^2	25 J/cm^2	110	~5.00	>4	Smith et al. (2002)
Milk (skim)	*Escherichia coli*	Artificial	–	14.9 J/cm^2	–	~7.00	3.4	Miller et al. (2012)
Milk (whole)	*Escherichia coli*	Artificial	–	8.4 J/cm^2	–	~7.00	>2.50	Miller et al. (2012)
Water	*Klebsiella terrigena*	Artificial	0.25 J/cm^2	–	2	5.5	>7	Huffman et al. (2000)
	Rotavirus SA11	Artificial	0.25 J/cm^2	–	2	5	>4	
	Poliovirus type 1	Artificial	0.25 J/cm^2	–	2	4.5	>4	
	Cryptosporidium parvum	Artificial	0.25 J/cm^2	–	2	4.25	>4	

Chatterley and Linden (2010) evaluated the efficacy of UV-LED at 265 nm for inactivation of *E. coli* in water and compared it to conventional low-pressure UV lamps. The result showed that both systems provided an equivalent level of inactivation, which indicates UV-LED has the potential to be an alternative to traditional UV lamps.

Song et al. (2016) summarized the results from the published studies on UV-LED decontamination of water. Some selected results from this review article is shown in Table 19.6. The data represented in the following tables focuses on a small portion of published data to provide a general range of microbial reduction in liquids.

Song et al. (2018) compared continuous and pulsed UV-LED irradiation for the inactivation of pure *E. coli* and coliphage MS2 in buffered lab water, as well as *E. coli* and total coliform in wastewater, based on the equivalent UV fluence and exposure intensity. The results of this study showed that with equivalent UV fluence of continuous and pulsed UV-LED treatment at 265 nm, comparable inactivation was obtained for coliform, *E. coli*, and virus (MS2). The study also indicated that pulsed UV-LED has a better thermal management of high energy UV-LED.

Li et al. (2010) also evaluated the germicidal effects of low-frequency pulsed ultraviolet light emitting diode on *Candida albicans* or *E. coli* biofilms. The results showed that a 20 min of pulsed irradiation (100 Hz) greatly damaged both microbial species. Moreover, over 99.9% of the microorganisms were inactivated at 60 min of pulsed irradiation (100 Hz). This study concluded that pulsed irradiation had significantly greater germicidal effect than continuous irradiation.

19.5 Effects of UV, PUV, or UV-LED on Food Quality

The use of UV light as an effective microbial reduction intervention in food processing is only beneficial if there are no significant negative quality changes associated with the treatment. UV, PUV light, and UV-LED involve no chemical agents and therefore expected to have minimal impact on the flavors and aromas. However, UV, UV-LED, and PUV light have the potential to promote photochemical changes in food products. The following sections highlight the effect of UV, PUV light, and UV-LED on quality changes in food.

19.5.1 UV Light

Photochemical changes in a food product is dependent on the absorption of photons and the amount of energy that is delivered to the surface of the products. The UV wavelength with the greatest germicidal response is delivered at 253.7 nm. At this specific energy wavelength, photons contain 112.8 kcal/Einstein (1 Einstein equals 1 mol of photons) of radiant energy which has the potential to disrupt O-H, C-C, C-H, C-N, H-N, and S-S bonds (Spikes 1981). Depending on the chemical structure

Table 19.6 Summary of published data for microbial inactivation of liquid foods by UV-LED

Food type	Microorganism	Contamination method	Wavelength (nm)	UV dose (mJ/cm^2)	Log_{10} inactivation	Reference
Apple juice	*Escherichia coli* K12	Artificial	254	707.2	3.58–3.92	Akgün and Ünlütürk (2017)
	Escherichia coli K12	Artificial	280/365	771.6	3.54–3.72	
Water	*Bacillus subtilis*	Artificial	250	59.2	3.00	Morris (2012)
	Mesophilic bacteria	Artificial	254	0.73	0.80	Chevremont et al. (2012)
	Mesophilic bacteria	Artificial	280/365	5.59	3.50	
	MS2	Artificial	255	60	2.30	Bowker et al. (2011)
	Escherichia coli	Artificial	255	9	2.70	
	Escherichia coli	Artificial	265	20	3.40	Chatterley and Linden (2010)
	Escherichia coli	Artificial	265	10.8	4.00	Oguma et al. (2013)

of foods there may be greater sensitivity to UV wavelengths that are correlated to nutrients that lend themselves to be "light sensitive." These nutrients include vitamin A, carotenes, cyanobalamin (vitamin B_{12}), vitamin D, folic acid, vitamin K, riboflavin (vitamin B_{12}), tocopherols (vitamin E), tryptophan, and unsaturated fatty acids. It is reported that vitamin D is the specifically altered by UV wavelengths. Certain natural pigments are also light sensitive. Carbohydrate tend to be unaffected by light (Spikes 1981). UV light has not been reported to have any negative effects on water quality.

Wallner-Pendleton et al. (1994) treated chicken carcasses with 82,560–86,400 μWs/cm^2 of ultraviolet energy and reported that the treatment had no deleterious effects on color or lipid oxidation values. After 10 days of storage at 7 °C, Thiobarbituric acid (TBA) values of thigh meat were 1.3 mg malonaldehyde/kg meat compared with 1.7 for controls. UV light treatment has also been associated with sensory defects and oxidation of milk fats (Reinemann et al. 2006). Coufal et al. (2003) treated hatching eggs with UV to evaluate the surface decontamination and did not observe any adverse effects on the fertility of eggs or hatchability. UV light treatments can enhance several nutraceuticals in fruit (Koutchma et al. 2018). UV light treatment increased the quantities of antioxidants and other health-beneficial compounds in fruits including grapes, pears, apples, blueberries, strawberries, pepper fruits, tomatoes, peaches, mangos, oranges, and grapefruits.

More research is needed to further understand both the positive and negative effects of UV light on food products.

19.5.2 PUV Light

19.5.2.1 Effect of PUV Light on Lipid Oxidation

The visible and infrared light portion of pulsed light can increase the temperature of the food causing undesirable quality changes including potential increase in microbial load. UV light also serves as an activation agent of lipid peroxidation. Oxidation in food products can lead to generation of off flavor and increased rancidity. High amounts of lipid peroxidation were observed on frankfurters treated with pulsed light when the distance from the lamp was reduced, which correlates to an increase in total energy exposure (Keklik et al. 2009).

UV light induces oxidation processes in meat which affects the sensorial properties of the product. This disadvantage is also observed with PUV light but is limited due to the short pulsation. Paskeviciute et al. (2011) observed lipid oxidation in meat and reported that 0.204–1.019 mg of malonaldehyde (MDA) per kilogram of meat was produced when treated with up to 2.7 J/cm^2 of PUV light. In the same study, there was no change in flavor or taste observed in raw meat, chicken broth, or cooked meat treated with 5.1 J/cm^2 of PUV light. A similar study by Keklik et al. (2010a) reported the effects of PUV light treatment on lipid oxidation of unpackaged chicken breast. Reported values were 5.87 and 12.43 μg of MDA/10 g of meat after a

5 second treatment at 13 cm and a 60 second treatment at 5 cm, respectively. Untreated controls were reported to have 5.42 μg of MDA per 10 g of meat. In another study, milk was treated with PUV light at a distance of 4 cm from the xenon lamp for 1, 3, 5, 7, and 10 pulses (2.2 J/cm^2/pulse). After treatment it was reported that PUV light did not cause the oxidation of fatty acids (Elmnasser et al. 2008). PUV light can potentially promote oxidation, but it is also possible to minimize the negative effects by optimization and control.

19.5.2.2 Effect of PUV Light on Protein Oxidation

Protein oxidation is identified as one of the leading causes of quality deterioration in meat, including poultry. The progression of protein oxidation stems from the presence of high concentrations of unsaturated lipids, heme pigments, metal catalysts, and other free oxidizing radicals. The sensory changes associated with protein oxidation in meat and poultry include deterioration of flavor, discoloration, nutrient destruction, and formation of toxic compounds. Fernández et al. (2014) treated vacuum-packaged cheese slices with PUV light at fluences of 0.7, 2.1, 4.2, 8.4. and 11.9 J/cm^2. Treatments of 4.2 J/cm^2 and less did not promote protein oxidation, but samples treated with 8.4 and 11.9 J/cm^2 resulted in significantly higher carbonyl concentrations. This study indicates that the high PUV doses contributes to protein oxidation in cheese. In another study, PUV light was evaluated for its effect on milk proteins and lipids. Samples of milk proteins and hydrolysates were treated with 1, 3, 5, 7, or 10 pulses of PUV light at 4 cm below the quartz window of the PUV light unit with a fluence of 2.2 J/cm^2. After treatments, no significant changes in amino acid composition were reported (Elmnasser et al. 2008). More studies are needed to clarify the impact of UV and PUV light on the promotion of protein oxidation.

19.5.2.3 Effect PUV Light on Color

Color is one of the important quality attributes of food along with flavor and texture which determines the overall consumer acceptability of a food product. With respect to meat and poultry, discoloration can be associated with the gaseous environment including the presence or absence of oxygen. Discoloration in food products is a natural chemical change that occurs over time. Processing interventions can increase the overall rate of color change (Wrolstad and Smith 2010). Keklik et al. (2010a) reported the change in the CIE L∗a∗b∗ color space of boneless/skinless chicken breast after treatment with PUV light. Changes in L∗, a∗, b∗ values reported after a 5 second treatment at 13 cm were 0.59, −0.77, and 0.70, respectively. After a 60-second treatment at 5 cm, changes in L∗, a∗, and b∗ values were reported as 23.43, 3.46, and 7.70, respectively. The results by Keklik et al. indicate that increasing proximity and duration (total energy) result in a significant change in the lightness and color of the surface of chicken. Isohanni and Lyhs (Keklik et al. 2009) treated both lean and skin-on chicken fillets with 100 seconds of ultraviolet

light and reported L∗, a∗, b∗ values over a period of 12 days. After 12 days untreated skinless chicken fillets had L∗, a∗, and b∗ values of 56.45, 4.49, and 1.66 while treated fillets had L∗, a∗, and b∗ values of 53.29, 1.74, and −0.33, respectively. After 12 days skin-on chicken fillets had L∗, a∗, and b∗ values of 70.19, 2.92, and 3.40 for untreated product and 72.86, 4.02, and 7.98 for treated products, respectively. The results suggest that there were no significant differences between treated and untreated samples. Gómez et al. (2012) treated apples with PUV light at two different distances from the lamp (5 or 10 cm) with exposures durations of 2–100 s (2.4–221.1 J/cm^2). After treatments of 221.1 J/cm^2 of PUV light, lower $L*$ values and higher $a*$ values were reported on the cut-apple surfaces. On the contrary, the application of 2.4 J/cm^2 maintained the original color of apples slices during storage. In another study, similar results were reported for raspberries and strawberries, where L∗a∗b∗ values did not change significantly due to PUV light treatment (Bialka and Demirci 2007).

19.5.3 UV-LED Light

LED has low heat emission, which reduces heat associated changes on food quality (D'Souza et al. 2015). In addition, it is known that less exposure to light can maintain the quality of foods by delaying senescence and improving phytochemical and nutrient content (D'Souza et al. 2015). Barnkob et al. (2016) reported that certain UV-LED exposure conditions are able to increase the vitamin D_3 content in pig skin. A vitamin D_3 content of 3.5–4.0 μg/cm^2 was produced with 20 kJ/m^2 of UV-LED treatment. This study concluded that the content of vitamin D_3 in food products containing pork skin could be increased by UV-LED exposure. Lante et al. (2016) evaluated the use of UV-LED for reducing enzymatic browning of fresh-cut fruits. Their study concluded that UV-A LED technology has the potential to reduce the browning of minimally processed products as an eco-friendly alternative to traditional methods. The UV-A illuminator prototypes for 9 LEDs and 30 LEDs utilized in this study are shown in Fig. 19.7.

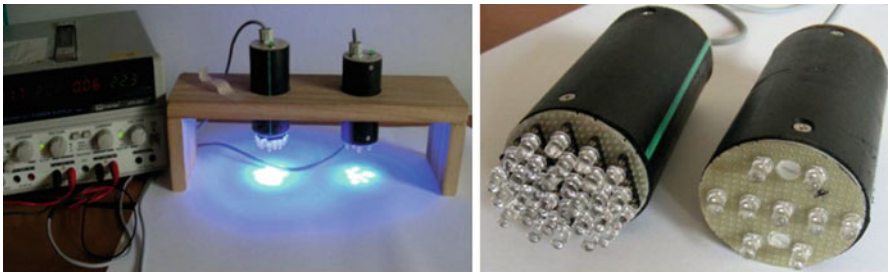

Fig. 19.7 UV-A illuminator prototypes (Lante et al. 2016)

19.6 Limitations and Challenges

The major limitation of UV, PUV, and UV-LED light technologies is that they are primarily surface decontamination treatments. Studies have supported that the high intensity delivered by PUV light has the capacity to penetrate below the surface on certain food matrices though penetration can still be limited. This limitation reduces the effectiveness of the light technologies on products that are reformed or ground (ex. comminuted meat) with effective germicidal response limited to the surface of a product. The complexity of the food surface matrix also influences the overall effectiveness of the light technologies. Pores and crevices on a food surface can protect the microorganisms from UV or PUV light exposure. Liquid treatment by UV and PUV light becomes challenging if the liquid is turbid and casts shadows caused by particulates (Xenon 2006). This shadowing effect reduces the overall germicidal response and effectiveness of the treatment. Uniformity of exposure is a continued challenge for UV and PUV light treatments (Sauer and Moraru 2009; Demirci and Ngadi 2012).

UV lamps pose another challenge due to the risk associated with the use mercury. Leakage of mercury from a UV lamp would be hazardous to individual's health and the environment. PUV light typically use inert gases such as xenon which pose less risk. The visible and infrared wavelengths associated with the broad spectrum produce a significant amount of heat that can increase the surface temperature of the food product. The increase in temperature is not only a confounding variable in microbial inactivation, but also effects product quality undesirably. PUV light wavelengths in the range of 100–240 nm produce ozone. Atmospheric ozone is regulated and must be maintained below 0.1 ppm atmospherically (OSHA 2018).

UV-LED possesses unique advantages compared to conventional lamps and can potentially be used for water treatment (Chen et al. 2017). However the effectiveness of UV-LED for inactivation of microorganisms in food is limited due to the optical density of the food (D'Souza et al. 2015). In addition, studies on the applications of UV-LED in food are limited and need to be discovered. Another limitation is the costs of scale-up of UV-LED system. An economic and new design of the UV-LEDs treatment system is desired since currently many of them are based on traditional UV lamp designs (D'Souza et al. 2015).

19.7 Conclusions and Future Trends

UV, PUV, and UV-LED can effectively inactivate microorganisms and can potentially be used as food decontamination interventions. The application of these technologies in a commercial setting is challenging, mainly due to the complexity of the food types. The microbial reduction obtained by these technologies for ready-

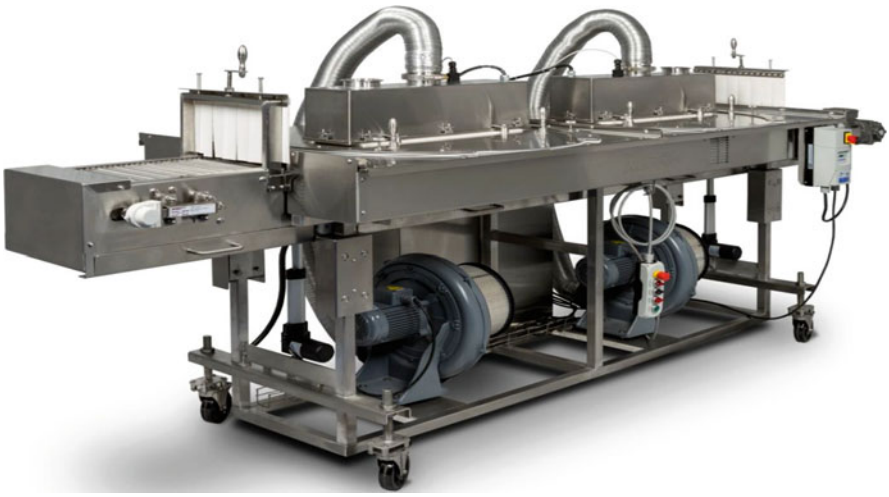

Fig. 19.8 Model Z-500 Pulsed Light System with conveyor (Xenon 2018)

to-cook (RTC) foods is comparable to the $\log_{10}$ reductions currently achieved by chemical antimicrobial interventions. Continued research is needed to better understand the change in organoleptic properties and quality attributes of the products after these treatments. Furthermore, development of lamps that limit the production of heat and ozone would be necessary to ensure that there are minimal effects on food quality and operator safety, respectively. Designing pilot scale systems such as the PUV conveyor system (Fig. 19.8) can provide the opportunity to investigate the effectiveness of the technology in applications similar to commercial settings.

Since the UV-LEDs treatment is mainly effective on food surfaces, researchers have mainly focused on water disinfection systems (Oguma et al. 2013). UV-LED lamps have many advantages such as energy savings, device durability, low environmental impact, high luminous efficiency, and minimal thermal effect (Barnkob et al. 2016). However, the application of UV-LED treatment is limited to water decontamination. More studies on the efficacy of UV-LED on various food products is warranted. In addition, the widespread use of UV- LED is currently severely limited due to the high costs of installing the lighting system (Chen et al. 2017). The performance of LED is expected to continue to improve and costs are expected to fall in the future to meet the demands of the food processing industry.

It is the responsibility of the food producers/manufacturers to produce wholesome, healthy, and safe food. Ultimately, food safety is a shared responsibility of both the food producers/manufacturers and the consumers. No process is perfect and though interventions such as those described in this chapter take steps to ensure a safer food product, there is always risk. Consumers should follow the safe food handling and preparation instructions provided with the product.

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Chapter 20
High Pressure Food Process Design for Food Safety and Quality

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20.1 Introduction

Thermal processing has predominantly been the choice of preservation method for the food industry for pasteurization or sterilization of foods. While thermal processing enables the food processors to ensure microbiological safety of the processed products, prolonged thermal treatment often degrades product quality and heat sensitive nutrients. With the increased consumer awareness on the role diet plays on health and wellness, consumers desire wholesome minimally processed nutritious foods. Consumers are also interested in processed products with minimal or no synthetic chemicals and preservatives. Thus, the food processors have been investigating a number of alternative "nonthermal" lethal agents (including use of high pressure, electric field, gases, cold plasma, irradiation, UV among others) with or without addition of heat to ensure product microbiological safety while having reduced or minimal impact on product quality and nutrients.

Among the nonthermal technological choices, high pressure processing (HPP) has emerged as a technology adapted by food processors to satisfy consumer demand for minimally processed food. The technology is also known as high-hydrostatic pressure processing or ultra-high pressure processing. Earliest

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investigation on food applications of pressure treatment began in the late nineteenth century (Bridgman 1931; Hite 1914).

During high pressure processing, elevated pressures (400–600 MPa), is used at ambient or chilled temperatures (for ~ 3 min holding time) to inactivate variety of microorganisms (non-spore forming bacteria, yeasts, mold, and virus), thereby extending the shelf-life with quality and sensorial properties preserved (Rastogi et al. 2007). Such pasteurization treatment does not destroy product covalent bonds and minimally influence the product chemistry, ultimately retaining its natural freshness (Balasubramaniam and Farkas 2008). The reduced-thermal exposure imparts additional opportunities to process heat-sensitive foods and nutrients (Table 20.1). First pressure pasteurized product (guacamole) was successfully introduced in the U.S. supermarkets by 1997. To date, high pressure pasteurization is nearly 12-billion-dollar market for pasteurization of variety of value-added products (including ready meal, juices, fruits and vegetable products, deli meats, salads, and seafood) (Balasubramaniam et al. 2019).

Bacterial spores are highly resistant to pressure treatment alone (Black et al. 2007b). High pressure in combination with modest heat is needed to inactivate bacterial spores. The process is often called as pressure-assisted thermal process (PATP), which can be used for the commercial sterilization of shelf-stable low-acid foods that can be stored and distributed at ambient temperatures. Food and Drug Administration (FDA) in 2009 & 2015 issued letters of no objection for two industrial petitions for the preservation of two low-acid products by PATP (Juliano

Table 20.1 Key benefits and limitations of high pressure processing

Description	Advantages and limitations
Isostatic pressure	Uniform pressure distribution through product's volume. Treatment time independent of sample volume
Thermal effects	Pressure effects help to reduce thermal exposure; reversible temperature change
Products type	Treatment effective against high moisture content liquids and solids. Due to difference in compressibility, the products containing air pockets may not be good candidate for pressure treatment
Water activity	Treatment efficacy diminishes with products that have low water activity
Process time	Independent of product geometry. Reduced treatment time compared to conventional process
Reaction rate	Pressure and heat can have synergistic, additive or antagonistic reaction rates.
Microbiological efficacy	Pressure treatment in general is effective against various vegetative bacteria, yeast, virus etc. Microbiological efficacy of pressure treatment diminishes with decreasing water activity of the test matrix. Pressure treatment at ambient or chilled conditions cannot be effective against bacterial spores.
Pressure effects against enzymes	Pressure treatment has variable efficacy on the inactivation of various enzymes
Throughput	Batch nature of technology limit throughput

et al. 2012; Stewart et al. 2016). However, at the moment, there are no commercial products treated by PATP available in the market.

Like temperature, pressure is a fundamental thermodynamic variable and has applications beyond food pasteurization and sterilization. This include infusion, freezing and thawing, crystallization, extraction, hydrolysis among others. This chapter provides an overview about key principles, equipment, and packaging options relevant to high-pressure process design and development. The microbiological safety of the pressure treated products is also summarized.

20.2 Basic Governing Principles

Isostatic principle, Le Chatelier's principle, principle of microscopic ordering, and Arrhenius relationship are some of the basic principles govern the high pressure processing of foods.

20.2.1 Isostatic Principle

During HPP, the pressure is transmitted quasi-instantaneously and homogeneously within the product through a hydrostatic effect (Rasanayagam et al. 2003; Ting et al. 2002). The term hydrostatic refers to the transport of force within the fluid at equilibrium. Pressure effects and processing time are independent of product's shape and size. Additionally, having high water activity (a_w) provides effective microbial destruction through high pressure. On the contrary, food with air pockets (marshmallows, leafy vegetables, etc.) or low-water activity products are not suitable candidates for pressure treatment.

20.2.2 Le Chatelier's Principle

Le Chatelier-Braun principle states that with increase in pressure, system's equilibrium will be shifted to a state that occupies the smallest volume (Hamann 1957; Balny and Masson 1993). Therefore, pressure enhances chemical/physical phenomenon that requires a concomitant volume decrease but encumbers certain reactions that require an increase in volume.

According to Arrhenius principle, thermal effects generally accelerate various reactions. Thus, combined pressure-thermal treatments may have synergistic, additive, or antagonistic effects on various reactions (Gupta et al. 2011). Gibb's definition of free energy (G) provides the relationship between pressure (P), temperature (T) and volume (V)

$$G \equiv H - TS \tag{20.1}$$

where H and S are enthalpy and entropy, respectively. Enthalpy constitutes of internal energy of system (U) and product of pressure and volume.

$$H \equiv U + PV \tag{20.2}$$

Combining Eqs. (20.1) and (20.2) illustrates the change in Gibb's definition of free energy:

$$d(\Delta G) \equiv \Delta V dP - dT\Delta S \tag{20.3}$$

For example, an increase in pressure reduces the volume, but increases the temperature of the substance. Application of pressure reduces product volume; however, the resulting thermal effects can influence energy changes as well.

20.2.3 Principle of Microscopic Ordering

Volume or free space of a given food substance decreases as a result of increase in pressure. This physical compression increases the degree of ordering of molecules of a given substance at a constant temperature. Interestingly, pressure and temperature expend antagonistic forces on molecule structure and chemical reactions. The principle of microscopic ordering states that at constant temperature, the degree of ordering of molecules of a given substance increases with the increase in pressure (Balny and Masson 1993).

20.3 Equipment

Industrial scale high pressure processing (HPP) essentially a batch process that comprises of the following five components (Fig. 20.1):

1. Pressure vessel: Pressure vessel is where pre-packaged product receives the target pressure-(thermal) treatment. Pressure vessels can be fabricated as monolithic, multiwall or wire-wound vessels. Monolithic vessels are less expensive and are fabricated from low-alloy steel with high tensile strength. They operate at moderate pressures (<400 MPa) with maximum internal diameter of 15 cm. Such vessels are not generally employed for operating pressures greater than 400 MPa as the vessels may be damaged as a result of plastic deformation and/or crack formation.

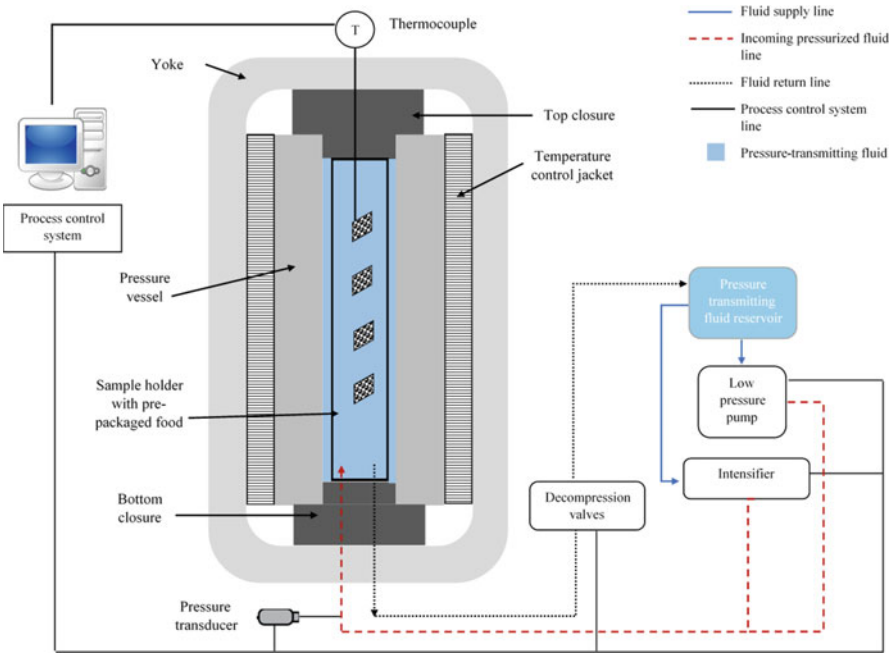

Fig. 20.1 Schematic diagram illustrating key components of a batch high pressure processing system

Shrink fitting of series of concentric cylinders on each other makes multiwall vessel. This limits crack propagation in a particular vessel layer as opposed to all layers.

Finally, wire-wound vessels are fabricated using a wire-wound technology. The high-strength wire is layered (under tension) on a thin wall core of a pressure vessel. Pressure vessels available in the food manufacturing operations are typically made using wire-wound technology as this enables the food processors to operate the vessel for thousands of cycles. Wire wound technology also has built in 'leak-before-break' safety mechanism, which minimizes the chances of catastrophic failure. Further, unlike monolithic, wire-wound option enable fabrication of larger diameter pressure vessels that can withstand higher operating pressures (~600 MPa).

Industrial scale high pressure equipment is commercially available as vertical or horizontal configurations with varying volumes, from 35-L to 525-L (Fig. 20.2). They are often housed and operated in a chilled environment. Laboratory or pilot scale pressure vessels are typically fabricated using monolithic or multi-wall option.

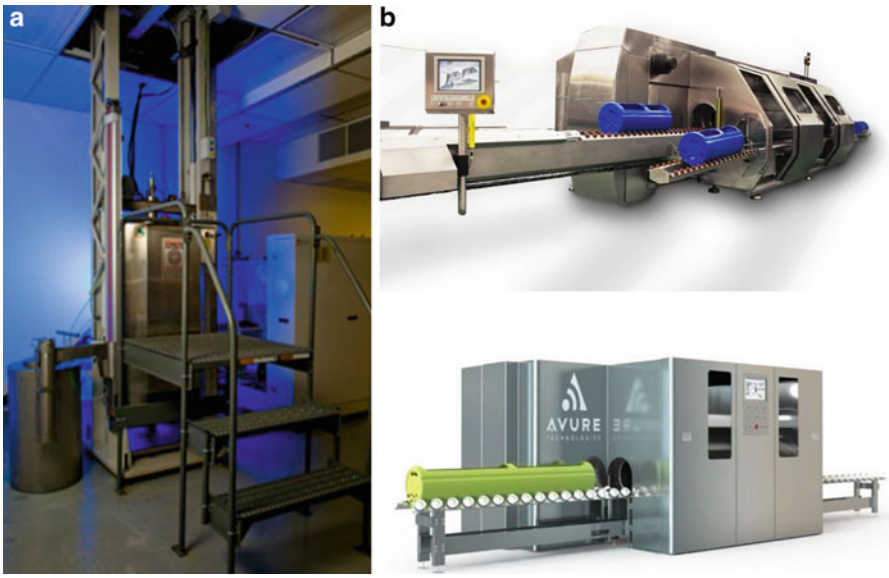

Fig. 20.2 (**a**) Pilot-scale 5 L high pressure equipment and (**b**) commercial high pressure equipment

2. End closures: The content of the pressure vessel during pressure treatment is secured by a set of top and bottom end closures.
3. Yoke: Yoke is an external frame that encompasses pressure vessel with two end closures and securely keep them together during operation. Yoke can be made from wire-wound steel frame or laminated steel plates.
4. Pressure pump and intensifiers: External pump and intensifier mechanisms is employed to pressurize the content of the pressure vessel to target pressure. Larger pressure vessels use indirect pressurization method where the vessel is first filled with pressure transmitting fluid through low-pressure pump. Water is commonly used as the pressure-transmitting fluid in industrial scale vessel while solutions such as propylene glycol are used in lab- or pilot-scale equipment. Balasubramanian and Balasubramaniam (2003) demonstrated that the composition of pressure-transmitting fluids can alter the heat of compression characteristics of the pressure transmitting fluid and thus have further influence on microbiological inactivation.
5. Process control system: A process control computer is used to control high pressure operations, monitor process (pressure, temperature and holding time) parameters, and store electronic processing records. The hardware must be compatible to be operated in a food plant environment which is user-friendly and can be easily sanitized. The sensors should be periodically calibrated. The industrial scale equipment also employs redundant sensors (Ting 2011). Pressure transducer drift or failure modes should be foreseen and addressed.

20.4 Packaging Materials and Containers

During HPP, the product is typically packaged using high barrier flexible or semi-rigid containers. This accommodates transient volume reduction (typically 15%) with application of pressure. At least one interface of the package should be flexible enough to transmitting the pressure to the foods (Balasubramaniam et al. 2004). Thus, rigid metal cans or glass bottles are not suitable packages for HPP. Some of the common polymer or copolymers packages commercially used are polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP) and ethylene vinyl alcohol (EVOH) (Juliano et al. 2010; Ayvaz et al. 2012). Other types include co-extruded films with polymeric barrier layers, vacuum deposited coating or polymer-metallic laminated films (Richter et al. 2010).

Selection of packaging materials depends on its composition, geometry, sealing and barrier (water, oxygen, and light) properties, and heat transfer. PP and PE packages exhibited higher compression heating than water (Schauwhecker et al. 2002; Knoerzer et al. 2010), which can create thermal differences within the process vessel. Packaging properties are subject to change under combined pressure-thermal treatment. For instance, 70% decrease in oxygen-transmission rate (OTR) and 25% decrease in water vapor transmission rate (WVTR) were observed by Kovarskii (1994) that analyzed HPP treatment effects on PET package. Alternatively, various metalized-polymer packages were damaged with concomitant barriers losses when treated at 600 or 800 MPa, 45 °C for 5–20 min. Pressure-assisted thermal processing (PATP) studies reported delamination of PP-Al layers in meals, ready-to-eat (RTE) pouches processed at pressures above 200 MPa, at 90 °C for 10 min. Similarly, the alterations of barrier properties in AlOx or SiOx laminates processed at 600 MPa, 110 °C for 5–10 min (Bull et al. 2010). Thus, such changes in barrier properties of the packaging material during pressure-thermal treatment can also adversely impact the shelf-life and quality attributes of the product during extended storage (Ayvaz et al. 2012).

It is also important to minimize headspace air in the package as oxygen can become reactive under pressure (Okamoto 1992) and induce adverse reactions. Thus, vacuum packaging is the preferred step to protect food during storage and allows maximum number of products to be processed per cycle. In summary, high barrier flexible pouches are commonly used in high pressure pasteurization applications. However, more research is required to identify suitable packaging polymers that withstand PATP conditions. Potential application of nanocomposite packaging material for such applications is also worth further investigation. While not a commercial practice, research is on-going to develop ‘continuous’ high pressure system for the preservation of liquid foods. Here the processed liquid product is packaged in pre-sterilized containers under a clean room environment like an aseptic packaging systems (Ayvaz et al. 2016).

20.5 Typical Process

A typical batch high pressure process operation begins with loading pre-packaged food samples (at certain initial temperature) into a polymeric carrier basket. The pressure transmitting fluid fills rest of the empty void space in the carrier basket. The sample carrier basket with the loaded contents are transferred into the high pressure vessel, which also contains pressure-transmitting fluid. The vessel is then sealed with end closures. A yoke secures the pressure vessel to repress top and bottom closures under pressure. The target pressure is achieved through compression of pressure-transmitting fluid through simultaneous action of pump and intensifiers. Depending upon the intensity of the target process (pasteurization and sterilization), care must be exercised to appropriately control the initial and process temperature of the food matrices as well. The product is held for a set amount of time at the target pressure-temperature (usually less than 10 min) and depressurized at the end of holding time.

20.6 Representative Pressure-Thermal History

Figure 20.3 depicts a typical pressure-temperature curve for a food sample undergoing a high pressure treatment. First, the vacuum packaged product is thermally pre-conditioned at the desired initial temperature (from T_0 to T_1) during pre-conditioning time (t_1). The carrier basket containing the product is subsequently loaded inside the pressure vessel over certain loading time (t_2).

Depending on the desired treatment intensity (pasteurization or commercial sterilization), the pressure vessel is also pre-conditioned to desired process temperature and subsequently filled with thermally preconditioned pressure-transmitting fluid. Two end closures are used to seal the pressure vessel. The end closures are further secured using a yoke mechanism during pressure treatment. The vessel is then pressurized from atmospheric pressure (P_1) by pumping pressure-transmitting fluid into the pressure chamber until the vessel reach the target pressure (P_2). The typical come-up time (t_3) for commercial scale equipment is about 2 min (to reach a target pressure of 600 MPa) and is a function of horsepower of the pumping mechanism used. During the pressure come-up time, the temperature of the test samples increases to T_3 as a result of isostatic compression. The samples are held at desired process intensity for specified time (holding time, t_4). Shorter pressure holding time (30 s to 10 min) is preferred in industrial practice.

Subsequently, the vessel is depressurized to atmospheric pressure (P_1) within few seconds (t_5) through a set of decompression valves which brings the sample temperature to the original value (T_4).

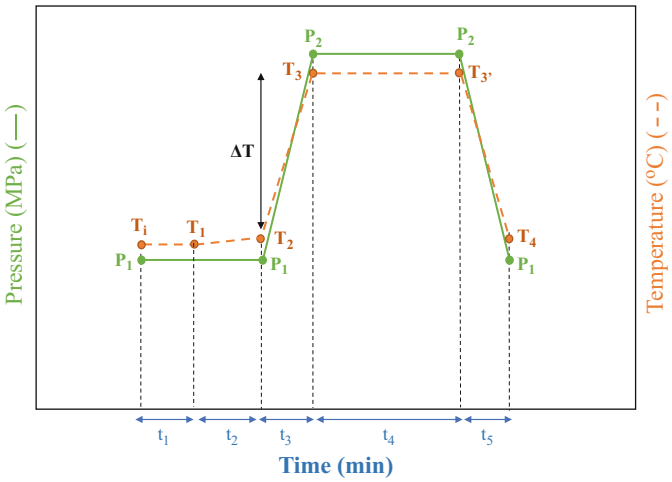

Fig. 20.3 Schematic diagram of pressure-temperature profile of a test sample during high pressure processing. P_1, and P_2 are the atmospheric and target process pressure, respectively. T_i (initial sample temperature), T_1 (temperature after pre-treatment), T_2 (sample temperature just prior to pressurization), T3 (sample temperature immediately after pressurization) T3' (final sample temperature prior to depressurization), and T_4 (final temperature after decompression). t_1, t_2, t_3, t_4 and t_5, are the pre-conditioning, sample loading, pressure come-up, pressure holding time, and decompression time, respectively; ΔT is the transient change in product temperature during pressurization

20.7 Process Uniformity

High pressure processing offers a unique advantage and quasi-instantaneous transmittance of pressure through the product volume. The resulting concomitant temperature change with pressure reduces the severe thermal exposure to the product during processing. Although pressure is assumed to be transmitted uniformly throughout the product, variations in both process pressure and temperature within the treatment chamber can contribute to the process nonuniformity, especially in pressure-assisted-thermal processing (PATP). Therefore, identification of least processed volume is critical during PATP. Thermal gradient generated at elevated processing temperatures under pressure can develop pronounced nonuniformity (Ting et al. 2002). This is influenced by engineering properties of food, packaging and pressure transmitting fluid as well as the design and insulation characteristics of pressure chamber.

During high pressure treatment, the temperature of food and pressure-transmitting fluid increases transiently because of the heat of compression and returns to the initial value upon decompression. Researchers have extensively investigated the heat of compression of various food composition, packaging materials and other fluids

(Bridgman 1931; Otero et al. 2000; Rasanayagam et al. 2003; Patazca et al. 2007). Heat of compression (δ) of water and most high-moisture foods is 3.00 °C/100 MPa at 25 °C, initial temperature. Fats and oils have a higher value of 8.00 °C/100 MPa at 25 °C due to the higher compressibility of long fatty acid chains and lower specific heat (Rasanayagam et al. 2003). While δ of water and most high moisture foods increase as a function of initial temperature, δ values of fatty foods are not influenced by initial temperature. The thermal effects from heat of compression of materials at elevated pressure-thermal conditions can create heat flux between the treated samples, pressure-transmitting fluid and through the pressure chamber, disrupting the uniform process lethality. Density changes in presence of thermal gradient and its resulting free convection must be acknowledged (Hartman 2002; Otero et al. 2007).

Previous researchers have investigated process nonuniformity through various heat-transfer and process lethality models. Hartman (2002) observed that inactivation rate increased with larger vessel volumes, although more than one log variation of surviving cell population was evident, depending on the packaging type and its location in pressure vessel. Nguyen et al. (2014) formulated an integrated process lethality model to assess the bacterial spore inactivation at PATP conditions. Spatial temperature variation within the sample carrier basket resulted in 3.5 log variation in surviving bacterial spores.

Process nonuniformity can be mitigated though many approaches that will control the temperature of constituents during high pressure processing. Maintaining certain uniform initial temperature of the packaged food and pressure transfer fluid (after taking into consideration respective heat of compression values) can help to minimize process non-uniformity during target treatment intensity. Having an external jacket as a part of the pressure vessel can also help to maintain certain process temperature. Adjusting factors such as ratio of sample to vessel volume, insulation characteristics of packaging material, carrier basket and the pressure vessel wall can also further help to maintain uniform process conditions.

20.8 Process Development with Pressure-Based Technologies and Its Microbiological Safety

20.8.1 Pasteurization

High pressure pasteurization involves pressure treatment of the prepackaged foods to 400–600 MPa at chilled or ambient conditions (for about 3–10 min). The process can inactivate variety of pathogenic and spoilage non spore forming bacteria, yeasts, molds, and viruses. However, this treatment is inadequate against bacterial spores and has variable effect on enzymes. Therefore, it is recommended to distribute and store pressure-pasteurized products under refrigeration (Balasubramaniam et al. 2016) .

Similar to thermal processing, the magnitude of microbial reduction is a function of food composition, pH, a_w, and type of microorganism. The ability to resist pressure can vary between microorganisms and its strains and depends on the stages of growth cycle. Water activity (a_w) of foods has a major influence on high pressure treatment. High moisture foods are suitable for high pressure processing. Examples of high pressure pasteurized products commercially available in United States, Europe, Japan, Australia China, India include guacamole, salad, seafood, deli meat, oysters, fruit juices and ready-to-eat meals, salsa, smoothies (Norton and Sun 2008; Mujica-Paz et al. 2011; Tonello 2011).

20.8.2 Commercial Sterilization

Pressure-assisted thermal processing (PATP) is an emerging commercial sterilization technology for producing shelf-stable low-acid foods. PATP requires preheating the prepackaged food to 75–90 °C and with subsequent combined application of pressure (400–600 MPa) with heat (90–120 °C). The combined treatment intensity can be used to sterilize low-acid foods within modest time (3–15 min) as opposed to 30–60 min needed for traditional retort process. Although not commercially available yet, PATP offers great benefits in producing shelf-stable low-acid products such as sauces, soups egg- and milk-products, ready-to-eat foods and vegetables (Juliano et al. 2012). In 2009, FDA issued no objection to an industrial petition for PATP sterilization of mashed potatoes (Stewart et al. 2016).

Pressure-ohmic-thermal sterilization (POTS) that combines application of elevated pressure with ohmic heating to produce shelf-stable low-acid foods or extended shelf-life foods. The collective effort of heat of compression effects under pressure and ohmic heating reduces thermal exposure on product quality (Park et al. 2013, 2014). Thus, this technology avoids the need for preheating the pressure vessel and saves time and energy required for processing. Increasing product temperature during treatment holding time can be adjusted by controlling Ohmic heating under pressure.

20.8.3 Extended-Shelf-Life

Extended-shelf-life (ESL) foods are defined as those foods that receive intermediate treatment intensity between those received by pasteurized and commercially sterilized products. Accordingly, they have longer shelf-life than its pasteurized counterpart when stored refrigerated (Balasubramaniam et al. 2016). This technology can be applied on low-acids foods with refrigerated storage post-treatment or can produce shelf-stable acidified foods. There are limited studies that investigated the efficacy of combined pressure-thermal treatments to produce ESL foods. For example, Legan et al. (2008) reported higher log reductions of non-proteolytic *Clostridium botulinum*

spores treated at combined pressure-thermal treatment than thermal treatment. Daryaei et al. (2013) showed that pressure level of 600 MPa and temperatures of 60–85 °C yielded more than 7-log reductions of *Bacillus cereus* spores in cooked rice. This combined treatment can be effective against psychrotrophic spores (including non-proteolytic *Clostridium botulinum* spores), vegetative bacteria, molds, yeasts and virus. Researchers recommended using strains of non-proteolytic *Clostridium botulinum* spores for validation studies of combined pressure-thermal treatment. Kamat et al. (2018) demonstrated shelf-stability of extended shelf life, acidified vegetables treated by modest pressure-thermal treatment conditions. At present, there are no commercial ESL products, but further research is needed to investigate the kinetics of destruction of various modest pressure-thermal resistance bacterial spores as well as storage stability of the treated ESL products.

20.8.4 Continuous High Pressure Processing for Liquid Foods

While liquid beverages processed by high pressure pasteurization is commercially available, the batch nature of the technology impedes adaptation of HPP for commodity oriented liquid beverages that requires a higher throughput. A more continuous high pressure based approaches are desired.

Ultra-shear technology (UST), also referred as high pressure homogenization (HPH) involves forcing a pressurized fluid through confined gap to an area of lower pressure (Fig. 20.4). Raw product is filled inside the pressure chamber to

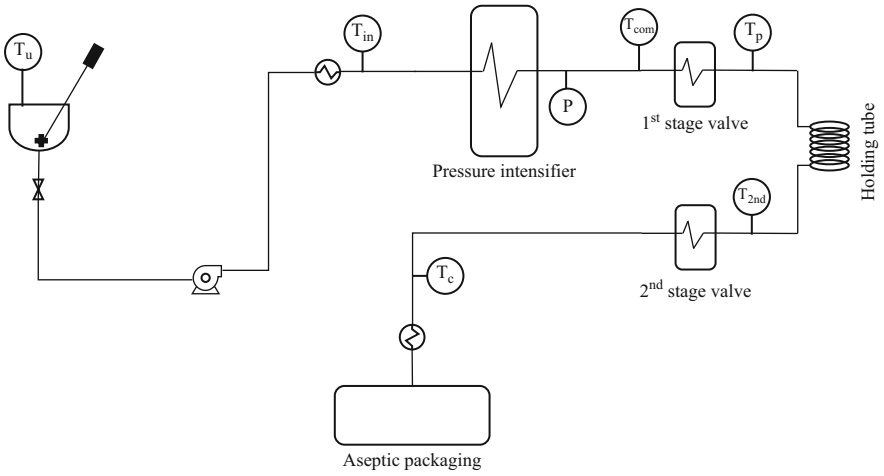

Fig. 20.4 Schematic drawing of continuous high pressure flow process (referred as high pressure homogenization or ultra shear technology). (Figure adapted from Martínez-Monteagudo et al. 2017)

amplify the atmospheric pressure of product to target pressure with the help of the intensifier. The product is then forced through the throttling valve where the fluid migrates through the minute gap. As the product travels through the gap, it experiences shear, cavitation, and turbulence that not only disrupt the particle size, but dissipate heat. Furthermore, this technology has potential applications in nanoemulsion and modifications of molecular structures, viscosity and sensorial properties. However, microbial safety and alterations in product quality are influenced by pressure intensity, process temperature, residence time, valve geometry, and product parameters (Martínez-Monteagudo et al. 2017). More research is needed to understand the impact of pressure, temperature, shear, and valve geometry on food safety and quality attributes of the treated beverages.

20.8.5 Factors Influencing Microbiological Safety and Stability of Pressure Treated Products

Microbial inactivation in pressure-treated products is governed by both process parameters (pressure, process temperature, holding time) and product parameters (food composition, pH, a_w) (Fig. 20.5).

Generally, vegetative cells of pathogens and spoilage microorganisms (bacteria, yeasts and mold) can be inactivated at 400–600 MPa pressure at chilled or ambient temperature. However, variations in pressure resistance exists between microorganisms, strains as well as different stages of growth cycle. Gram-positive bacteria are more pressure resilient than Gram-negative bacteria due to their greater rigidity of cell envelope (Table 20.2). Conversely, complex cell membrane structure of Gram-negative bacteria effortlessly inclines them to high pressure inactivation. The physiological state of the microorganism can also influence the response to pressure treatment. Cells in the exponential (log) phase are more sensitive to pressure than cells in the stationary phase. There is no correlation between thermal and pressure resistance of microorganisms as the inactivation mechanisms exerted by the lethal treatments are distinctive.

Bacterial spores (*Clostridium*, *Bacillus,* and *Alicyclobacillus*) and ascospores are highly pressure resistant but can be inactivated through combined treatment of pressure (400–600 MPa) and temperature (90–120 °C) (i.e. pressure-assisted thermal processing, PATP) (Table 20.3). Within the bacterial spores, pathogenic spore strains of *Clostridium botulinum* have been identified to be greatly resistant to pressure-temperature treatment (Stewart et al. 2016).

Moderate pressure (100–400 MPa) combined with 20–50 °C process temperature can trigger spore germination, if any present, through inactivation of nutrient germinant receptors (Black et al. 2007b). Such approach does not provide assurance for 100% spore germination and treatment conditions that render target level inactivation of bacterial spores are desired.

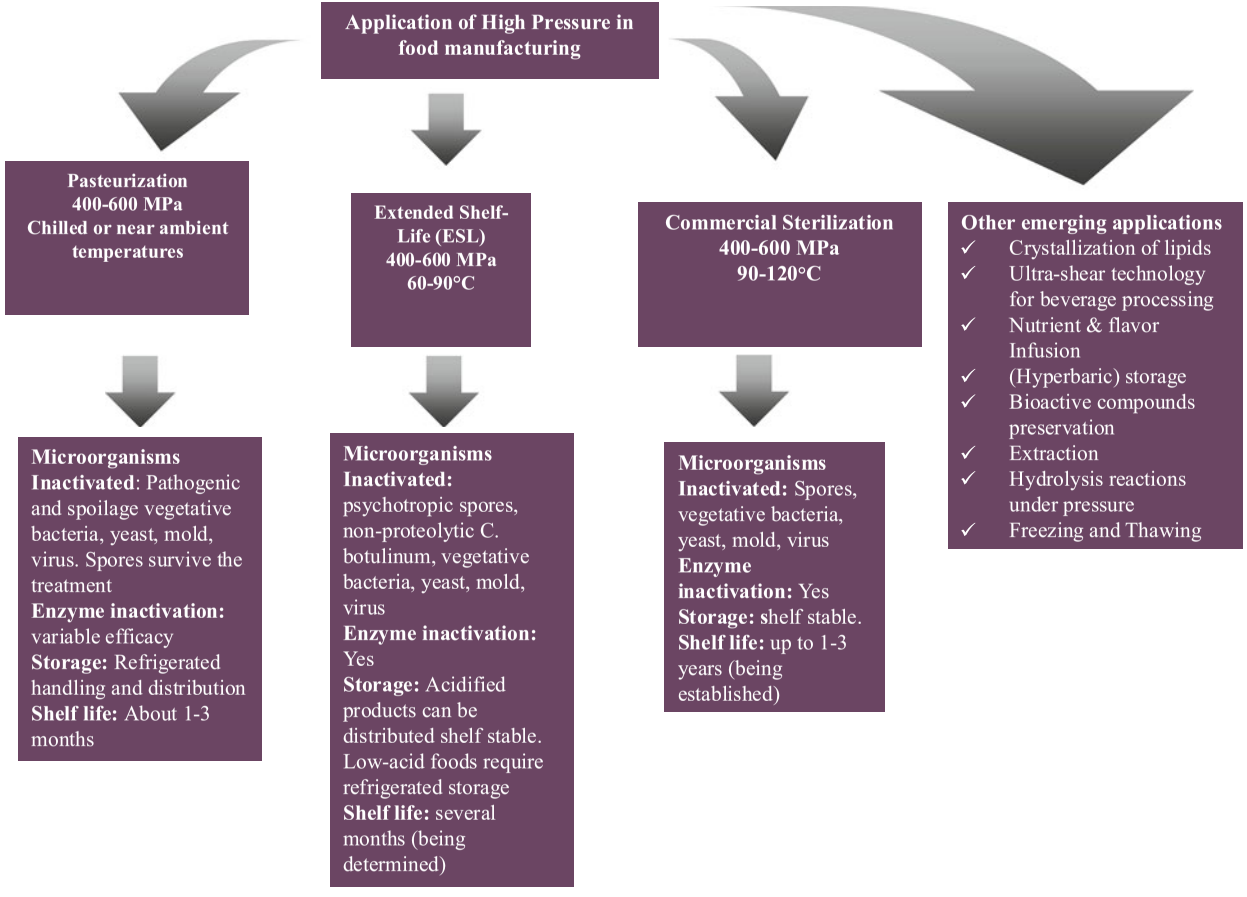

Fig. 20.5 Various applications of high pressure based technology in the food industry

Table 20.2 Inactivation of different spoilage and pathogenic vegetative microorganisms during high pressure treatment at ambient or chilled process conditions

Microorganism type		Suspending medium	Process conditions	Inactivation (log_{10} reduction)	Reference
Spoilage or pathogenic vegetative bacteria	*Campylobacter jejuni*	Pork slurry	300 MPa, 25 °C, 10 min	6.0	Shigehisa et al. (1991)
	Escherichia coli O157:H7	Ultra-high temperature (UHT) milk	600 MPa, 20 °C, 15 min	≤2.0	Patterson et al. (1995)
		Poultry meat	600 MPa, 20 °C, 15 min	3.0	Patterson et al. (1995)
		Ground beef	400 MPa, 12 °C, 20 min	4.4	Morales et al. (2008)
	Lactobacillus viridescens	Ham	500 MPa, 25 °C, 5 min	4.0	Park et al. (2001)
	Lactococcus	Fresh lactic curd cheese	300–600 MPa, ≤22 °C, 5 min	≤7.0	Daryaei et al. (2006, 2008)
	Listeria monocytogenes	Orange juice	600 MPa, 25 °C, 10 min	6.5	Erkmen and Dogan (2004)
		Frankfurters	500 MPa, 31 °C, 3 min	5.0	Lucore et al. (2000)
	Pseudomonas fluorescens	Minced beef	200 MPa, 20 °C, 20 min	5.0	Carlez et al. (1993)
	Salmonella Enterica	Navel and Valencia orange juices	600 MPa, 20 °C, 1 min	≤7.0	Bull et al. (2004)
Yeasts	*Saccharomyces cerevisiae* (vegetative cells) *Penicillium roqueforti* (vegetative cells)	Pork slurry Cheese	300 MPa, 25 °C, 10 min 500 MPa, 20 °C, 20 min	<1.0 >6.0	Shigeshia et al. (1991) O'Reilly et al. (2000)
Mold	*Aspergillus niger* (vegetative cells)	Tomato	350 MPa, 10 °C, 20 min	4.0	Arroyo et al. (1997)

Table 20.3 Inactivation of different bacterial spores during combined high pressure-thermal treatment

Bacterial spores	Suspending medium	Process conditions	Inactivation (log_{10} reduction)	Reference
Alicyclobacillus acidoterrestris NZRM 4447	Malt extract broth	600 MPa, 65 °C, 7 min	2.8	Uchida and Silva (2017)
Bacillus cereus (strains NZ 3, NZ 4, NZ 5, NZ 6, and NZ 7)	9.5% reconstituted skim milk	600 MPa, 72 °C (initial temperature), 1 min	3.6–6.1	Scurrah et al. (2006)
Bacillus amyloliquefaciens TMW 2.479 Fad 82, TMW .482 Fad 11/2	Deionized water	700 MPa, 121 °C, 1 min	8.0	Ahn et al. (2007)
Bacillus amyloliquefaciens fad 82	Egg patty mince	700 MPa, 121 °C, 1 min	6.0	Rajan et al. (2006a)
Geobacillus stearothermophilus ATCC 7953	Egg patties	700 MPa, 105 °C, 5 min	4.0	Rajan et al. (2006b)
Bacillus coagulans 185A	Tomato juice	600 MPa, 85 °C, 7 min	≥7.0	Daryaei and Balasubramaniam (2013)
Bacillus cereus ATCC 9818	Cooked rice	600 MPa, 85 °C, 4 min	≥7.0	Daryaei and Balasubramaniam (2013b)
Clostridium botulinum nonproteolytic type B (strains ATCC 25765 and TMW 2.518)	Mashed carrots (pH 5.15)	600 MPa, 80 °C, 1 s	>5.5	Margosch et al. (2004)
Clostridium botulinum proteolytic type A (strain ATCC 19397)	Mashed carrots (pH 5.15)	600 MPa, 80 °C, 12 min	>5.0	Margosch et al. (2004)
Clostridium sporogenes	Deionized water	700 MPa, 121 °C, 1 min	8.0	Ahn et al. (2007)

20.8.5.1 Process Parameters

Process parameters such as pressure level, process temperature, process come-up time, and treatment time (holding time) influence the microbial inactivation efficacy of the pressure treated foods. These parameters are discussed below:

(i) *Pressure*: Low to moderate pressure creates a tailing effect in microbial inactivation (decrease in inactivation with time) (Hoover et al. 1989; Earnshaw et al. 1995; Smelt 1998). Above a certain pressure threshold, death rate of microorganisms becomes prominent at increasing pressure. Some of pressure-induced damages include cell membrane disruption, inactivation of critical proteins responsible for replication and alteration of cell morphology.

(ii) *Temperature*: Pressure applied at low (<20 °C) or moderate temperatures (~45 °C) is more effective to inactivate vegetative microorganisms. The resistivity of microorganisms to pressure treatment becomes dominant at room temperature (~25 °C). Bacterial endospores require even more severe treatment: high pressure (400–600 MPa) combined with high process temperatures (90–120 °C) for inactivation (Rajan et al. 2006a, b; Ahn et al. 2007; Bull et al. 2009; Daryaei et al. 2013b).

(iii) *Process come-up time*: Come-up time refers to the amount of time required to increase pressure of food from ambient pressure to a target pressure. This is often depended on the compressibility of the packaged food and pressure-transmitting fluid, pressure generating pump, and the sample to volume ratio. Typical process come-up time is 2–4 min. Longer come-up times can add to the total process time, which can affect the kinetics of microbial inactivation. Although microbial inactivation occurs during a set time at process-thermal treatment, a fraction of bacterial spore inactivation has been reported during the come-up time (Margosch et al. 2004; Koutchma et al. 2005; Ahn et al. 2007). Rajan et al. (2006a) observed about 1.2-log reductions of *Bacillus amyloliquefaciens* in egg patty mince for come-up time of 0.7 min for 700 MPa at 121 °C treatment while only 0.1-log reduction was observed for 500 MPa, 95–105 °C with come-up time of 0.5 min. The extent of spore inactivation during come-up time varies among different vegetative microorganisms as well as bacterial spores. Thus, it is important to pay attention to process come-up time (Margosch et al. 2004; Ahn et al. 2007).

(iv) *Processing time*: Processing time (also known as pressure holding time) specifies the amount of time imposed onto the food to ensure sufficient lethality. It refers to the time between come-up time and decompression which is usually 3 to 10 min. In general, increasing the holding time at higher pressures leads to greater inactivation of vegetative microorganism (*Salmonella typhimurium* and *Listeria monocytogenes*) and spores (*Bacillus amyloliquefaciens*) (Chapleau et al. 2006; Ahn et al. 2007; Ratphitagsanti et al. 2009; Kaur et al. 2016). For example, a treatment of 700 MPa, 105 °C, 5 min holding time resulted in 6-log reductions of *Bacillus amyloliquefaciens* than 2 min of holding time at same pressure-temperature conditions which resulted in 3-log reductions. Depending on the pressure level and type of microorganism of concern, the holding time is adjusted to provide sufficient time for the lethal effect to be adequate to destroy any surviving viable cells.

20.8.5.2 Product Parameters Affecting High Pressure Treatment

Product parameters such as food composition, a_w and pH of the foods can affect the microbial efficacy of pressure-thermal treated foods.

Degree of microbial inactivation can vary depending on the food medium used. When comparing against buffer system typically used in microbiological research, food composition (lipids, carbohydrates, proteins, and minerals) may provide

additional baroprotective effect on microorganisms to counteract the physical stress caused by pressure (Hauben et al. 1998; Black et al. 2007a). Therefore, the more nutrient rich the food medium is, the more difficult it is for inactivation. García-Graells et al. (1999) reported a 7-log reduction of *Escherichia coli* MG1655 in phosphate buffer treated at 400 MPa, 20 °C, 15 min while only 3-log reduction was achieved in milk treated at the same conditions.

Foods with high water activity (a_w) are more suitable for pressure treatment. Reducing a_w have shown to increase survival rate of spoilage yeasts such as *Rhodotorula rubra* (Oxen and Knorr 1993), *Escherichia coli* and *Saccharomyces cerevisiae* (Takahashi et al. 1993). Cell shrinkage and thickening of cell membrane as a result of decreased water activity resists the pressure treatment (Knorr 1993; Oxen and Knorr 1993; Takahashi et al. 1993; Palou et al. 1997; Molina-Höppner et al. 2004).

Acidity of food medium plays a vital role in microbial inactivation. Reducing pH has shown to promote lethal effects of pressure treatment on microorganisms such as *L. monocytogenes*, *Escherichia coli*, *Salmonella enterica* (Mackey et al. 1995; Alpas et al. 2000). pH of the substrate can be lowered by two possible ways: (i) transient pH shift under pressure or (ii) acidification of the food substrate.

Since high pressure affects non-covalent bonds, isostatic pressure induces ionic dissociation of water molecules causing a transient shift in pH (Cheftel 1995; Smelt et al. 2002). Under pressure, the pH displaces towards acidic values succumbing the microorganisms to process treatment. pH returns nearly to its initial value upon depressurization.

Alternatively, foods that are naturally acidic or acidified can enhance the lethal effect of pressure-thermal treatment on microorganisms and prevent outgrowth of sublethal injured cell during storage. The advantage of acidifying the food substrate is also observed on inactivation of bacterial spores. A 2.5-log reduction of *Clostridium sporogenes* PA3679 spores was achieved at 400 MPa, 25 °C for 30 min at pH 4.0, while only <0.5 log reduction was reported at same treatment in pH 7.0 (Stewart et al. 2000). Kamat et al. (2018) demonstrated shelf-stability of pressure-thermal treated acidified pickled vegetables.

20.8.5.3 Kinetic Models for Microbial Safety

There are certain key differences needs to be considered while developing kinetic models for microbial destruction through the application of high pressure. Unlike thermal processing, depending upon the type of microorganisms, there may be certain microbiological population reduction during pressure-come-up time. Thus, it is prudent to separately consider pressure-come up microbiological reduction efforts when using both linear and non-linear models.

In addition, conventional linear model may not adequately describe the experimentally determined microbiological inactivation data. Some of the reasons for the lack of fit may include injured or resistant cells, cells clumping and target of multiple microorganisms or non-uniformity during process treatment. Thus, researchers

proposed non-linear models including Weibull model (Peleg and Cole 1998), log-logistic (Chen and Hoover 2003a, b), and modified Gompertz model (Patterson and Kilpatrick 1998) to better describe microbial inactivation behavior.

Rajan et al. (2006a) and Ahn et al. (2007) utilized log-linear and Weibull model to characterize the inactivation curves of various bacterial spores at PATP conditions. One of the key limitation of non-linear models is that they often rely upon simple curve fitting of the experimental data and provide limited physical insight about the underlying process. On-going research is to gain better appreciation for physical significance of various non-linear model parameters. For example, Weibull model kinetic parameter *b* generally increased with increasing pressure and temperature with a decrease in D-value. For example, *Bacillus sphaericus* NZ 14 spores treated at a process condition of 700 MPa at 105 °C yielded b value of 1.5 (D-value =0.6 min) which increased to 2.1 at 700 MPa at 121 °C (D-value = 0.5 min) (Rajan et al. 2006a).

Similarly, combined pressure-thermal survival curves generally exhibited an upward concavity ($n < 1$) with a rapid decline in the beginning followed by a tailing towards the end. Interestingly, as the pressure increased at constant temperature, *n* value decreased further. For example, at 121 °C processing temperature, changing pressure from 500 to 700 MPa decreased the *n* value from 0.76 to 0.47 for *Bacillus amyloliquefaciens* (Rajan et al. 2006a).

Log logistic model has been applied for inactivation patterns of *L. monocytogenes* Scott A in whole milk (Chen and Hoover 2003a), *Salmonella* Typhimurium DT 104 (Guan et al. 2005) and *Bacillus coagulans* spores (Wang et al. 2009), and is beneficial for longer pressure treatment time where the experimental data actually follows a sigmoidal curve. A drawback of this model is lack of predicting the pressure inactivation and the need for longer processing times (Guan et al. 2005).

Modified Gompertz model provided the advantage of calculating pressure-dependent primary kinetic parameters such as maximum death rate (μ_M), lag phase duration (t_{lag}) and tailing ratio (q_G) from the Gompertz parameters and secondary kinetic parameters (z_{HP}) from the linear relationship of μ_M and applied pressure with the limitation of the need of large amount of data. As pressure level changed from 325 MPa to 400 MPa, μ_M increased with a decrease in t_{lag} and q_G values for *Listeria innocua*, indicating the increasing sensitivity of *Listeria innocua* at higher pressures (Saucedo-Reyes et al. 2009). Cells in exponential phase yielded higher z_{HP} values of 625 MPa while cells in stationary phase resulted in lower value of 200 MPa demonstrating the rate of change of kinetic parameters for stationary phase cells are greater than that of exponential phase. Although, Gompertz model was helpful in determining the primary and secondary kinetic parameters (z_{HP}) to predict the inactivation of microorganisms at various pressure treatment conditions, the model requires large amounts of data.

Although the synergistic effect of pressure and heat on bacterial spore inactivation has been established, very limited studies have related the accumulated lethality *(F-value)* during pressure-assisted thermal processing as a function both the lethal agents (pressure and heat). Most authors describe PATP accumulated lethality based on thermal effects only (Koutchma et al. 2005; Bull et al. 2009). Nguyen et al. (2014)

developed an integrated process lethality model to assess the combined pressure-thermal lethality ($F_{T,P}$) on *Bacillus amyloliquefaciens* spore inactivation. The model used n^{th}-order kinetics and published data on pressure-thermal inactivation of *Bacillus amyloliquefaciens* spore (Rajan et al. 2006a) to determine kinetic parameters. Predicted log reductions generated from the accumulated lethality ($F_{T,P}$) model were in reasonable agreement with the experimental values for deionized water and carrot pureé. More studies are needed to develop a comprehensive database of kinetic model parameters of various microorganism under defined process conditions are needed.

20.8.5.4 Pressure Injury and Recovery of Microorganisms During Storage

Although the process conditions are selected to significantly reduce the population of microorganism of concern, there is a possibility that a fraction of cells are sub-lethally injured. Post-treatment, these injured cells can recover during storage under optimal growth conditions and in presence of nutrient-rich foods.

Bozoglu et al. (2004) highlighted two types of pressure-induced injury of four foodborne pathogens (*Salmonella* Enteritidis, *Staphylococcus aureus*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*) in pressure treated milk stored at three different temperatures (4, 22, 30 °C). First type of injury, I1, refers to the structural damage that can recover in selective media, while second type of injury (I2) signifies metabolic injury that preferably grew on nonselective media than in selective media. Potential growth of injured cells was possible only after full recovery to I1 injury state.

The presence of injured cells post HPP treatment raises two concerns:

(i) Possible recovery of survivors after some time lag after treatment.
(ii) Overestimation of process lethality as the injured cells may not be detected in selective conditions to enumerate the survivors.

Balamurugan et al. (2018) detected the recovery of *L. monocytogenes* in high pressure treated cooked sausages (final count: 10^4 CFU/g) at 21 days of storage at 10 °C, despite a 7-log reduction post pressure treatment. Reducing the storage temperature to 4 °C seemed to control the outgrowth of the pathogen to below the detection limit extending the storage to 35 days. Hayman et al. (2004) observed a similar lag in the outgrowth of *L. monocytogenes* in 98-day storage at 4 °C in ready-to eat (RTE) meat treated at 600 MPa, 20 °C for 180 s. Recovery was not detected until day 91.

Recovery of injured cells during storage can be inhibited in foods that are acidic, lower a_w or contain antimicrobial additives which can improve the microbial stability (Patterson et al. 1995; Gänzle et al. 2001). PATP treatment at 700 MPa, 105 °C, 5 or 15 min significantly reduced *Bacillus amyloliquefaciens* spores in carrot purée to below detection limit ($<10^2$ CFU/mL) (Ratphitagsanti et al. 2010). The population remained below the detection limit at longer holding time of 15 min in PATP carrot

puree, while an outgrowth to $>10^7$ CFU/mL was detected with 5 min holding time. The addition of organic acids (citric acids, lactic acid) in carrot puree combined with PATP conferred an inhibition step to suppress the growth of any injured *B. amyloliquefaciens* regardless of holding time.

A quantitative analysis using plating method has been used to quantify the recovery of injured cells in limited products at selected pressure treatment conditions. There is a need to evaluate the extent of microbial injury through various pressure-thermal treatments and enumerate survivors on nonselective media at varying incubation temperatures to ensure microbiological safety of treated foods during storage (Balasubramaniam and Farkas 2008).

20.8.5.5 Storage Stability of Pressure-Treated Products

Typically, high pressure pasteurized products can be stored 60–90 days under refrigerated temperature. Chilled storage is required to inhibit outgrowth of potential spores and enzymes that were unaffected by pressure treatment. Residual population and type of bacteria inclusive of other factors such as food matrix, pH, storage temperature and packaging environment regulates the type of flora to initiate spoilage (ICMSF 2005).

Lactic acid bacteria (LAB) has been identified as the main spoilage microorganism during refrigerated storage of high pressure pasteurized foods (Han et al. 2010; Peñas et al. 2010; Rodríguez-Calleja et al. 2012). During the 90-day storage study of high pressure treated sliced vacuum-packed cooked ham, a slow recovery of LAB, from initial population of 10^3 CFU/g after treatment to final population of 10^8 CFU/g at day 90, was detected during 4 °C storage (Han et al. 2010). The ham eventually developed a slimy layer on the surface similar to the untreated vacuum-packed ham that spoiled within 15 days of refrigerated storage. Additionally, a corresponding reduction in pH from 6.38 to 5.20 during storage was observed in both untreated and pressure treated ham as a result of acids produced by the lactic acid bacteria.

Thus far, *Pediococcus acidilactici* was determined as one of the most pressure-resistant microorganisms amongst other bacteria, *Lactobacillus brevis* and *Torulaspora delbrueckii*, in pressure treated inoculated ranch dressing (Waite et al. 2009). Despite more than 6.4 log reduction immediately after treatment of 600 MPa for 3 min with a final population of $<1.2 \times 10^1$ CFU/g, *Pediococcus acidilactici* manage to proliferate to 1.3×10^5 CFU/g at the end of 3 weeks at 26 °C storage. Extending the holding time to 10 min at 600 MPa seemed to emanate similar trend post treatment and during storage as 3 min holding time. It is evident that LAB have moderate tolerance to pressure treatment resulting in sub-lethal injured cells, but the recovery of these injured cells is favored by the surrounding food matrix and appropriate storage temperature.

Apart from treatment intensity and microorganisms' inherent resistance to treatment, packaging properties can influence the growth of microorganism during refrigerated storage as well. Adequate oxygen, water and light barrier properties are some of the characteristics that the packaging material must possess to support

the quality of food during storage. These barrier properties can be modified under high pressure treatment that may impact the quality of the product during storage (Kovarskii 1994). Innovation in packaging materials such as nanocomposite, modified atmosphere packaging (MAP), and active packaging are combined with high pressure to protect the food quality and microbiological shelf-life, especially for meat products (Jofré et al. 2008; Marcos et al. 2008; Rodríguez-Calleja et al. 2012; Lerasle et al. 2014). Rodríguez-Calleja et al. (2012) investigated the combined effect of antimicrobial coating, high pressure and MAP on shelf-life of fresh chicken breast fillets. Natural microflora of LAB, *Pseudomonas* spp., *Escherichia coli*, *Brochothrix thermosphacta* were effectively inactivated by the synergistic effect of antimicrobial coating and high pressure, extending the shelf-life of chicken fillets up to 28 days under refrigeration. However, storing the pressure treated chicken fillets in modified atmosphere packaging with 30% CO_2/70% N_2 had no further control on microbial recovery. LAB still managed to grow gradually during storage. Modifications in CO_2 and O_2 in the headspace was attributed to the microbial metabolism, muscle tissue respiration and dissolution of CO_2 into meat. Similar phenomenon has been observed by other authors (Ruiz-Capillas and Jiménez-Colmenero 2010; Ščetar et al. 2013; Lerasle et al. 2014).

Alternatively, combining high pressure treatment with antimicrobial packaging seemed to augment the efficacy of the lethality on many pathogens such as *L. monocytogenes* and *Salmonella* in ready-to-eat processed meats (Jofré et al. 2008; Marcos et al. 2008). Antimicrobial packaging is a type of active packaging that requires an integration of antimicrobials into the polymeric package to reduce or impede food-borne pathogenic or spoilage microbial growth in packaged foods (Appendini and Hotchkiss 2002). The direct contact between package and food product facilitates the migration of antimicrobial components towards the surface of meat during storage for effect and are refrained from deterioration by food enzymes or other constituents. Several antimicrobial agents such as organic acids and their salts, sulfite, nitrites, antibiotics and bacteriocins have been impregnated into polymeric films. These antimicrobial agents can be incorporated into product formulation (Daryaei and Balasubramaniam 2012). As of now, nisin is the only bacteriocin used as a food additive (EC 1995; FSIS 2002). Enterocins A and B, sakacin K and lactate salts are used as food ingredients in meat industries (Aymerich et al. 2000; Hugas et al. 2002; Vogel et al. 2006). More research is needed to identify natural ant microbiological ingredients that can be incorporated in the pressure treated formulated products.

Jofré et al. (2008) examined the effect of several antimicrobial interleavers alone or with high pressure (400 MPa, 17 °C, 10 min) on inactivation of *Salmonella* spp. inoculated in sliced cooked ham. Interleavers are layers of packaging that consists of a non-woven polyamide layer sandwiched between perforated and non-perforated polypropylene layers. Perforated polypropylene layer is then sprayed with the antimicrobial solution. Amongst the antimicrobial packaging, nisin and nisin+lactate combined with high pressure declined *Salmonella* count to below detection limit of <10 CFU/g (initial load: 10^4 CFU/g) which was sustained during 3 months of storage at 6 °C. On the contrary, a progressive reduction in microbial load was evident with

antimicrobial interleavers alone. Combined application of antimicrobial packaging, high pressure treatment, and refrigerated storage can be effectively used to eliminate microorganism to produce value-added products with extended shelf-life (Aymerich et al. 2005; Arqués et al. 2005; Liu et al. 2012).

20.9 High Pressure Equipment Cleaning Sanitation, and Maintenance

Manufacturing facility that houses HPP equipment follows standard GMP and HACCP protocols. General Clean-In-Place (CIP) series of alternating water rinse, alkaline and acidic cleaning solutions is employed. Primarily, pressure transmitting fluid (water) is drained from the reservoir and through the system. The reservoir is then filled with alkaline detergent solution (≤70 °C) that is recirculated within the system for 15–30 min. Subsequently alkaline reidue is removed through a water rinse followed by circulation of acid cleaning solution for 15–30 min. (Fig. 20.6).

With the exception of seafood, most of the pressure-treated products are currently pre-packaged. Thus, the food evades direct contact with the equipment surface. Therefore, only components such as conveyors, basket, vessel and pressure transmitting fluid reservoir are cleaned once a week. Carrier baskets that are used to pressure treat seafood meat (where there is a direct contact between food and the basket) are recommended to be cleaned daily.

Seals and plugs mounted onto the end closures are prone to wear and tear and thus, they must be inspected, cleaned and lubricated daily. It is also a good practice to inspect packages before and after each HPP cycle for any packaging ruptures. In

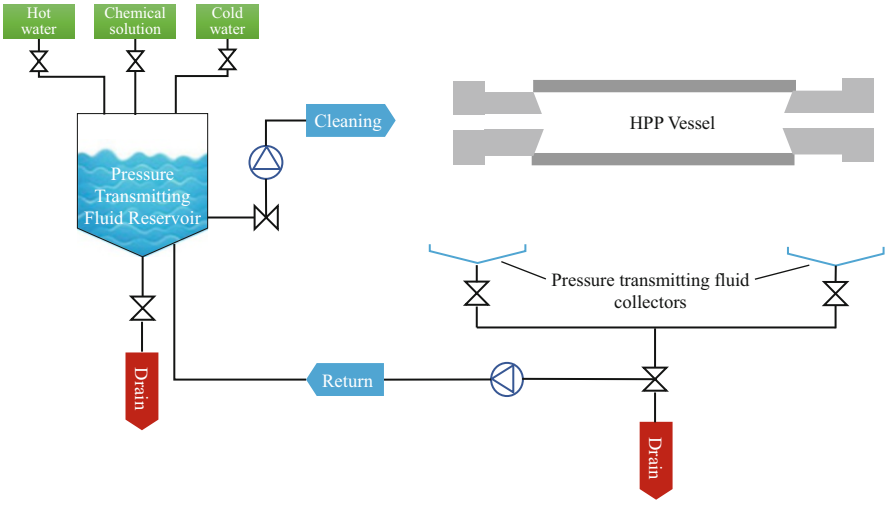

Fig. 20.6 Schematic diagram for a CIP system for high-pressure pasteurization system

the event of obvious package failure, the components must be sanitized immediately with cleaning cycle along with the drain opening.

High pressure process is a mechanical intensive process, wherein various equipment components undergoes significant wear and tear. Therefore, a scheduled maintenance must be employed as part of the Good Manufacturing Practice (GMP). Depending upon the intensity of the treatment, components such as seals available within the top/bottom closure, decompression valves, and high pressure pumps needs to be periodically inspected and replaced. Food processors should scan the pressure vessels for any corrosion marks as this factor can affect the strength of the vessels. Conducting a thorough and preventive maintenance will lengthen the life of the HPP equipment and optimize the productivity of the manufacturing plant.

20.10 Limitations and Challenges

Due to emerging nature of the high-pressure technology, the technology is primarily limited to high value added products with limited throughput. The technology may not be cost justified for commodity type products that requires high throughput. Efforts are needed to develop continuous high pressure processing methods, particularly for beverage processing, to increase the throughput and reduce the cost.

The pressure treatment is more effective with high moisture content, non-porous solids foods as well as liquids. Products containing low-water activity (e.g., peanut butter) or products containing air pockets (e.g., leafy greens) may not be suitable for pressure treatment (Grasso et al. 2010).

High pressure treatment at ambient or chilled conditions is not sufficient for bacterial spore inactivation. Thus, adequate precaution must be made to maintain refrigerated storage and handling of pressure pasteurized products.

20.11 Conclusions and Future Trends

Over the past two decades, high pressure technology has gained status as a commercially viable technology to produce variety of value-added pasteurized products. Food processors employ pressure as the lethal agent at ambient or chilled conditions to extend shelf-life of foods, while satisfying consumer demands of preservative-free mildly processed foods. Development of natural antimicrobial compounds that can be added as a part of formulated clean-label foods are needed. Since pressure pasteurized products do not inactivate bacterial spores, the products must be stored under refrigerated storage conditions. Simultaneous application of pressure with modest heat can help to inactivate bacterial spores and preserve ambient stable shelf-stable low-acid foods. While the entrenched kinetic models on HPP microbial inactivation explain HPP treatment efficacy, more studies are needed to develop a comprehensive database on combined pressure-thermal effect on various

microorganisms suspended in different food matrices. Similarly, kinetics of destruction of microorganisms as a function of pressure, temperature, and shear are needed towards development of a continuous high pressure methods for preserving liquid beverages. Despite the high cost and batch nature of HPP, consumer's demand of minimally processed clean-labeled foods has boosted the HPP equipment manufacturing sector to install more than 120 HPP units throughout North America, Europe, Asia, Australia and many more. The ability to simplify the product ingredient statement has allowed consumers to welcome pressure pasteurized products as part of their healthy lifestyle and still continues to do so. With the innovative applications and the potency of HPP to deliver minimally processed, clean-labeled products, HPP is constantly on the move to change the perception of processing and retail marketing.

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Chapter 21
Pulsed Electric Fields for Pasteurization: Food Safety and Shelf Life

Tony Z. Jin and Howard Q. Zhang

21.1 Introduction

Consumer demand for minimally processed and fresh-like food products has led to an increased interest in innovative non-thermal processing technologies, which aim to achieve similar microbial inactivation with reduced or no application of heat, while keeping the quality of foods at the highest possible level.

Pulsed electric field (PEF) processing is a non-thermal method of food preservation that uses short bursts of electricity for microbial inactivation and has minimal or no detrimental effect on food quality attributes. The technology of pulsed electric fields was proposed by Sale and Hamilton (1967) in the late sixties as a way to inactivate microorganisms in foods. Its application in food pasteurization has been studied extensively worldwide, resulting in the publication of thousands of articles regarding PEF treatment of foods. Research groups working on PEF technology have made tremendous progress towards understanding its principles, identifying key aspects of the industrialization process, and the commercialization of this technology in the juice and beverage industry (Jin 2017).

The inactivation of microbial populations by PEF depends on the various interdependencies of different treatment parameters, including PEF treatment parameters (electric field strength, treatment time, pulse frequency, pulse width, and treatment temperature), PEF treatment system (batch/ static or continuous chamber; coaxial or

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co-field; square wave, exponential decay, or oscillatory pulses), properties of the food product (electric conductivity, density, viscosity, pH, temperature), and microbial characteristic (bacteria or mold/yeast, Gram-positive or negative, vegetative cell or spores) (Jin et al. 2015; Min et al. 2007).

Although the majority of PEF studies involved lab scale PEF systems, pilot and commercial scale PEF processing systems are available and have been evaluated for orange juice, tomato juice, and applesauce (Jin et al. 2009a, b; Min et al. 2003a, b). In the late 1990s, the PEF Consortium for Technology Commercialization, consisting of food processors, equipment manufacturers, universities, energy suppliers, and the US Army was formed. Diversity Technology Inc. (DTI) built the world's first commercial-scale PEF food processing system in association with the Ohio State University (OSU), USA. The OSU/DTI system demonstrated that PEF processing can be directly scaled to meet processing volumes up to 50,000 L/h or more (DTI 2019). In 2005, PEF processed organic fruit juice products were sold in the commercial market in Oregon, USA (Mosley 2005). In Europe, the first commercial PEF operation was started in 2009 with the installation of a 1500 L/h juice preservation line (Toepfl 2012). PEF technology has been shown to have great potential for enhancing the safety, improving the quality and extending the shelf life of various types of liquid foods.

PEF technology has been extended to other food applications, such as drying, osmotic dehydration, and extraction (Yu et al. 2017a, b, c). PEF pasteurization faces more challenges than other applications because a higher PEF intensity is required to achieve a desirable level of microbial inactivation, particularly in food. This book chapter focuses on PEF pasteurization in real food products, and covers the equipment used in a PEF processing system, the mechanisms by which PEF treatment inactivates microorganisms, the use of surrogate microorganisms, and on its application for food safety (Inactivation of foodborne pathogens) and shelf life (Inactivation of spoilage microorganisms). Challenges and possible solutions are also briefly discussed.

21.2 PEF System

A typical PEF processing system or unit is composed of four major components: pulse generator, PEF treatment chamber, fluid handling system, and control and monitoring devices (Jin 2017). Based on the flow rate, PEF systems can be classified into laboratory or bench-top scale, pilot scale, or commercial/production scale. Figure 21.1 illustrates PEF processing flow charts of lab and pilot scale operations.

A pulse generator provides pulses to food samples in the treatment chamber and is a core component for PEF technology. A pulse generator mainly consists of DC power supply that converts wall voltage to high voltage and pulse modulators (capacitors, switches, etc.) that provide special pulses to the sample in a treatment chamber. The design of a pulse generator with a wide range of pulse parameters has been reported by several researchers (Flisar et al. 2014; Loeffler 2006; Puc et al.

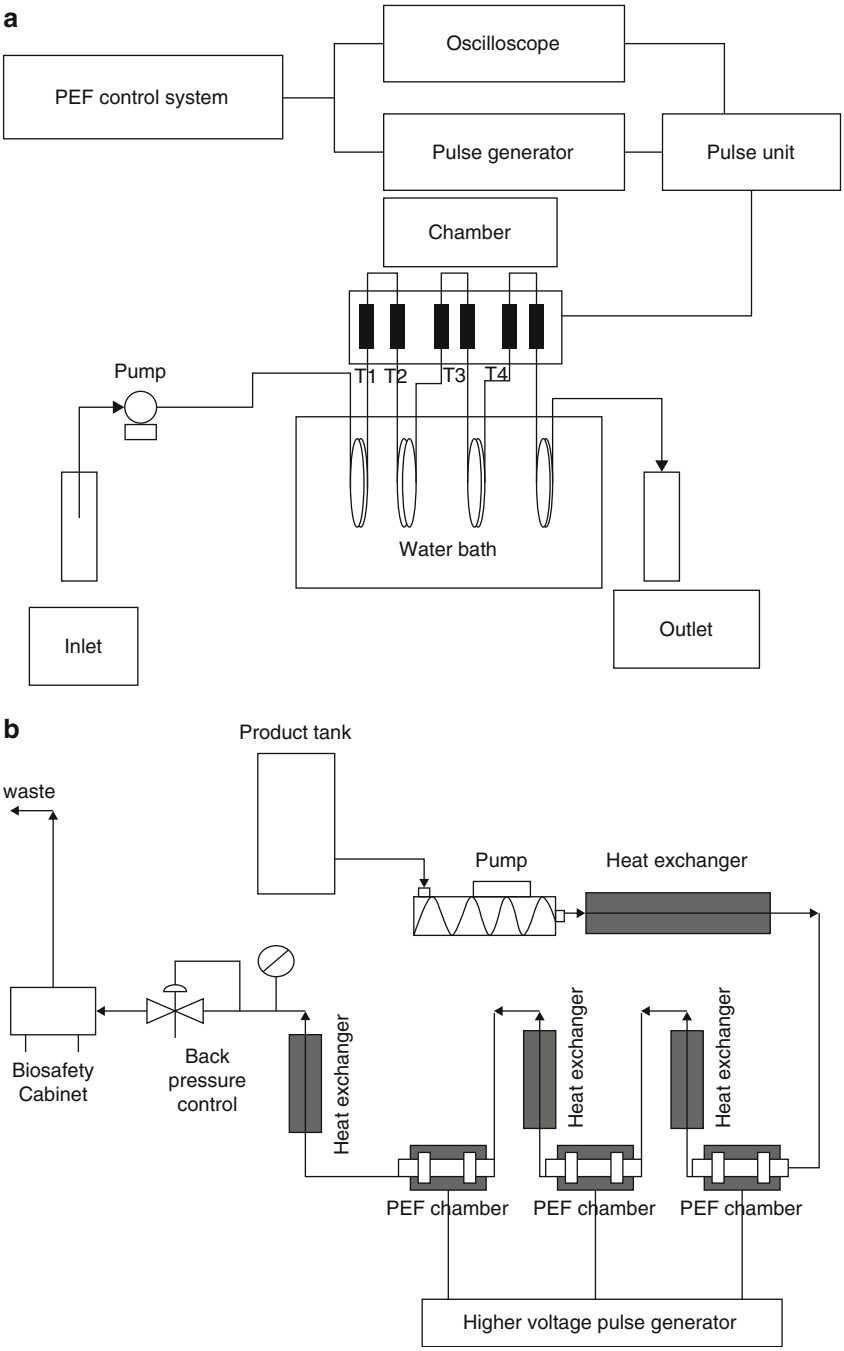

Fig. 21.1 Continuous PEF processing flow chart. (**a**): Lab scale; (**b**): Pilot scale. (Adopted from Jin et al. (2015), used with permission)

Fig. 21.2 PEF processed foods packaged in cup, glass bottle, PET bottle and glass jar

2004; Rebersek et al. 2014). The processing parameters associated with a pulse generator include output power, pulse shape, pulse width, pulse frequency, and pulse polarity.

The treatment chamber is a key component of any PEF system; it is where the food product receives electric pulses. One treatment chamber consists of two electrodes, one for high voltage connection and the other for ground connection. An insulator provides a gap (Treatment Zone) between the electrodes for samples passing through and receiving pulse treatment.

Several types of chambers have been studied over the years. The treatment chambers that have been studied the most are the parallel plate and the coaxial and co-field treatment chambers. The parallel plate treatment chamber has been used for small scale and bench studies, whereas the coaxial and co-field continuous treatment chambers have been used for small or large-scale studies (Flisar et al. 2014; Jin and Zhang 1999; Min et al. 2003a, b). Figure 21.2 shows their geometric configurations.

A fluid handling system for a continuous PEF pasteurization system is similar to other thermal food pasteurization methods in the juice and beverage processing industry. It includes an input/feed tank, a pump, heating or cooling, and a receiving tank. The fluid handling system ensures that PEF pasteurization can be done on a production scale and food to be properly processed. Figure 21.1 shows two examples of a fluid handling system for small and pilot scale continuous PEF processing systems.

Control and monitoring devices include temperature (cooling and heating), voltage, current, flow rate, and back pressure controllers and monitors. These records are important for validation and certification when filing a PEF process with an agency for authorization.

Extra equipment, such as an air trap (for lab scale units) or deaerator (for production scale units), may be needed for a specific product. For instance, a mixing step is required for food with multiple ingredients such as sugar, starch, food colorings, and flavoring agents. The mixing prior to PEF treatment, particularly for viscous foods, such as apple sauce, rice pudding, or liquid egg, could entrap air in the product and cause possible arcing or affect the uniformity of field strength distribution. Jin et al. (2009a, b) and Li et al. (2005) installed a deaerator and operated it at 740 ~ 750 mmHg vacuum for apples sauce and salad dressing after the products were mixed with other ingredients and before the products entered the PEF treatment chambers.

A packaging unit is a piece of add-on equipment for juice and beverage production, which can be integrated with a pilot plant or production scale PEF system. An aseptic packaging machine (Benco Asepak/2) was used to package the PEF treated apple sauce or tomato juice in tri-laminate plastic cups thermoformed by the packaging machine (Jin et al. 2009a, b; Min et al. 2003a). A bottle filler was also used to package PEF-treated juice (Min et al. 2003b). Figure 21.2 shows PEF treated samples packaged in different containers.

21.3 PEF Processing Parameters

Unlike thermal processing or other nonthermal processing, PEF processing involves many parameters (Jin et al. 2015). Among them, the electric field strength, pulse width/during time, pulse frequency, and treatment time are considered to be as the most important parameters involved in PEF pasteurization. The use or report of those parameters in PEF studies and applications are very important; they must be well defined (Jin 2017).

Electric field strength or electric field intensity is associated with pulse generator (output power) and treatment chamber design, and its uniformity in distribution is also influenced by fluid delivery system (flow rate), control devices (temperature), and other factors, such as air bubbles. Therefore, electric field strength is one of the critical processing parameters and also a most complex parameter.

The intensity of the applied pulses is given as electric field strength [kV/cm], which is defined as applied voltage divided by the gap distance between two electrodes in a treatment chamber. For a parallel treatment chamber, electric field strength is calculated as shown in Eq. 21.1,

$$\mathrm{E} = \frac{\mathrm{U}}{\mathrm{D}} (kV/cm) \tag{21.1}$$

where, E – field strength (kV/cm); U – applied voltage (kV); and D – gap distance (cm) between two parallel plates (Fig. 21.3a).

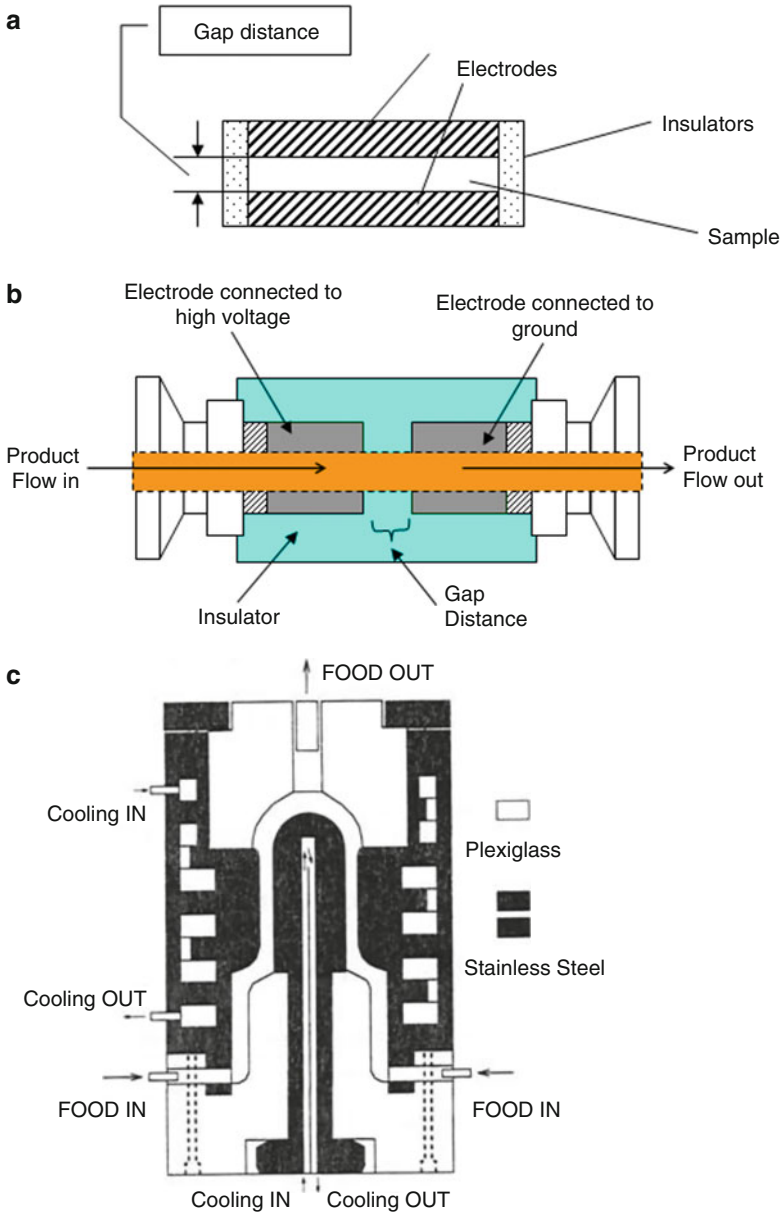

Fig. 21.3 Parallel batch treatment chamber (**a**), co-field continuous treatment chamber (**b**), and coaxial continues treatment chamber (**c**). (Adopted from Qin et al. (1995b), used with permission)

For a co-field treatment chamber, Eq. 21.1 is also used for calculating and reporting the average field strength. The gap distance (D) is between two tips of tubular electrodes (Fig. 21.3b).

For the coaxial PEF treatment chamber (Fig. 21.3c), the maximum electric field strength E can be determined by Eq. 21.2 (Fernández-Molina et al. 2001):

$$E = \frac{U}{r\left[\ln\left(\frac{R_2}{R_1}\right)\right]}(kV/cm) \tag{21.2}$$

where, U – applied voltage; r – radius where the voltage is measured; and R_1 and R_2 – inner and outer radii.

Generally, the applied voltage is used for the calculation, which is measured between two electrodes. For exponential decay pulse, the average or effective voltage is approximately 37% of peak voltage. Few published papers have presented results using peak voltage or field strength, Therefore, attention should be paid to the parameters when results are compared.

Although many different waveforms are applicable for PEF technology, the pulse shapes most commonly used are either exponential decay or square wave pulses (Toepfl et al. 2007). Figure 21.4 presents typical waveform of exponential decay pulse (A) and square wave pulse (B).

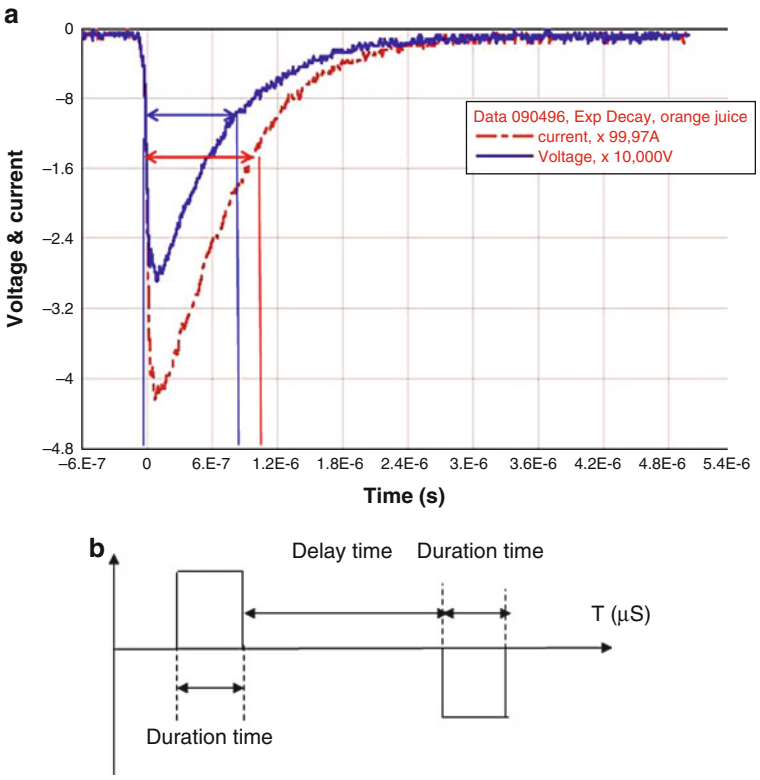

Fig. 21.4 Typical waveforms and pulse width (duration) measurement. Exponential decay pulse (**a**) and square wave pulse (**b**)

Pulse width or pulse duration is the amount of time a pulse is held at an effective voltage. It is determined depending on the waveform applied. For square waveform, effective pulse width/duration is measured at 50% of peak voltage if a perfect square shape is not formed; for exponential decay pulses, the effective pulse width is defined as the time until the electric field decreases to 37% of peak voltage (Rebersek et al. 2014), as shown in Fig. 21.4.

Pulse polarity includes monopolar and bipolar pulses. During PEF treatments, the food product can receive all positive or all negative pulses (monopolar) or receive half of positive pulses and half of negative pulses (bipolar). The time between the positive pulse and the negative pulse, known as "delay time", can be adjusted so that the pulses can be evenly or unevenly distributed throughout the treatment time. Figure 21.4b shows biopolar square wave form.

Pulse frequency or pulse repetition rate is defined as pulse per second (PPS). For monopolar polarity, PPS is equal to frequency (Hz), while for bipolar polarity, PPS is equal to 2× frequency. All pulse parameters (shape, width, frequency, and polarity) can be determined or monitored using oscilloscopes connecting to the system and can be recorded using a special interface and software.

PEF treatment time is associated with fluid handling system (flow rate), also with pulse generator (pulse width, shape, and frequency), and treatment chamber (gap distance and gap volume).

Treatment time is the time the food product is exposed to PEF. The total treatment time depends on the residence time in each chamber, the number of pulses received in each chamber, and the number of total treatment chambers. They are calculated as shown below:

Residence time in one chamber T_r:

$$T_r = \frac{Volume\ of\ one\ chamber}{Flow\ Rate} = \frac{V}{F} \tag{21.3}$$

Number of pulses received in each chamber n_p:

$$n_p = T_r \times f \tag{21.4}$$

Total treatment time T_t:

$$T_t = n_p \times n \times \tau_c \tag{21.5}$$

Or

$$T_t = n \times \tau_c \times f \times \frac{V}{F} \tag{21.6}$$

where, T_r – residence time in one chamber; F – average flow rate (cm^3/s); V – gap volume of one chamber (cm^3); n_p – Number of pulses received in each chamber; T_t –

Total treatment time; τ_c – effective pulse width (s); f – pulse frequency (Hz); and n – number of treatment chambers.

Similar to electric field strength, total treatment time is also an important process parameter. In general, longer treatment times allow for greater rates of microbial inactivation. However, as seen in Eq. 21.6, total treatment time depends on pulse width, pulse frequency, and number of treatment chambers. Jin et al. (2015) reported that even though the same total treatment time and field strength were used, changing the combinations of pulse frequency and pulse width resulted in different microbial inactivation rates. For some cases, multiple fluid circulations throughout the same PEF unit can be used to increase the treatment time, which have been demonstrated in the early bench scale studies.

Treatment temperature is generally defined as the temperature of food measured before entering the PEF treatment chamber (inlet temperature). If multiple treatment chambers are used, the inlet temperature may be different during PEF processing as PEF pulses input energy to each chamber causing a temperature increase that depends on the field strength, food conductivity and the cooling/heating capacity between two treatment chambers.

During PEF treatment, electrical current flows through the food product, causing the temperature of the food product to increase; hence, the temperature of the food product after treatment in the chamber (outlet temperature) is higher). The temperature difference between the outlet and inlet can be estimated as shown in Eq. 21.7:

$$\Delta T = \frac{E^2 \times \sigma \times T_t}{\rho \times C_p \times F} \tag{21.7}$$

where, ΔT – total temperature change in sample ($^\circ C$); E – average electric field strength (V/m); T_t – total treatment time (μs); σ – conductivity of the sample to be processed (S/m); ρ – density (g/m^3); C_p – specific heat ($J/g\,^\circ C$); and F – flow rate (m^3/s).

The temperature calculated in the above formula is the total temperature change in the sample. Since the sample temperature is regulated after flowing through each treatment chamber, the actual temperature increase in the sample during processing is $\Delta T/n$, n being the number of chambers in use, assuming there is no heating or cooling between two chambers.

Some studies have used the term product temperature, and this term can be confused with inlet temperature. Product temperature is the temperature of the product in a feed tank or before a feed pump. Without preheating or cooling procedures, the product temperature should be the same as product inlet temperature. However, it is not necessary for the product temperature to be the same as the treatment temperature (inlet). The inlet treatment temperature can be controlled by cooling or heating the product before entering the treatment chamber and after PEF treatment as shown in Fig. 21.1. To control the treatment temperature, stainless steel

coils with water bath are normally used for a continuous lab scale PEF system and tubular or plate cooing/heating units are used for continuous pilot or production scale PEF systems.

Treatment temperature is an important factor in relation to microbial reduction. An increase in treatment temperature during PEF processing generally corresponds to increased inactivation of microorganisms at a particular electric field strength. However, it is important to consider the following phenomena: as product temperature increases, product conductivity increases; as resistance of the treatment load decreases, the electric field strength applied becomes lower than expected.

21.4 Theory or Mechanism of Microbial Inactivation

Pulsed electric field processing is a physical process that uses high voltage pulses with durations in the range of microseconds to milliseconds to generate an electric field between the two electrodes which confine the treatment gap of the PEF chamber where a food product is located (either in a static or a continuous design).

PEF treatments induce electroporation of cell membranes in microbes, plant, and animal cells and this can affect cellular functions. Electroporation refers to the permeabilization of cell membranes triggered by an external electric field (Zimmermann 1986; Rems and Miklavcic 2016). Electroporation can be reversible or irreversible. During reversible electroporation, the pores which are formed in the cell membrane can reseal after the application of PEF treatment. During irreversible electroporation, however, the pores which are formed are permanent, allowing for the movement of extracellular as well as intracellular molecules across the cell membrane. This can disrupt cellular homeostasis and eventually lead to cell death (Kranjc and Miklavčič 2017). In 1998, researchers at the Ohio State University developed a microscopic real time imaging PEF system (Fig. 21.5). The images from the system provided the first-time evident that yeast cell membranes were broken,

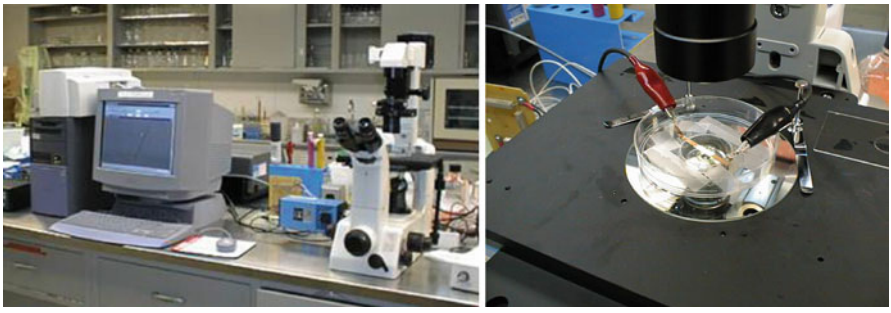

Fig. 21.5 Microscopic real time imaging PEF system. Left: High speed microscopy imaging system including Nikon Diaphot 300 Inverted Microscope and Cooke FlashCam high speed camera (maximum 1,000,000 exposures per second); Right: Mini PEF treatment chamber under the camera

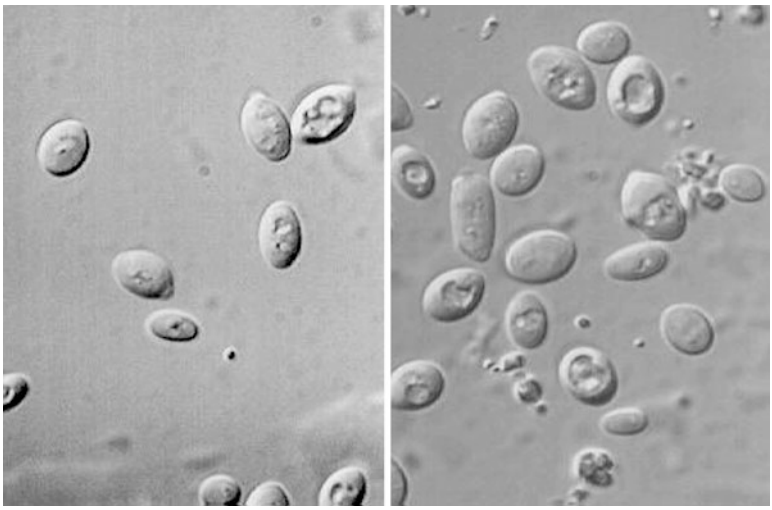

Fig. 21.6 Yeast cells (*S. cerevisiae*) visualized under PEF by optical camera. Yeast cells in 0.1% peptone solution before PEF treatment (Left) and after PEF treatment (40 pulses with 2 μs pulse width and 20 kV/cm field strength) (Right)

and intracellular components were released after PEF treatment (Fig. 21.6). The external electric field used needs to be above a critical value, the transmembrane potential – which is different for each cell type – to induce membrane electroporation. Based on the membrane dielectric breakdown theory, when the electric field strength is applied above this threshold, irreversible cell membrane breakdown happens (Zimmermann 1986). Grahl and Markl 1996 defined a critical term, EC, as the electric field strength, from which on a significant reduction of viable cell counts could be observed. Exemplary values of E_C for different microbial species in different matrices are given in Table 21.1. Critical electric field strength or minimum electric field strength is required to inactivate microorganisms during PEF food pasteurization. The critical electric field strength value is within the range of 4–14 kV/cm, depending on the types of microorganisms, treatment media, and treatment time (Álvarez et al. 2006; Castro et al. 1993; Grahl and Markl 1996; Toepfl et al. 2007).

Commercial exploitation of PEF for liquid food pasteurization requires a sufficient level of microbial inactivation to guarantee safety. Current guidelines developed by the United Stated Food and Drug Administration (FDA) require that fruit juice processors achieve a 5 log reduction of target foodborne pathogens in fruit juices prior to distribution, regardless of the pasteurization method applied. (Code of Federal Regulations 21CFR120.1). To achieve that level of microbial reduction, long treatment times (i.e., >100 μs) at high electric field strengths (i.e., >30 kV/cm) are required for PEF applied in food pasteurization.

Table 21.1 Critical electric field strength (E_C) of selected microorganisms in kV/cm

Organism	Gram behavior	E_C	Solution	References
S. cerevisiae	n/a	4.7	Skim milk	Grahl and Märkl (1996)
S. cerevisiae	n/a	4.7	Orange juice	Grahl and Märkl (1996)
S. cerevisiae	n/a	5.4	Na-alginate	Grahl and Märkl (1996)
E. coli	Negative	10.3	Whole milk	Zhao et al. (2013)
L. monocytogenes	Positive	11.2	Whole milk	Zhao et al. (2013)
P. fluorescens	Negative	11.5	Na-alginate	Grahl and Märkl (1996)
L. brevis	Positive	12.1	Na-alginate	Grahl and Märkl (1996)
E. coli	Negative	12.7	Skim milk	Grahl and Märkl (1996)
L. plantarum	Positive	13.0	Model beer	Ulmer et al. (2002)
S. aureus	Positive	13.3	Whole milk	Zhao et al. (2013)
S. enterica Dublin	Negative	13.8	Skim milk	Sensoy et al. (1997)
E. coli	Negative	14.0	Na-alginate	Grahl and Märkl (1996)

Adopted from Schottroff et al. (2017)

21.5 Sublethal Injury, Recovery, Resistance and Surrogates

In the case of reversible electroporation, cells with membrane damage are known as sublethally injured cells, which refers to a physiological state in-between life and death. The degree of sublethal injury is dependent on the properties of the matrix, especially the pH, the types of microorganisms, electric field strength and treatment time (Garcia et al. 2003, 2005a; Zhao et al. 2013).

Sublethal injury is of great importance for PEF applications in food pasteurization where PEF is used for the inactivation of pathogenic and spoilage microorganisms. In general, lowering the pH facilitates the inactivation of microorganisms by PEF, due to the intrusion of acid molecules into the cytoplasm through the emerging pores in the membrane (Schottroff et al. 2017). Thus, acidic foods are of little concern considering the occurrence of sublethal injury in microorganisms. For the treatment of foods with a pH close to neutral, however, sublethal injury has to be thoroughly investigated.

As most commercially produced foods are subject to storage and transportation before consumption, it is a safety concern that injured foodborne pathogens after a PEF treatment could be recovered during storage. Hence, it is necessary to investigate the occurrence of sublethal injury and recovery after PEF treatments. Garcia et al. (2005b) investigated the influence of treatment time, electric field strength, and the pH of the treatment media on the occurrence of sublethal injury in two species of Gram-positive bacteria (*Bacillus subtilis* ssp. *niger*, *Listeria monocytogenes*) and six species of Gram-negative bacteria (*Escherichia coli*, *Escherichia coli* O157:H7, *Pseudomonas aeruginosa*, *Salmonella senftenberg* 775 W, *Salmonella typhimurium*, *Yersinia enterocolitica*) after PEF treatment. They found that Gram-positive bacteria were more resistant to PEF treatment at pH 7.0 and Gram-negative bacteria were more resistant to PEF treatment at pH 4.0, as shown by the detection of a large proportion of sublethally injured cells. The proportion of sublethally injured

cells was also greater when longer treatment times and higher electric field strengths were applied. No sublethal injury was detected when Gram-positive bacteria were treated at pH 4.0 and Gram-negative bacteria at pH 7.0. Based on these results, they concluded that bacterial inactivation by PEF is not an 'all or nothing' event (Garcia et al. 2005b).

Mosqueda-Melgar et al. (2007) stated that *L. monocytogenes* was more resistant to PEF than *S.* Enteritidis and *E. coli* in melon and watermelon juices when treated at the same processing conditions. However, other researchers found that microbial characteristics such as cell size, shape or type of the cell envelopes did not exert the expected influence on microbial PEF resistance, but depended on the pH of the treatment medium. *L. monocytogenes*, which showed the highest PEF resistance at pH 7.0, was one of the most sensitive at pH 4.0. The most PEF resistant strains at pH 4.0 were the Gram-negative bacterial strains *E. coli* O157:H7 and *S. senftenberg*.

Food pilot plant and in-factory bacterial challenge studies are often conducted with biosafety level 1 microorganisms, i.e. surrogate, to model the inactivation characteristics of pathogens. The National Advisory Committee on the Microbiological Criteria for Foods (2010) recommends the use of surrogate microorganism in place of target pathogens for in-plant inactivation studies. The FDA (2000) also states that a microorganism described as a surrogate bacterium must be "a nonpathogenic species and strain responding to a particular treatment in a manner equivalent to a pathogenic species and strain." Therefore, it is important to select and evaluate suitable indicator strains and nonpathogenic surrogates, which can accurately fit the stress responses, sublethal behavior and resistance of pathogenic microorganisms for PEF pasteurization.

Gurtler et al. (2010) compared the inactivation of Enterohemorrhagic *E. coli* O157:H7 (EHEC) and two strains of *Salmonella* Typhimurium in orange juice (pH 3.4) by PEF treatment against twenty strains of non-pathogenic bacteria (NPEC). They found that the higher populations of both species of *Salmonellae* were inactivated (2.81 and 3.54 log CFU/ml) in comparison with the reduction of EHEC (2.22 log). When tested under the same conditions, inactivation of EHEC was slightly greater than that of a non-pathogenic *E. coli* (NPEC) (2.02 $\log_{10}$). They suggested that the higher PEF resistance of non-pathogenic *E. coli* may provide a desirable margin of safety when used in pilot plant challenge studies in place of *E. coli* O157:H7 (Fig. 21.7).

Following up on the previous study, Gurtler et al. (2011) compared the inactivation of the non-pathogenic *E. coli* with *E. coli* O157:H7 in strawberry juice (pH 3.4) subject to PEF treatment. Inoculated juice was treated at outlet temperatures of 45, 50 and 55 °C at a field strength of 18.6 kV/cm for 150 μs with a laboratory-scale PEF unit. They found that PEF treatment inactivated surrogate *E. coli* at 45, 50, and 55 °C by 2.86, 3.12, and 3.79 log CFU/ml, respectively. They also discovered that PEF treatment inactivated *E. coli* O157:H7 under the same conditions by 3.09, 4.08, and 4.71 log CFU/ml, respectively. Hence, non-pathogenic *E. coli* could be used as a surrogate strain for *E. coli* O157:H7 in juices.

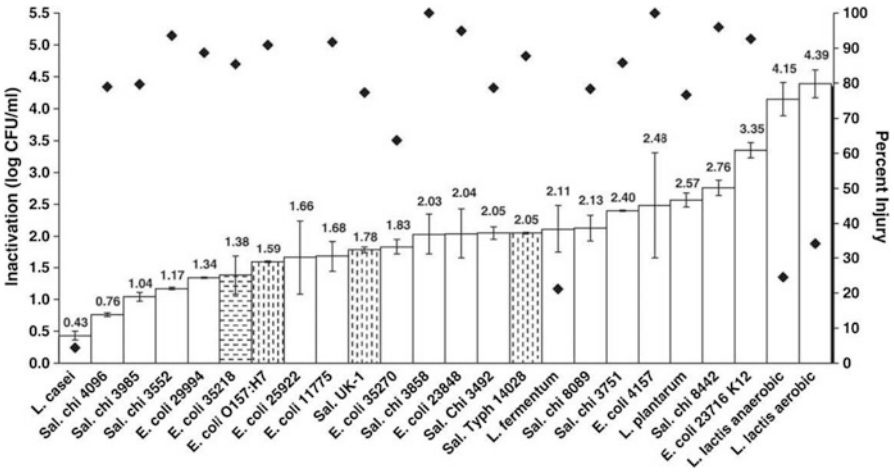

Fig. 21.7 Comparison of inactivation of pathogenic and non-pathogenic bacteria in orange juice treated with pulsed electric field (22 kV/cm) at 45 °C and a treatment time of 59 μs. Diamonds (◆) indicate percent (%) injury of surviving bacteria. Percent of bacterial injury (reported as a percent of the raw number of cells injured) was determined by the formula 21.1 −(raw number of cells recovered on selective agar/ raw number of cells recovered on non-selective agar) × 100%. Vertically-etched bars indicate pathogenic bacteria. Horizontally-etched bar represents the selected surrogate, non-pathogenic *E. coli* ATCC 35218. (Adopted from Gurtler et al. 2010, used with permission)

21.6 PEF Applications for Enhancing Food Safety

Many PEF studies focusing on the inactivation of foodborne pathogens have been conducted in media, such as peptone water or other bacterial growth media, particularly for kinetic studies. However, the efficacy of PEF for microbial inactivation in real food is often different from that in a growth medium. Even same fruit juice but different brand not always shows the same microbial reduction by the same PEF treatment conditions (Jin et al. 2015). Therefore, use of real food in study is necessary for the commercial application of PEF technology. The most studied foods are liquid foods, such as fruit juices and beverages and liquid egg as shown in Table 21.2, because they are easier handle by a continuous pump system; followed by semisolid foods, such as apple sauces, strawberry purée etc. Few studies have focused on the antimicrobial effectiveness of PEF treatment on whole fruits.

Amiali et al. (2006) investigated the effects of PEF intensity and temperature on the inactivation of *E. coli* O157:H7 and *S.* Enteritidis artificially inoculated in liquid egg white. Liquid egg white inoculated with each pathogen was treated using a continuous-flow PEF system. The PEF treatment with 60 pulses resulted in a reduction of 0.2 to 2.8 log and 0.3 to 3.6 log for *E. coli* O157:H7 and *S.* Enteritidis, respectively. For both bacteria, increasing treatment temperature tended to increase the inactivation rate. At constant electric field strength, more inactivation was obtained at 30 °C compared with 10 °C for the 2 bacteria investigated in this study.

Table 21.2 Reduction of foodborne pathogens or surrogates in food by PEF treatments

Food	Microorganism	PEF treatment condition	Log reduction	Reference
Apple juice	*E. coli* O157:H7	29 kV/cm, square wave	5	Evrendilek et al. (1999)
Cranberry juice	*E. coli*	0–40 kV/cm, 69–80 μs treatment time	6.4	Gupta et al. (2003)
Orange juice	*L. mesenteroides, E. coli, L. innocua*:	30 kV/cm, 100 L/h	5	McDonald et al. (2000)
Skim milk	*E. coli*	50 kV/cm, 62 pulses, square wave, < 30 °C	2.5	Qin et al. (1995b)
UHT milk	*E. coli*	22.4 kV/cm, 300 μs of treatment time	4.8	Grahl and Markl (1996)
Skim milk	*E. coli*	40 kV/cm, exponential decay, 15 °C	6	Martin et al. (1997)
Skim milk	*S. dublin*	30 °C, 50 °C, 25 kV/cm, 100pulses	1, 2	Sensoy et al. (1997)
Pasteurized whole, 2%, and skim milk	*L. monocytogenes* Scott A	30 kV/cm, 600 μs of treatment time, square wave, 50 °C	4	Reina et al. (1998)
Fat free milk	*E. coli*, *L. innocua*	2.5 μs pulse width, 3 Hz frequency, 0–60 pulses 41 kV/cm	2.3–6.5 (*E. coli*), 0.7–2.8 (*L. innocua*)	Dutreux et al. (2000)
Skim milk	*L. innocua, P. fluorescens*	50 V/cm for 200 μs	26~2.7	Fernandez-Molina et al. (2001)
Skim milk	*S. aureus*	3.7 μs pulse duration time, 250 Hz frequency, 35 kV/cm, 450 μs of treatment time	3.3 ~ 3.5	Evrendilek et al. (2004)
Liquid whole egg	*L. innocua*	2 μs pulse duration, 3.5 Hz, 10.6, 21.3 and 32 pulses, 30, 35, and 40 kV, exponentially decay pulse	3.5	Calderon-Miranda et al. (1999)
Liquid whole egg	*S.* Enteritidis	200pps frequency, 2.12 μs pulse duration, 25 kV/cm, 250 μs total treatment time, PEF + 55C for 3.5 min	1 and 4.3	Hermawan et al. (2004)
Liquid egg	*E. coli*	26 kV/cm, 37 °C	6	Martin-Belloso et al. (1997)

Adopted from Min et al. (2007), used with permission

Zhao et al. (2007) conducted a similar study investigating the effects of PEF intensity and temperature on the inactivation of *S. enteritidis*, *E. coli*, and *S. aureus* in liquid egg white. The treatment temperatures used in this study were 40 and 20 °C. After PEF treatment at electric field strength of 30 kV/cm for 800 μs, the survival fraction of *S.* Enteritidis, *E. coli* and *Staphylococcus. aureus* decreased by 4.3, 3.8 and 3.0 logs, at 20 °C, while those bacterial populations decreased by 6.3, 5.0 and 3.8 log at 40 °C. Bazhal et al. (2006) also investigated the effects of PEF intensity and temperature, individually or in combination with each other, on the inactivation of *E. coli* O157:H7 in liquid whole egg. They found that a combined increase in the treatment temperature to 60 °C and PEF treatment (15 kV/cm) resulted in 4 log reduction of *E. coli* O157:H7 populations in liquid whole egg, whereas thermal treatment alone only achieved a 2 log reduction at 60 °C.

Additional to the combination PEF and temperature, Jin et al. (2009a, b) also investigate the pH as a factor to contribute bacterial inactivation. In their study, effects of PEF, temperature, pH and PEF on the inactivation of *Salmonella typhimurium* DT104 cells in liquid whole egg (LWE) were investigated. The PEF inactivation of *S.* Typhimurium cells at 15 or 25 °C was pH dependent. There were 0.6 log (15 °C) and 1.3 log (25 °C) reductions of *S.* Typhimurium in LWE at pH 8.2, 1.0 (15 °C) log and 1.8 log (25 °C) at pH 7.2, and 1.1 log (15 °C) and 1.1 log (25 °C) at pH 6.6. In the tested range, liquid whole egg at pH 7.2 and PEF treated at 25 °C demonstrated the highest reduction in *S.* Typhimurium populations.

Inactivation of three pathogens in melon and watermelon juices were investigated by Mosueda-Melgar et al. (2007). PEF treatment at 35 kV/cm, 4 μs pulse duration in bipolar mode and square shape were applied on *S.* Enteritidis, *E. coli* and *L. monocytogenes* populations inoculated in melon and watermelon juices, the outlet temperatures of last PEF treatment did not exceed 40 °C. Although PEF treatment reduced the populations of the three microorganisms, *L. monocytogenes* was more resistant to PEF than *S.* Enteritidis and *E. coli* in both juices when treated at the same processing conditions. Evrendilek et al. (2000) investigated the effect of PEF processing on the inactivation of *E. coli* O157:H7 in apple juice through a bench scale PEF system with electric field strengths of 34, 31, 28, 25, and 22 kV/cm and a mean total treatment time of 166 μs. They found that PEF processing using the bench scale PEF system resulted in a 4.5 log reduction in *E. coli* O157:H7 populations in apple juice.

Gurtler et al. (2011) investigated the effects of PEF treatment and the antimicrobials sodium benzoate, potassium sorbate, and citric acid on the inactivation of *E. coli* O157:H7 in strawberry juice. *E. coli* O157:H7 was inoculated into single-strength strawberry juice with or without 750 ppm sodium benzoate (SB), 350 ppm potassium sorbate (PS), and 2.7% citric acid (CA). The juice was treated at outlet temperatures of 45, 50 and 55 °C at a field strength of 18.6 kV/cm for 150 μs with a laboratory-scale PEF unit. *E. coli* O157:H7 populations in juice with antimicrobials and 2.7% CA (pH 2.7) subject to PEF treatment were reduced by 2.60, 4.32 and 6.95

log CFU/ml at 45, 50 and 55 °C). Geveke et al. (2015) investigated the effects of PEF treatment on the survival of *E. coli* ATCC 35218 populations in fresh strawberry purée. Fresh strawberry purée (pH 2.4) was inoculated with *E. coli* (ATCC 35218) and processed using a pilot plant PEF system at a flow rate of 100 L/h. *E. coli* populations were reduced by 7.3 log in strawberry purée at 24 kV/cm and a outlet temperature of 52 °C.

Effects of pulse polarity and pulse delaying time on inactivation of *E. coli* O157:H7 inoculated into apple juice and skim milk samples were determined by Evrendilek and Zhang (2005). They observed that an inactivation effect as the inoculated food samples were subjected to mono and bipolar square wave pulses. Bipolar pulses with different pulse delaying times were also applied. The results in skim milk yielded a significant difference between mono (1.27 log CFU/ml) and bipolar (1.96 log CFU/ml) pulses with bipolar pulses being significantly more efficient. Among different pulse delaying times, 20 μs caused a significantly higher inactivation than the others in apple juice and skim milk. However, there was no significant difference between mono (2.56 log CFU/ml) and bipolar (2.63 log CFU/ml) pulses on the inactivation of *E. coli* O157:H7 inoculated into apple juice (Evrendilek et al. 2000).

Commercial exploitation of PEF for liquid food pasteurization requires high level of microbial reduction, that is, long treatment times (>100 μs) at high electric field strengths (>30 kV/cm) are required for PEF-only treatments, i.e., very intense treatments for a continuous flow, which carries several technical and economic difficulties. However, there are additional hurdles, which can be combined with PEF that potentially allows a reduction in the intensity of the PEF treatment applied. Besides the combination of mild heat with antimicrobials and/or packaging, a new approach is use of a two-stage PEF process.

In the first stage, the PEF pretreatment could be applied to whole fruits to achieve a ca. 2 log reduction, allowing for the production of juice with high nutritional value. In the second stage, the juice would be subject to a second PEF, or by other nonthermal processing treatments, to achieve an additional 3-log reduction. In this way, less severe processing conditions could be applied for each treatment, not only allowing for the preservation or improvement of quality and nutritional properties, but also meeting the FDA 5-log reduction requirement for juice pasteurization. Similarly, PEF has the potential for flexible applications in other derived fruit products.

Jin et al. (2017) treated whole fresh blueberries using a parallel pulsed electric field (PEF) treatment chamber and a sanitizer solution (60 ppm peracetic acid [PAA]) as PEF treatment medium with square wave bipolar pulses at 2 kV/cm electric field strength, 1 μs pulse width, and 100 pulses per second for 2, 4, and 6 min. The combination of PEF and PAA was able to achieve up to 3 log reduction of *E. coli* K12 and *Listeria* innocua as well as 2 log/g reduction of native microbiota. The results demonstrate the potential of PEF applications to enhance the safety and improve the quality and nutritional value of fruits and their derived products.

21.7 PEF Applications for Improving Shelf Life

In addition to the inactivation of pathogenic microorganisms, PEF can be used to inactivate spoilage microorganisms to extend the shelf life of treated food products. Some of those studies are summarized in Table 21.3 and some PEF treated food samples are shown in Fig. 21.2.

Evrendilek et al. (2000) conducted a study to see if PEF treatment could extend the shelf life of fresh apple cider and reconstituted apple juice. Through a pilot-plant scale PEF system (85 L/h), fresh apple cider and reconstituted apple juice were processed using a 35 kV/cm electric field strength and 94 μs total treatment time, and aseptically packed into 213 g (6 oz) plastic cup containers. PEF treatment extended the shelf life of apple juice and apple cider to 70 days.

Jin et al. (2009a, b) investigated the effects of PEF + HTST treatment on the sensory quality and shelf life of applesauces. Fuji applesauce and blueberry applesauce from fresh apples were processed with PEF (39 kV/cm field strength and 82 μs total treatment time) followed by high temperature short time (HTST, 70 °C for 24 s) pasteurization (PEF + HTST) and aseptically packaged. PEF + HTST processed applesauce demonstrated high and stable sensory scores throughout 9 months storage at 27 °C, and had aerobic plate counts and mold & yeast counts of <10 cfu/ml throughout storage.

The quality and shelf life of PEF treated tomato juice and apple juice were evaluated by Min et al. (2003a, b) using a commercial scale PEF processing unit (500 L/h). After PEF treatment at 40 kV/cm for 57 μs, the PEF-processed tomato juice showed microbial shelf life at 4 °C for 112 days. While processed by PEF at 40 kV/cm for 97 μs, the PEF-processed apple juice had microbial shelf life at 4 °C for 196 days. Sensory evaluations indicated that flavor and overall acceptability of PEF processed juice were preferred to those of the thermally processed juices.

Yeom et al. (2004) formulated a yogurt-based product which was subject to processing by mild heat and PEF treatment in order to investigate the effects of these treatments on aerobic bacterial populations and yeasts and molds. PEF treatment was accomplished using a pilot plant scale PEF system. The following parameters were used for this study: product flow rate of 100 L/h, electric field strength of 30 kV/cm and total PEF treatment time of 32 μs. The pulse duration time was fixed at 1.4 μs and repetition rate was 500 pulses per second (pps). The PEF treatment combined with mild heat (60 °C for 30 s) significantly decreased the populations of total viable aerobic bacteria and total mold and yeast of yogurt-based products during storage at both 4 and 22 °C for 90 days. Sensory evaluation indicated that there was no significant difference between the control and processed products.

Pomegranate juice was processed in a commercial scale PEF processing system by Guo et al. (2014). The juice was processed at 35 and 38 kV/cm for 281 μs at 55 °C with a flow rate of 100 L/h. PEF treatments significantly inhibited the growth of total aerobic bacteria, which remained at <2.5 log CFU/ml throughout the 12-week storage period. No yeast and mold were detected (<0.69 log CFU/ml) in PEF treated

Table 21.3 Reduction of spoilage microorganisms in food by PEF treatments

Food	Microorganism	PEF treatment condition	Log reduction	Source
Apple juice	*S. cerevisiae*	50 kV/cm, square wave, 29.6 °C	6.3	Qin et al. (1995a)
Apple juice	*S. cerevisiae*	40 kV/cm, 64 pulses, exponential decay, 15 °C	3.3	Harrison et al. (1997)
Apple juice	Aerobic microorganisms, yeasts & molds	Pilot plant-scale: 35 kV/cm, 94 μs of treatment time, 85 L/h, 952 Hz	Bacteria −2.1 Y & M – 1.5	Evrendilek et al. (2000)
Apple juice	*S. cerevisiae*	20 kV/cm, 10.4 pulses, square wave	4	Cserhalmi et al. (2002)
Beer	*S. cerevisiae*	40 kV/cm, 46 pulses, 70 μs, square bipolar	Up to 2.2	Milani et al. (2015)
Cranberry juice	Aerobic microorganisms, yeasts & molds	40 kV/cm, 150 μs of treatment time, square wave	Bacteria-4.8, Y & M- 4.9	Jin and Zhang (1999)
Orange juice	Aerobic microorganisms	29.5 kV/cm, 60 μs of treatment time, square wave	4.2	Qiu et al. (1998)
Orange juice	Aerobic microorganisms, yeasts & molds	30 kV/cm, 240 μs of treatment time, 2 μs of pulse width, 1000 Hz, 2 mL/s	Bacteria-2.5 Y & M- 2.5	Jia et al. (1999)
Orange juice	Aerobic microorganisms, yeasts & molds	35 kV/cm, 59 μs of treatment time, 1.4 μs of pulse width, 600 pps, 98 L/h	Bactera-7 Y&M- 7	Yeom et al. (2000)
Orange juice	Aerobic microorganisms, yeasts & molds	40 kV/cm, 97 μs of treatment time, 2.6 μs of pulse width, 1000 pps, 500 L/h	Bacteria-6 Y& M- 6	Min et al. (2003b)
Orange juice	*L. brevis*	8 chambers, 1–10 μs pulse width, 1000 Hz, 1–12 kV electric field strength, mono-bipolar pulse	5.8	Elez-Martines et al. (2005)
Grape juice	Naturally occurring microorganisms	20 pulses of 65 kV/cm	PEF only-5.9 PEF + nisin (400 U/mL) treatment-6.2	Wu et al. (2005)
Whey protein fortified orange juice	Aerobic microorganisms, yeasts & molds	32 kV/cm, 92 μs of treatment time, 3.3 μs of pulse width, 800 Hz, 79 L/h	Bacteria-0.5 Y & M-3.5	Sharma et al. (1998)
Yogurt	*S. cerevisiae*	18 kV/cm, 55 °C	3	Dunn and Pearlman (1987)

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juices during storage up to weeks 10 and 12, and these results are similar to those the thermally processed juice. PEF-treated juice had the same consumer satisfaction scores as the unprocessed juice, which were significantly ($p < 0.05$) higher than those from thermally processed juice samples.

Li et al. (2005) developed a model ranch salad dressing with modified corn starch, cane sugar, whey protein powder, citric acid powder, table salt and tap water and artificially inoculated the salad dressing with *Lactobacillus plantarum* 8014. They investigated the effect of both PEF treatment and a combination of PEF and mild heat treatment on *L. plantarum* populations and on the shelf life and found that PEF-only treatment at 34 kV/cm for 45.7 μs resulted in a more than 7 log reduction of *L. plantarum* populations and significantly extended the microbial shelf life of the model salad dressing to 8-weeks when stored at refrigerated conditions. PEF processing at 31.8 kV/cm for 45 μs followed by a mild heat processing at 67.2~73.6 °C for 24 s resulted in a shelf stable product. No *L. plantarum* 8014 cells were recovered in the model salad dressing at room temperature for at least 1 year.

21.8 Conclusions and Future Trends

Numerous PEF studies in lab scale PEF systems, pilot and commercial scale PEF processing systems have been conducted and evaluated and cost analyses of a commercial scale PEF system using orange juice as a model have been reported by Jin and Zhang (2002) and Sampedro et al. (2013). These studies have demonstrated this technology as a promising application for enhancing food safety, improving food quality and extending food shelf life. Unfortunately, despite such achievements, the movement of this technology from lab scale to full industrial production and its commercialization has been very slow, and currently there are few commercial food production lines using PEF pasteurization technology. There may be multiple reasons that prohibit the commercialization of PEF pasteurization. One reason may be due to the high initial investment cost of PEF equipment, although the PEF operation cost for long term operation is competitive with thermal processing. Other major reasons may be due to the complexity of PEF processing technology and lack of consistent data in microbial reduction, as required by the FDA for a 5-log reduction of a target pathogen.

There are many interdependencies among process parameters (electric field intensity, pulse shape, treatment time, temperature, flow rate, etc.). In other words, the effectiveness of PEF treatment in terms of microbial inactivation depends on the various relationship between the different processing parameters. Numerous studies have demonstrated this complexity (Jin et al. 2015; Min et al. 2007; Ortega-Rivas 2011; Saldaña et al. 2014). In addition to PEF processing conditions, it has been widely described that PEF lethality is also associated with media/food properties (pH, presence of antimicrobial and ionic compounds, conductivity and medium ionic strength) and microbial characteristics. Most PEF studies were performed using

different equipment and under different conditions in various laboratories around the world. The results obtained from these studies were not consistent and sometimes even contradictory, which have made it difficult to obtain general conclusions on the main control parameters affecting microbial inactivation by PEF.

The complexities of PEF treatment are a challenge for potential users of PEF pasteurization, especially for those in industry, who have less research experience with this technology. A processor must validate that the PEF processing conditions being used are able to achieve the desired microbial reductions for a targeted pathogen in their specific product. In other words, the same processing parameters that may work on product A, may not work on product B. The complexity of PEF processing and the lack of information exchanged between industry and researchers may be the main reasons why the food industry is reluctant to adopt this technology. Therefore, it is necessary to understand the defined processing conditions and then simplify these conditions so that PEF processing will be similar to thermal processing with few buttons to be pressed for food pasteurization. PEF equipment manufacturers should team up with university, food industry, and government agencies to "standardize" the processing conditions and procedures with their equipment, such as pulse generator and treatment chambers. When done this way, the results and conclusions would not be misinterpreted, preventing confusion amongst potential users of this technology without PEF research background.

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Chapter 22
Ultrasonic Processes

Hao Feng, Junzhou Ding, and Bin Zhou

22.1 Introduction

Sound waves are mechanical vibrations that are created by a vibrating object. In 1794, Lazzaro Spallanzani, an Italian physiologist and biologist, discovered that bats navigated in the dark by the reflection of high frequency sounds. His discovery becomes the basis of ultrasound physics. Six years after that, brothers Pierre and Jacques Curie, both are French physicists, demonstrated the important piezoelectric effect, e.g. the ability of certain materials to generate an electric charge in response to applied mechanical stress, where "piezo" is for "push" in Greek. The first practical application for piezoelectric devices was proposed and tested during the World War I in sonar devices. The applications of ultrasound in food to secure microbial food safety are based on the bactericidal effect of high intensity ultrasound. The lethal effect of ultrasound was first reported in 1929 by Harvey and Loomis (Harvey and Loomis 1929). Thereafter, many studies have been published documenting the application of ultrasound in the treatment of liquid foods and fresh fruits and

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vegetables, among other food types, for securing microbial food safety. This chapter provides a concise summary of selected ultrasonic processing systems and technologies, followed by a literature review of the research findings reported in a period of past 10 years with regard to application in food safety.

22.1.1 Engineering Principles of Power Ultrasound

22.1.1.1 Generation of Ultrasound

Ultrasound refers to sound waves with frequencies above the human hearing threshold (~20 kHz). In practical applications, ultrasound can be divided into two categories depending on frequency and the sound intensity: high frequency ultrasound and power ultrasound. Power ultrasound (high intensity ultrasound) operates at the frequency range of 20 to 100 kHz with a sound intensity in the range of 10 to 1000 W/cm^2. The high frequency ultrasound operates at frequency ranging from 2 MHz to 20 MHz with low sound intensities of 100 mW/cm^2 to 1 W/cm^2. The relatively high sound intensity of power ultrasound allows it to be used in a spectrum of processing applications, including food safety applications. Piezoelectric effect or piezoelectricity is the underlying physical concept for generation of ultrasound. A typical ultrasound transducer, a device that produces sound waves, contains a piece of piezoelectric ceramic material, such as barium titanate, lithium sulfate, lead metaniobate, or lead zirconate titanate, sandwiched by two electrodes (Feng and Yang 2011). When a high frequency alternate voltage is applied to the electrodes, the piezoelectric material starts to vibrate rapidly and generate ultrasonic waves.

The fundamental effect of ultrasound on a continuum fluid is to impose an acoustic pressure (P_a) to it in addition to a hydrostatic pressure already acting on the medium. The acoustic pressure is a sinusoidal wave defined by time (t), frequency (f) and the maximum pressure amplitude of the wave ($P_{a,max}$). The maximum pressure amplitude of the sound wave ($P_{a,\ max}$) is directly proportional to the electrical power input of the transducer (Patist and Bates 2008).

22.1.1.2 Propagation of Ultrasonic Waves

As a type of mechanical wave, ultrasound can be transmitted in air, liquid, or solid. Most power ultrasound applications involve a special transmission mode, e.g. longitudinal waves, in which the displacement of molecules is in the same or opposite direction of wave propagation. Under such a transmission, the ultrasonic waves propagate via a series of compression and rarefaction waves induced on the molecules of the medium (e.g. liquid) (Feng and Yang 2011). Since most ultrasound applications involve a liquid, the speed of sound in liquids becomes of interest. The speed of sound in selected liquids are shown in Table 22.1. The speed of sound

Table 22.1 Sound speed in air, and liquids and solids

Liquid	Sound speed (m/s)	References
Air (as a reference) at 0 °C 100% relative humidity	331.77	Zuckerwar (1998)
Air (as a reference) at 0 °C 0% relative humidity	331.45	Zuckerwar (1998)
Water at 5 °C	1425.6	Baltasar et al. (2011)
Water at 20 °C	1481.8	Zuckerwar (1998)
Water at 35 °C	1514.3	Baltasar et al. (2011)
Water at 65 °C	1547.4	Baltasar et al. (2011)
Skim milk at 40 °C	1548.0	Elvira and Rodrigues (2009)
Sea water (with electrolytes) at 30 °C	1545.8	Zuckerwar (1998)
Fruit juices (density from 1040.3 to 1061.5 kg/m^3)	1521.4 to 1547.9	Contreras et al. (1992)

increases with increasing pressure and temperature, and with addition of electrolytes (Zuckerwar 1998, Hidalgo Baltasar et al. 2011).

Like for the propagation of any waves, the transmission of ultrasound in a medium undergoes attenuation in the intensity of the wave or diminish of intensity of a wave front. At an interface, the sound wave is partially reflected and partially transmitted. This behavior has an important implication in food safety applications. In a container (or beaker), for instance, the reflected waves from the wall(s) of the container (or beaker) would form complicated standing wave patterns resulted in regions with lowest sound intensity and others with highest sound intensity. This non-uniform sound intensity distribution in a treatment chamber has to be addressed in order to achieve effective microbial inactivation. Similarly, food pieces in an ultrasonic treatment chamber would "block" the sound wave so that each piece of food, such as leaves of fresh produce, would receive different exposure of ultrasound. Consequently, the microbial reduction on each food piece would be different. Therefore, for food safety applications, measures must be taken to ensure a relative uniform acoustic field in a treatment chamber, or among the food items in the chamber.

22.1.1.3 Acoustic Cavitation

The mode of action for most ultrasound applications, especially those related to food safety applications is attributed to a phenomenon called acoustic cavitation. As shown in Fig. 22.1, when an ultrasonic wave travels through liquid, a cavity can be formed at the rarefaction portion of the sound wave when the negative pressure exceeds the local tensile strength of the liquid. Cavitation normally emerges at locations where gas nuclei and other impurities reside. The formation-growth-implosion behavior of the cavitating bubbles depends on the physical and chemical

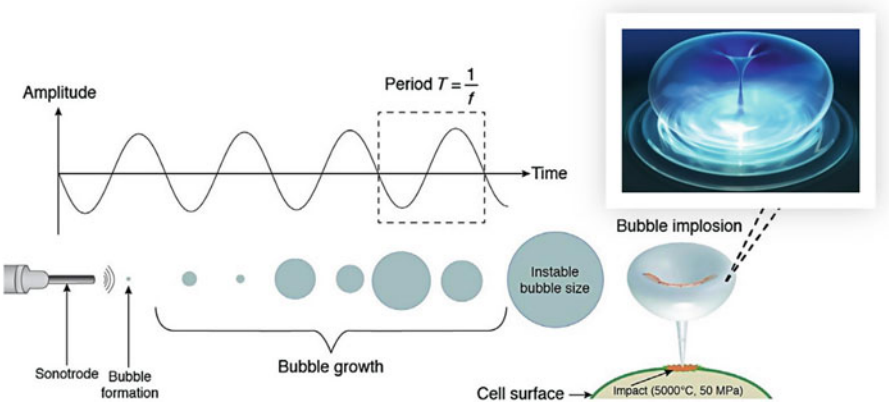

Fig. 22.1 Formation of acoustic cavitation bubbles, adapted from (Roohinejad et al. 2018), with a picture representing liquid jet formation during cavitation bubble collapse from www.mondolithic.com

properties of the media, frequency of the sound wave, and amplitude of the sound wave (Feng and Yang 2011).

Stable (static) cavitation can be generated at a sound intensity of 1–3 W/cm^2, where the bubbles can grow to a size big enough to allow them to float to the surface, which is called as ultrasonic degassing. Transient cavitation bubbles are generated when the sound intensity exceeds 10 W/cm^2, where the cavitating bubbles normally have an effective residence time of less than 100 ns. The implosion of transient bubbles results in localized extreme physical conditions such as very high temperatures (e.g. 5000 K) and pressures (e.g. 1000 atm), high heating and cooling rates, high shear, and formation of shock waves (Leighton 1994; Suslick and Price 1999). Many of these cavitation-induced physical conditions have a lethal effect on microorganisms.

22.1.1.4 Measurement of Acoustic Power

The characterization of a sound field in a liquid can be achieved by measuring the sound pressure distribution with a device called a hydrophone. A hydrophone is a sound-to-electricity transducer that takes real-time measurements of the acoustic pressure in a liquid, which converts sound signals to electrical signals in real time (Feng and Yang 2011). Currently, ultrasonic cavitation meters are used to measure the intensity of the acoustic emission, using two channels-cavitation signals (High frequency 400 kHz −1.2 MHz) and the sonication signal (Low frequency 20 kHz–50 kHz). The cavitation meter provides point-to-point measurement of cavitation distribution and hence the ultrasound field distribution, which is useful for validation of numerical simulation results. Both sound intensity level described in decibels (dB) and ultrasound emitting surface intensity (W/cm^2) are used in acoustic studies.

However, for processing applications, it is more useful to characterize the intensity of an ultrasonic treatment so that the experiment by any research group can be repeated and validated by a third party. For that purpose, relative cavitation intensity determined by hydrogen peroxide formation and acoustic power density (W/L) have been used to describe the intensity of an ultrasound treatment.

The cavitation intensity method is mainly used with water as the medium (Raviyan et al. 2005). Therefore, for a liquid food treatment with ultrasound, it only provides the relative cavitation intensity when the volume and other treatment conditions of the liquid food is the same as that of water. Acoustic power density (W/L) is a more practical means to measure the acoustic energy input into a food system (Manas et al. 2000). The acoustic power (P) can be estimated by calorimetric method shown in Eq. (22.1) below:

$$P = mC_p \frac{dT}{dt} \tag{22.1}$$

where m is the mass of the liquid (kg), C_p is the specific heat of the liquid (J/(kg K)), dT/dt (K/s) is the initial slope of the temperature versus time curve. The acoustic power density (APD) is the amount of power per unit volume (W/L) of the sample to be sonicated. For processing control, APD is the appropriate measurement compared to the surface intensity measurement in W/cm^2. The later does not provide any clue on the sample treatment volume and thus cannot be used as a process design and control parameter.

22.1.2 Mechanism of Ultrasonic Inactivation of Microorganisms

When ultrasonic waves propagate in a liquid, stable and transient cavitation bubbles are generated. The localized high temperature, high pressure, high shear, shock waves, and high velocity water jets produced by the imploding cavitating bubbles are responsible for most of the bactericidal effects of ultrasound treatments (Feng and Yang 2011). Traditional thinking is that for ultrasonic inactivation of microorganisms, the lethal factors could be a combination of different chemical and physical effects related to cavitation activity, which imparts ultrasound inactivation an inherent complexity compared to other non-thermal processing technologies. However, the contribution of free radicals generated by acoustic cavitation (sonolysis) in microbial inactivation has been confirmed to be negligible by a number of reports (Pagan et al. 1999). Most studies have attributed the lethal effect of ultrasound treatment to physical actions related to cavitation (Tiwari and Mason 2012). It is now commonly agreed upon that the physical forces of ultrasonic waves cause mechanical rapture of cell envelopes (Condon et al. 2011). Detailed studies using flow cytometry analysis have revealed that bacterial cells contain several targets which are sensitive to ultrasound treatment, including cell wall, cytoplasmic

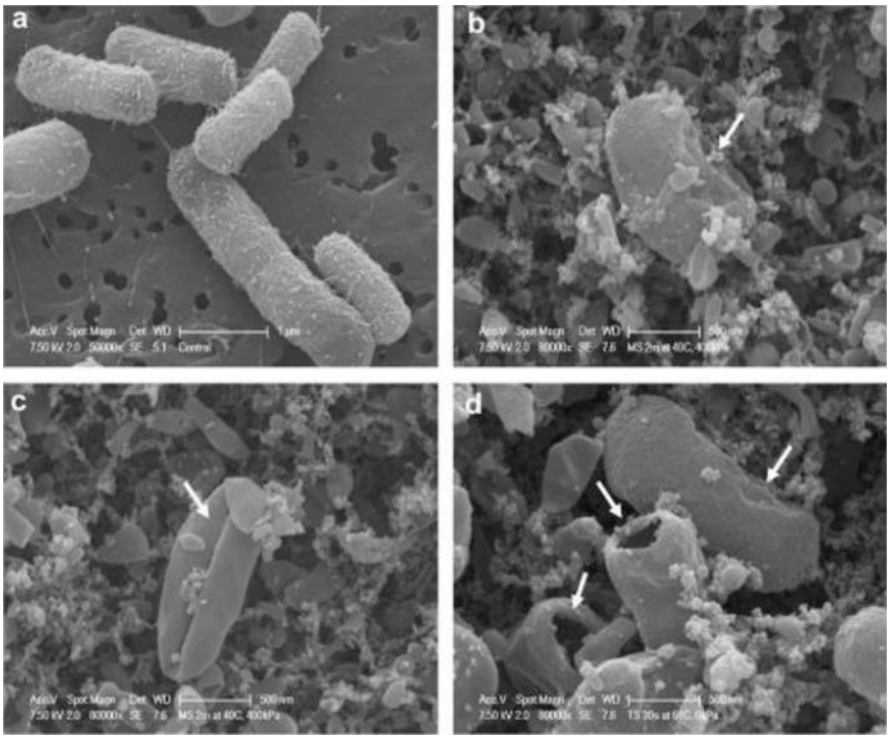

Fig. 22.2 *E. coli* K12 cells observed with environmental scanning electron microscopy (ESEM): (**a**) control (50,000 magnification), (**b**) and (**c**) manosonication at 40 °C and 500 kPa for 2 min (80,000 magnification), (**d**) thermosonication at 61 °C and 100 kPa for 0.5 min (80,000 magnification), selected from (Lee et al. 2009)

membrane, DNA, internal cell structures, and outer membrane. The primary damage that causes cell death is microorganism-specific. For instance, for *E. coli* cells, the rupture of outer membrane is responsible for the loss of viability (Alzamora et al. 2011).

The bacterial cell damage caused by sonication has been investigated in a number of studies using microscopic imaging techniques (Guerrero et al. 2001, 2005; Lee et al. 2009). *E. coli* O157:H7 cells treated with MTS exhibited extensive damages, including ruptured cells and breakage of cell membranes (Fig. 22.2). Obviously, the cell damage shown in the images is irreversible, which is an advantage of power ultrasound in microbial inactivation. (Kahraman et al. 2017).

During an ultrasound treatment, heat is produced, just like the application of many other, if not all, non-thermal technologies. In addition, ultrasound may be applied in combination with other factors, such as mild heat and hydrostatic pressure. However, since the mode of action in an ultrasound treatment is attributed to activities arisen from acoustic cavitation, not heat, ultrasound is referred to as a non-thermal technology in food processing applications.

22.1.3 Inactivation Kinetics and Kinetic Models

Kinetics have been used for understanding reaction rate and kinetic data, and the design of food processing unit operations. Pasteurization and sterilization are two typical processes where first-order microbial inactivation kinetics are often used to obtain D- and z-values. With the D- and z-values, the treatment time of a thermal process can be determined to achieve a target microbial count reduction. Scientists working on power ultrasound have also attempted to provide similar D- and z-values to guide the design of, for instance, an ultrasound-assisted liquid food pasteurization process (Feng 2011), as shown in Table 22.2.

22.1.3.1 First-Order Kinetic Models

Similar to the thermal processing counterpart, ultrasound inactivation of bacteria has attempted to use first-order inactivation kinetics to describe the response of microorganisms. An advantage for this method is that the well-established design procedure in thermal processing (pasteurization or sterilization) can be used in the design of an ultrasound-assisted treatment. For instance, the widely used D- and z-value concept can be used to define the survival count of a bacterium subjected to an ultrasound treatment.

It needs to be pointed out that for a specific ultrasound treatment, such as for sonication, thermo-sonication, mano-sonication, or mano-thermo-sonication (MTS), the definition of D- or z-value may not be as intuitive as in the case of thermal processing. For instance, D-value in the MTS treatment has to do with a number of parameters, such as the treatment time, sound intensity (acoustic power density), temperature, and hydrostatic pressure. As a result, more than one D-value would exist for an MTS treatment. It could be the time used to get 90% reduction in the population of a target organism at a fixed sound intensity, temperature, and hydrostatic pressure. Then for a different sound intensity or pressure, D-value should be different. In some other cases, ultrasound treatment may be combined with another physical or chemical treatment to enhance microbial inactivation. In ultrasound-assisted fresh produce treatment, for instance, it might be even more difficult to define D-value when ultrasound surface treatment is combined with a chlorine wash at certain concentration. Precaution must be taken to understand the physics of the process and clearly define the kinetic parameters.

22.1.3.2 Non-linear Inactivation Models

In many cases, ultrasonic inactivation of microorganisms has been shown to follow non-linear inactivation kinetics, such as those defined by the Weibull, modified Gompertz, or biphasic linear models. Table 22.3 tabulates a few selected non-linear inactivation models used to describe non-linear inactivation behavior

Table 22.2 Ultrasonic processes for inactivation of bacteria in liquid foods: studies in 2008–2019

Food matrix	Ultrasound device	Treatment	Microorganism Studied	Microbial reduction	References
Apple cider	MTS /probe system (Sonics & Materials	20 kHz/ 100 kPa/ 59 °C, 3.8 min	*E. coli* K12	5 log	Lee et al. (2013)
Apple-carrot juice	MTS /probe system (Sonics & Materials)	20 kHz/ 300 kPa/ 60 °C, 0.5 min	*E. coli* 0157:H7	5 log	Kahraman et al. (2017)
Carrot juice	Ultrasonic processor, UP400S (Hielscher USA)	24 kHz/ 58 °C, 10 min	Indigenous microorganisms	>4 log	Martínez-Flores et al. (2015)
Carrot juice	400 W ultrasonic device (Hielscher USA)	24 kHz/ 58 °C, 2 min	*E. coli* ATCC 11775	5 log	Pokhrel et al. (2017)
Mango juice	Ultrasonic Elma, cleaning bath (Model TI-H-10)	25 kHz/ 60 °C, 1.36 min	*E. coli* O157:H7	1 log	Kiang et al. (2013)
Milk	Ultrasonic processor, UP400S (Hielscher USA)	24 kHz/ 63 °C, 30 min	*L. innocua*	2.5 log (whole milk); 4.9 log (Skim milk)	Bermúdez-Aguirre and Barbosa-Cánovas (2008)
Milk/beef slurry	Ultrasonic processor, UP200S (Hielscher USA)	24 kHz/ 70 °C	*B. cereus* spores	D: 2.9 min (skim milk); 0.4 min (beef slurry)	Evelyn and Silva (2015a, b)
Milk/ orange juice		20 kHz/ 72 °C, 20 sec	Milk: Indigenous thermophilic bacteria (ITB) and Spores (S); Orange juice: *S. cerevisiae* (SC)	5.39 log (ITB); 1.66 log (S); 6.57 log (SC)	Ganesan et al. (2015)
PBS buffer	MTS /probe system, (Sonics & Materials)	20 kHz/ 50 °C/ 400 kPa, 5 min	*E. coli ATCC 2592/ S. aureus ATCC 25923*	6.25/4.55 log, respectively	Chantapakul et al. (2019)
PBS buffer	MTS /probe system	20 kHz/ 61 °C,	*E. coli* K12	5 log	Lee et al. (2009)

(continued)

Table 22.2 (continued)

Food matrix	Ultrasound device	Treatment	Microorganism Studied	Microbial reduction	References
	(Sonics & Materials)	pH = 7, 0.5 min,			
Strawberry juice	Ultrasonic processor (S-4000, Misonix Sonicators)	20 kHz/ 55 °C, 9 min	Aerobic mesophilic bacteria, Yeasts	100% killing	Herceg et al. (2013)
Sugarcane juice	Ultrasonic processor (Q Sonica)	20 kHz/ 50 °C vs	*E.* coli ATCC 25922 *B. cereus* F4810 total aerobic mesophilic	5.5/2.5/3.2 log, respectively	Garud et al. (2017)

with ultrasound (Feng 2011). Lee et al. (2009) reported that the biphasic linear, log-logistic, and modified Gompertz kinetic models fit the inactivation data for MTS, thermo-sonication, and mano-sonication treatments of *E. coli* K12 better than the first-order and Weibull models.

During the power ultrasound inactivation process, the non-linear inactivation behavior could be attributed to subpopulations of different resistance and physiological reactions of the cells to ultrasound treatment (Heldman and Newsome 2003). The break-up of cell aggregates (lumps) by acoustic cavitation may cause formation of shoulders on bacterial survival curves (Feng 2011). The tails may be caused by a gradual change in the properties of the liquid medium during sonication. For instance, in a probe system open to air, air may be entrained into the liquid, which would induce a progressive decrease in the cavitation activity and hence a reduction in inactivation rate. For a treatment lasting relatively long time, the output power of the ultrasonic generator system will decrease over time, which also cause a decrease in inactivation efficacy (Feng 2011).

22.2 Types of Ultrasonic Processing Systems

22.2.1 Probe System

One of the most widely used methods to deliver acoustic energy into a liquid phase to perform a target operation is a probe system (or ultrasonic horn). An advantage of a probe system is its ability to deliver large amounts of power (high-power dissipation per unit area) into a relatively small volume of liquid product (Gogate and Pandit 2015). The power intensity in the vicinity of the probe can be very strong, and as a result, pitting of the probe (cavitation-induced erosion) will occur after prolonged use of a probe, which has been an issue with the probe systems, especially for food grade or medical applications. The sound intensities in a probe system can

Table 22.3 Selected non-linear inactivation kinetic models

Model	Primary model equation	Reference
Weibull	$\log \frac{N}{N_0} = -\left(\frac{t}{\alpha}\right)^{\beta}$	Mafart et al. (2002)
Modified Gompertz	$\log \frac{N}{N_0} = \mathrm{a} \cdot \exp\left[-\exp\left(b + c \times t\right)\right] - a \cdot \exp\left[-\exp\left(b\right)\right]$	Linton et al. (1995)
Biphasic linear	$\log \frac{N}{N_0} = \log_{10}\left[(1-f) \cdot 10^{-\frac{t}{D_{sens}}} + f \cdot 10^{-\frac{t}{D_{sens}}}\right]$	Cerf (1977) Lee et al. (2013)
Log-logistic	$\log \frac{N}{N_0} = \frac{A}{1+e^{4\sigma(\tau - \log t)/A}} - \frac{A}{1+e^{4\sigma(\tau - \log t_0)/A}}$	Chen and Hoover (2003)
Modified Baranyi	$\frac{dN}{dt} = -k_{\max}\left(1 - \left(\frac{N_{\min}}{N}\right)^{m}\right)N$	Koseki and Yamamoto (2007)
K-L-M	$\log \frac{N}{N_0} = -2kt^{0.5}$	Klotz et al. (2007)
Double Weibull	$\log \frac{N}{N_0} = \log_{10}\left[f \cdot 10^{-\left(\frac{t}{\delta 1}\right)^{p1}} + (1-f)10^{-\left(\frac{t}{\delta 2}\right)^{p2}}\right]$	Coroller et al. (2006)
Lorentzian	$\log \frac{N}{N_0} = a + \frac{b}{1+\left(\frac{t-c}{d}\right)^2}$	Evelyn and Silva (2015a)
Log-logistic model	$\log \frac{N}{N_0} = \frac{A}{1+e^{4\sigma(\tau - \mathrm{Log}t)/A}} - \frac{A}{1+e^{4\sigma(\tau+6)/A}}$	Evelyn and Silva (2015b) Lee et al. (2013)
Modified biphasic shoulder model	$\mathrm{Log}_{10}(N) = N_0\{[f\exp(-\mathrm{K}_{\max 1}\mathrm{t}) + (1\text{-}f)\exp(-\mathrm{K}_{\max 2}\mathrm{t})]\exp\frac{\mathrm{K}_{\max 1}\mathrm{SL}}{1+[\exp(\mathrm{K}_{\max 2}\mathrm{SL})-1]\exp(-\mathrm{K}_{\max 1}\mathrm{t})}\}$	Inguglia et al. (2018)

Updated from Feng (2011)

Fig. 22.3 (**a**) Ultrasonic probe system and (**b**) a typical ultrasonic bath (Crest Ultrasonics)

easily reach several hundred Watts per square centimeter at the top of the probe. A laboratory scale unit with 500 to 600 W can be commercially purchased at relatively low prices that may be the reason for widely reported research works using a probe unit. Commercially, a probe system with 16,000 watts of ultrasonic power has been developed (Hielscher, Germany).

A probe system can be operated in batch or continuous mode. When operating at continuous made, a number of probe units can be connected in series or in parallel to increase the throughput of the whole process. As such, an ultrasonic system with a power of more than 40 kW can be achieved. Many probe systems provide an option to run the unit in pulsation mode to save energy and increase the durability of the equipment. Figure 22.3a shows typical probe systems.

22.2.2 Bath System

In an ultrasonic bath or tank system, a number of transducers are normally placed at the bottom surface of the bath, which emit sound waves into the bath filled with water (or disinfector added). Most ultrasound baths are used for cleaning applications at frequencies from 20 kHz to 1 MHz. A base model of an ultrasonic bath is shown in Fig. 22.3b. In cleaning applications, lower ultrasound frequencies produce larger cavitation bubbles with stronger implosions for heavy duty cleaning, whereas higher frequencies produce smaller cavitation bubbles good for more delicate cleaning or for submicron particulate removal. Ultrasonic baths have been widely used in cleaning of industrial parts (Awad 2011). In food applications, ultrasonic bath devices have the potential to be used for food produce surface decontamination, which is discussed in detail in Sect. 22.3.2. The acoustic power density in ultrasonic baths is significantly lower than that of probe systems. Therefore, the sonic cleaning process mainly removes microorganisms and other solid deposits from product surface while the killing of the detached bacteria is achieved by a sanitation agent in the washing solution.

22.2.3 Dual−/Multi-Frequency Ultrasound System

The acoustic field in a traditional fixed-frequency ultrasonic probe or bath system is not uniform, mainly due to the standing wave formation in a fixed-frequency system (Feng 2011). In addition, a single frequency sonication unit can only mobilize bubbles with sizes (R_r) at or near their natural resonance frequency (f_r) to generate cavitation, where f_r can be estimated with equation (22.2) given by Mason and Lorimer (2002).

$$f_r = \frac{1}{2\pi R_r}\left[\frac{3\gamma P_h}{\rho}\right] \tag{22.2}$$

where R_r is the radius of the bubble, ρ is the density of the liquid, P_h is the hydrostatic pressure, and γ is the ratio of the specific heats.

The use of multiple transducers at different frequencies was reported to increase the cavitation activity. Thus, increasing attention has been paid to apply multi-frequency techniques for improving the efficacy of a sonication treatment. The goal is to create a sonication environment under multiple frequencies so that cavitating bubbles with a wide range of sizes can go through implosion, hence increasing cavitation activity and improve uniformity. In a dual frequency system tested by Ciuti et al. (2003), the enhancement on ultrasonic cavitation, as measured by iodine release, is more than the addition of cavitation effects at the individual frequencies. In a waste water treatment study, Zou and Wang (2017) reported that the combination of two ultrasound frequencies, e.g. 17 kHz + 33 kHz or 70 kHz + 100 kHz with sodium hypochlorite (NaClO) enhanced the disinfection efficacy, and one more log cycle reduction in the population of *Bacillus subtilis* was achieved for the frequency pair of 17 kHz + 33 kHz, compared to the treatment with the individual frequency. In a recent study, shredded cabbage samples were washed at three frequencies (28, 40, 68 kHz) with a custom-designed washing unit (Alenyorege et al. 2018). Sweeping frequency (SF) (± 2 kHz from the central frequency) and fixed frequency (FF) ultrasound treatments were performed in washing tests. However, they reported that the treatment at a fixed frequency of 40 kHz was the most effective with >3 log CFU/g reduction in non-pathogenic *E. coli* counts, and approximately 20% improvement in total phenolic content. The non-uniform ultrasound field distribution in the treatment tank and the blockage of the sample to be treated to the propagation of sound wave may be responsible for the observed inconsistencies in the reports on ultrasound applications in microbial inactivation.

22.2.4 Airborne Ultrasonic System

Most microbial inactivation studies are performed in a liquid medium, and the process is also termed as *sonication* if the treatment temperature is in sub-lethal

ranges. In some situations, a treatment without the presence of a liquid is desirable. For instance, aerosolized anthrax spores (*Bacillus anthracis*) contaminated mails may be used by terrorists can pose a serious challenge to national security. Consequently, mails to governmental offices must be decontaminated before it can be delivered. Obviously, such treatment cannot be performed in a liquid. To address this challenge, Hoover et al. (2002) proposed the use of non-contact ultrasound (NCU) (70 kHz to 200 kHz) to treat *Bacllius thuringiensis kurstaki* (Bt) spores sealed in white paper envelopes. They reported a 99.9% destruction of dried bacterial spores in 30 s treatment at 93 kHz. A recent study by Charoux et al. (2019) explored the use of airborne ultrasound (26 kHz) for decontamination of black pepper grains inoculated with *Bacillus subtilis* vegetative cells and spores. After a 30-min treatment, 2.19 log CFU/g reduction was achieved in the population of bacterial cells while no significant reductions were observed in spore contaminated samples ($P > 0.05$). The significant difference on the destruction of bacterial spores in the above two studies may be attributed to the impedance mis-matching issue in all airborne ultrasound applications. The Hoover et al. (2002) group had taken special measures to minimize the impedance mis-matching in their transducer design, thus achieving an effective inactivation of spores.

22.2.5 Focused Ultrasound System

In an effort to develop high-intensity focused ultrasound (HIFU) to inactivate bacteria contained in the fluid pus of abscesses as an alternative to traditional drainage method, Brayman et al. (2017) used focused transducers (1.95 MHz) to treat non-pathogenic *E. coli* cell suspensions with an initial population of 1×10^9 cells/mL (Fig. 22.4). Using acoustic pressure as an indication of the treatment intensity, they observed a maximum of 2 log (99%) reduction of *E. coli* in 10 min, with a D value of 5, similar to most independent ultrasound treatments at sub-lethal temperatures reported in the literature (Feng 2011). The less effective inactivation should be attributed to the set up used, where the sound waves were transmitted through de-gassed water, passing through a polystyrene wall to reach the cell suspension in one of the 96-well microplate wells with round bottoms, all introducing losses in sound intensity. In a follow-up study (Brayman et al. 2018), the same group reported a scaled-up experiment in which 5- or 10-mL of *E. coli* cell suspension was treated with sound waves emitted from a 44.5-mm diameter, spherically focused single-element transducer (1.057 MHz). With the scaled-up unit, a less effective inactivation was reported; 2.5 min was required to achieve a 50% (or 0.3 log) reduction in 5 mL samples whereas it took 6 min to get a 50% (or 0.3 log) reduction in 10 mL samples. This method may find applications in the treatment of food systems.

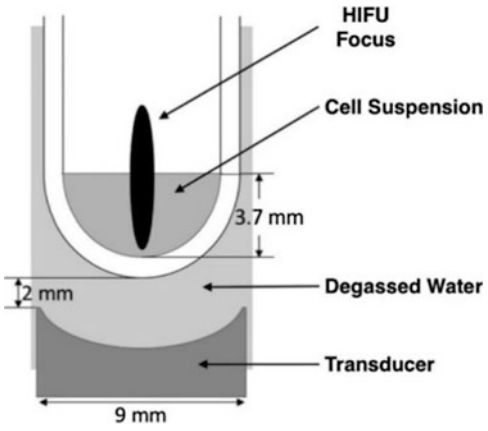

Fig. 22.4 Experimental setup representing one of the four transducers and sample wells. Each transducer has a diameter of approximately that of a microplate well, and situated 2 mm below the well bottom, with degassed water as the coupling medium. The bacterial sample suspension filled the well to a height of 3.7 mm. (Brayman et al. 2017)

22.3 Applications of Ultrasonic Processing for Enhancing Food Safety

22.3.1 Pasteurization of Liquid Food

22.3.1.1 Thermal Sonication (TS) and its Limitation

Ultrasound treatment of a liquid food is aimed at achieving a 5-log reduction in the population of a target pathogenic organism. Thermal sonication (TS) refers to the application of ultrasound in combination with heat at lethal temperatures. Compared with sonication at sub-lethal temperatures, TS can significantly reduce treatment times, while keeping the same lethality and improving food quality (Zenker et al. 2003). Ugarte-Romero et al. (2006) reported that TS treatment at 57 °C shortened the time to achieve a 5-log reduction of *E. coli* O157:H7 in apple cider by 25%. Lee et al. (2013) also found that TS (20 kHz, 59 °C) resulted in a 5-log reduction of *E. coli* K12 cells suspended in apple cider in 3.8 min, while keeping similar color parameters and key aroma compounds similar to raw apple cider during storage over a 3-week period at refrigeration temperature. Ganesan et al. (2015) examined the microbial survival in orange juice treated by a TS treatment (3 μm amplitude, 20 kHz), and concluded that a 3-min sonication at 84 °C achieved 5.5 log reduction of yeast cells. The study of Martínez-Flores et al. (2015) about the survival of natural flora in carrot juice showed that the TS-treated (sonicated at 58 °C) samples had much less growth of mesophiles (3.1-log), yeast and molds (4.6-log), and enterobacteria (2.0-log) after a 20-d storage, compared to that sonicated at 54 °C (5.8, 7.8, and 7.5 log, respectively). Another study reported that TS treatment (24 kHz, 120 μm, and 400 W) at 58 °C achieved more than 5 log reduction of *E. coli* in carrot juice within 2 min, but up to 10 min at 54 °C, while there was no significant difference ($P > 0.05$) on the physicochemical characteristics and color parameters between fresh and ultrasound treated samples (Pokhrel et al. 2017). Kiang et al. (2013) recorded a lower D-value of *E. coli* O157:H7 in mango juice

treated with TS at 60 °C than that sonicated at 50 °C (1.36 min vs 3.20 min). For aerobic mesophilic count in natural sugarcane juice (Garud et al. 2017), the TS treatment (50 °C) yielded a higher microbial reduction (3.9 log) than the sonication treatment at 10 °C (2.6 log), reduced the time required for 5 log reduction by 60% as compared to sonication. Evelyn and Silva (2015a, b) demonstrated that TS inactivated psychrotrophic *Bacillus cereus* spores in skim milk more effectively than thermal treatment alone, the D_{70} value in skim milk was 2.9 min for TS and 8.6 min for the thermal treatment. When TS is applied in milk treatment, fat content affected significantly the rate of inactivation. A 30-min TS treatment (24 kHz, 63 °C) achieved 2.5 log reduction of *Listeria innocua* in whole milk (3.47% fat content), 4.5 and 3.2 log reduction in reduced fat content milks (1% and 2% fat), and 4.9 log reduction in fat free milk (Bermúdez-Aguirre and Barbosa-Cánovas 2008).

It is known that an ultrasound treatment is often accompanied by heat generation. Therefore, a carefully designed TS system can utilize the heat produced by ultrasonication to achieve a target operation without the need for an additional heating system. A good temperature control will help to achieve the goal, and also minimize the potential quality loss caused by high temperatures (Abid et al. 2014; Herceg et al. 2015; Rawson et al. 2011; Wu et al. 2008).

There is a temperature upper limit for TS treatment above which the addition of heating would not introduce any additional killing, may be due to a cushioning effect when vapor-filled bubbles implode at a relatively high temperature (Feng 2011). This phenomenon was reported to be observed in the TS inactivation of yeast, spore formers, and Gram-positive and Gram-negative cells. Feng (2011) defined a cut-off temperature T_1 on the free-energy difference (ΔG) vs. temperature (T) curve for TS using non-equilibrium thermodynamic theory, and found that when the temperature is greater than T_1, the additional treatment by ultrasound will not cause the conditions to be more favorable than they are already. Consequently, no more additional killing will be obtained when the TS is performed at temperatures above T_1. Nevertheless, since ultrasound treatment in a liquid will generate heat, and if the heat generation is controlled to achieve a constant temperature, then a TS treatment is relatively easy to achieve. This should be the reason for widely reported TS studies. As a result, relatively abundant inactivation data are available in the literature for microbial inactivation with TS method.

22.3.1.2 Mano-Thermo-Sonication (MTS) and High Intensity, Short Time (HIST) Treatment

Noticeably, it takes a few minutes to up to 10+ minutes to achieve a 5-log reduction in the population of a target microorganism in a liquid food using a TS system. In a conventional thermal pasteurization operation, however, only 15 s are needed to achieve the FDA required 5-log reduction in many liquid foods. Consequently, a TS treatment compares unfavorably with conventional thermal counterparts with regard to treatment time, thus throughput and production costs. To address this issue, an ultrasonic treatment at lethal temperature and low hydrostatic pressure (200 to

500 kPa), termed mano-thermo-sonication (MTS) was introduced in the field of food preservation to enhance microbial inactivation efficacy. With this method, Pagan et al. (1999) found that static pressure significantly affected D values of mano-sonication (MS) (20 kHz, 40 °C) for *L. monocytogenes* inactivation (4.3, 1.5, and 1.0 min at 0, 200, and 400 kPa, respectively). In the study of Lee et al. (2009), MTS treatment resulted in a 5-log reduction of *E. coli* K12 in a buffer (pH = 7) at 61 °C in 0.5 min. In apple cider, a 5-log reduction of *E. coli* K12 was obtained in 1.4 min by MTS, compared to 3.8 min for a TS treatment (Lee et al. 2013). Kahraman et al. (2017) also reported that elevated treatment temperature (60 °C) and hydrostatic pressure (300 kPa) in the MTS system significantly enhanced the microbial reduction in apple-carrot juice, and achieved 5-log CFU/ml reduction of *E. coli* 0157:H7 in 30 s for MTS treatment at 60 °C, in comparison to 60 s at 50 °C.

In comparison with TS, not many microbial inactivation data are available in the literature for MTS treatments. One reason is the challenge in the development of laboratory scale MTS systems, especially continuous flow systems. Food technologists and microbiologists need to work closely with engineers for the development of such systems. In practical settings, however, an MTS treatment can be achieved by utilizing the heat produced by the ultrasound treatment and the hydrostatic pressure established when using a pump to transport the liquid food into a processing unit. A compilation of the studies in the period of 2008 to 2019 using ultrasound to inactivate microbe in liquid food is given in Table 22.2.

22.3.2 Surface Decontamination of Fresh Produce

22.3.2.1 Surface Decontamination with a Probe System

A few reports using probe system to inactivate or remove bacterial cells attached to product surfaces have been published. Mott et al. (1998) investigated the application of axially propagating ultrasound (APU) at frequencies ranging from 20 to 350 kHz to one end of water-filled glass tubes for the removal of mineralized *Proteus mirabilis* biofilm. The results showed that three 30 s pulses from the 20-kHz transducer removed 87.5% of the biofilm. Berrang et al. (2008) reported that 30-s ultrasonication improved the antimicrobial performance of both quaternary ammonium- and chlorine-based chemicals by 1.29 and 1.14 log CFU/cm^2 for *L. monocytogenes* from the inner wall surface of model polyvinyl chloride drain pipes, respectively. Baumann et al. (2009) used a probe system (20 kHz) to remove *L. monocytogenes* biofilms from stainless steel chips. They found that when ultrasound was combined with 0.5 ppm ozone, no recoverable *L. monocytogenes* cells can be detected from chip surfaces after a 60-s treatment. Zhou et al. (2009) tested the improvement of ultrasonic probe system for inactivation *E. coli* O157:H7 inoculated on spinach by chlorine. The results showed that ultrasonication

significantly enhanced the reduction of *E. coli* cells on spinach for all treatments by 0.7 to 1.1 log cycle over that of washes with chlorine alone. The probe system can deliver a large amount of acoustic energy into the treatment chamber (high APD). However, this method has common disadvantages: non-uniform ultrasound field in the treatment chamber (or beaker), difficulty in scale-up, single fixed frequency, the generation of radical species, the erosion of the tip with prolonged use, and the contamination of small metallic particles.

22.3.2.2 Surface Decontamination with a Tank System

Ultrasonic cleaning is a mature technology for surface cleaning of industrial parts, and commercial equipment for large scale cleaning operations is available in the market (Awad 2011). Its use in food safety applications, however, is a relatively new endeavor. An ultrasonic cleaning bath or tank is usually made of a stainless-steel tank with transducers installed on its base and side wall(s). The critical factors in a cleaning operation include acoustic power density (APD), the acoustic field uniformity in the tank, type of sanitizer, the design of ultrasonic vessel, and operation conditions such as agitation of liquid. As reported by Seymour et al. (2002), the combination treatment of ultrasound (32–40 kHz) with chlorinated water in an ultrasound tank for 10 min enhanced the removal of *Salmonella* Typhimurium attached to iceberg lettuce by 1 log CFU/g compared to chlorine wash alone. Huang et al. (2006) observed a 2.26 to 2.97 log reduction in *Salmonella enterica* and a 1.36 to 2.26 log reduction in *E. coli* O157:H7 inoculated on lettuce through the combined treatment of ClO_2 and 170-kHz ultrasonication. When apples are washed by a combination of ClO_2 and ultrasonication, the bacterial reductions were 3.12 to 4.25 log for *S. enterica* and 2.24 to 3.87 log for *E. coli* O157:H7, respectively. It has also been demonstrated that ultrasound enhanced the removal of *S. enterica* and *E. coli* O157:H7 inoculated on alfalfa seeds in a treatment with 1% calcium hydroxide (Scouten and Beuchat 2002). It should be noted that the above sanitation tests were performed with a bench-top batch washing tank. For industrial scale produce wash, the inactivation data from a continuous system would be more useful.

For that purpose, Zhou et al. (2012) developed a pilot-scale, continuous-flow washing system with ultrasonic capability (Fig. 22.5) for sanitation of fresh produce. The system was designed in such a way that a good spatial uniformity of ultrasound treatment can be achieved with a minimized ultrasound-strength non-uniformity and reduced dispersion in the residence-time distribution. In the unit, ultrasound transducer boxes with three frequencies, e.g. 25, 40, and 75 kHz, were used. Assessment of cavitation-driven damage to aluminum foil was used by Zhou et al. (2012) to examine the ultrasound distribution in the continuous-flow washing tank. Figure 22.6 shows how ultrasound-induced cavitation perforates an aluminum foil, and that there is no difference in damage at different spanwise locations in the channel. This indicates that the distribution of ultrasonic intensity across the 12-inch channel

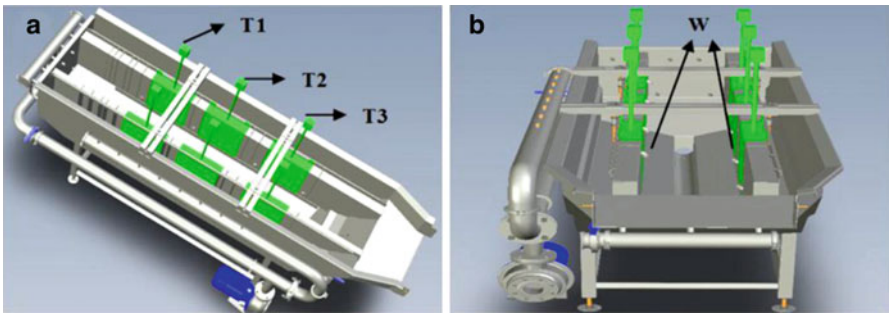

Fig. 22.5 (**a**) Top view of the ultrasonic washer with T1 (25 kHz), T2 (40 kHz), and T3 (75 kHz) denoting the three pairs of transducer boxes; (**b**) top-lateral view of the washer with W indicating the washing channel wall (Zhou et al. 2012)

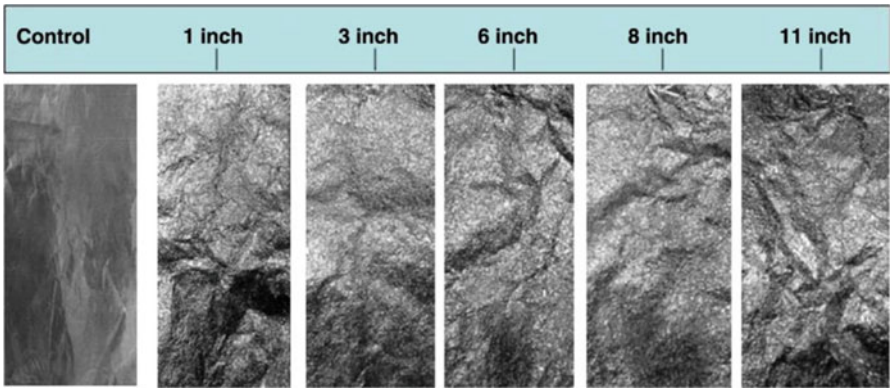

Fig. 22.6 Damage to aluminum foils treated for 30 s at five spanwise locations in the ultrasonic washing channel of a continuous-flow ultrasonic washing system with a channel width of 12 in. (304.8 mm)

used in this study is quite uniform. With the ultrasonic continuous-flow washing system, Zhou et al. (2012) reported that additional log reductions of 1.0 and 0.5 CFU/g for *E. coli* cells inoculated on spinach, for washing in single-leaf and batch-leaf modes, respectively, were achieved compared to treatment with chlorine alone. Using the same ultrasonic washing system, Salgado et al. (2014) examined the whole-head washing of iceberg lettuce (*Latuca sativa* L.) using combinations of water, chlorine, peroxyacetic acid, and ultrasound. They found that, compared with the traditional cut-before-wash method, the ultrasound-assisted wash-before-cut process achieved additional *E. coli* O157:H7 reductions of 0.37–0.68 log CFU/g from iceberg lettuce, reaching total reductions of 2.43 and 2.24 log CFU/g, respec-

tively. The findings from the study of Salgado et al. (2014) demonstrated that by simply changing the current washing procedure in the fresh cut produce industry, a noted improvement in the microbial safety of the fresh products can be achieved. Microbial decontamination studies (2008 to 2019) with ultrasound with both probe and tank units is summarized in Table 22.4.

22.3.3 Spoilage Microorganisms

Saccharomyces cerevisiae and other yeasts are often involved in spoilage of liquid foods with high sugar content and low pH values. Inactivation of *S. cerevisiae* and other spoilage microorganisms in juices with ultrasound was performed by a few research groups. Bevilacqua et al. (2014) inactivated *S. cerevisiae* and other spoilage yeasts (*Pichia membranifaciens*, *Wickerhamomyces ano*malus, *Zygosaccharomyces bailii*, *Zygosaccharomyces rouxii*, *Candida norvegica*) in five fruit juices with a probe unit (20 kHz, 130 W) with pulsation. The inactivation of *S. cerevisiae* varied from 1.5 to 2.2 log CFU/ml depending on the pulse (from 2 to 6 s) and the inactivation of the other yeasts was less effective than that of *S. cerevisiae.* In another study, the survival of *Alicyclobacillus acidoterrestris* spores and *S. cerevisiae* cells treated with 30 min in a probe unit at 30 or 44 °C was examined in commercial and natural fresh-squeezed apple juices (Ferrario et al. 2015). A 2.5- and 2.8-log decrease was observed in *S. cerevisiae* population after 30 min of sonication at 30 and 44 °C, respectively. No reduction in the count of *A. acidoterrestris* spores was observed. Generally, the inactivation of yeasts with ultrasound is less effective compared with Gram-positive and Gram-negative cells, as shown by the high D values of yeasts with ultrasound treatments (Fig. 22.7). Considering the short process times with thermal processing methods to treat juices, it is less efficient to use ultrasound to inactivate spoilage causing microorganisms in juices.

The spoilage organisms grown on wood surfaces were effectively inactivated or removed by an ultrasound treatment. Yap et al. (2007a, b) reported a study in which an ultrasound treatment not only removed the tartrate, but also killed the *Dekkera* spp. and *Brettanomyces bruxellensis* located deep in the pores of the wooden barrels. A commercial wine barrel cleaning and disinfection system was developed by an Australia company, as shown in Fig. 22.8 (Patist and Bates 2011).

Table 22.4 Effect of ultrasonic processing on surface decontamination of plant food: studies in 2008–2019

Food Matrix	Ultrasound device	Treatment conditions	Microorganism studied	Microbial reduction	References
Spinach leaves	Ultrasonic probe system	21 kHz/ chlorine, acidified sodium chlorite (ASC), peroxyacetic acid (POAA)/ 2 min	*E. coli* O157:H7	0.7–1.1 log more than sanitizers alone	Zhou et al. (2009)
Whole-head iceberg lettuce	Pilot-scale washing flume	25, 40, and 75 kHz/ chlorine, peroxyacetic acid (POAA), 2 min	*E. coli O157:H7*	0.37–0.68 log more than sanitizers alone	Palma-Salgado et al. (2014)
Brown Rice	Ultrasonic cleaner (JAC 4020, KODO Technical Research Co.)	40 kHz/acidic electrolyzed water, 3 min	*B. cereus*	3.29 log	Tango et al. (2014)
Fresh-cut coconut	Probe system	40 kHz/ high pressure carbon dioxide (10 MPa, 40 °C and 10 W), 20 min	*S. enterica* typhimurium	8 log	Ferrentino et al. (2015)
Fresh-cut carrot	Probe system	40 k Hz/ high pressure carbon dioxide (10 MPa, 35 °C and 10 W), 3 min	*E.* coli ATCC 25922	8 log	Ferrentino and Spilimbergo (2015)
Cherry tomatoes and strawberries	Ultrasonic chamber (KQ-250B, Kunshan Ultrasonic Instrument Co.)	40 kHz/slightly acidic electrolyzed water (SAEW), 10 min	Total aerobic bacteria/yeasts and molds	1.77/1.29 log (cherry tomatoes); 1.29/1.50 log (strawberries)	Ding et al. (2015)
Apples	Ultrasonic chamber (Ningbo Xinzhi Biological Sci. and Tech. Co. Ltd.)	28, 45, and 100 kHz/chlorine dioxide, 10 min	*Alicyclobacillus acidoterrestris* Spores	5 log	Cai et al. (2015)
Fresh-cut bell pepper	Ultrasonic cleaner (JAC-4020, KODO Technical Research Co.)	40 kHz/acidic electrolyzed water, 1 min	*L. monocytogenes*/ *S. enterica* serovar Typhimurium	3.0 log	Luo and Oh (2016)

Strawberries	Ultrasound bath (Model Soniclean 15, Sanders Medical®)	40 kHz/peracetic acid (PA), 5 min	*S. enterica* subsp. Enterica	2.1 log	do Rosário et al. (2017)
Chinese cabbage	Ultrasonic chamber (Wuxi Fanbo Biological Eng. Co., Ltd.)	28, 40, 68 kHz, 30 min	*E.* coli	>3 log	Alenyorege et al. (2018)
Mirabelle plum	Ultrasound chamber (Hielscher)	30 kHz (continuous)/60 min	Total plate count	1.47 log	Hashemi (2018)
Bagged romaine lettuce	Ultrasonic cleaner (Branson Ultrasonics)	42 kHz/Tween-20, sodium dodecyl sulfate (SDS), chlorine, 10 min	*L. innocua/E. coli* O157:H7/ *Pseudomonas fluorescens*	0.5 log (Ultrasound) for all strains; 1.5 log for *P. fluorescens* (Ultrasound+surfactant)	Huang et al. (2018)
Iceberg lettuce shreds	Ultrasonic tank (Digital Ultrasonic Cleaner PS-40A, Codyson)	40 kHz/10 ppm chlorine/pulsed light, 1 min	*S. enterica* (Montevideo, Newport, Saintpaul, Stanley)	>1.20 log	Huang and Chen (2018)
Soybean sprouts	Ultrasonic cleaner (KODO Technical Research Co.)	40 kHz/Slightly acidic electrolyzed water, fumaric acid/40 °C, 3 min	L. *monocytogenes E. coli* O157: H7	4 log	Ngnitcho et al. (2018)

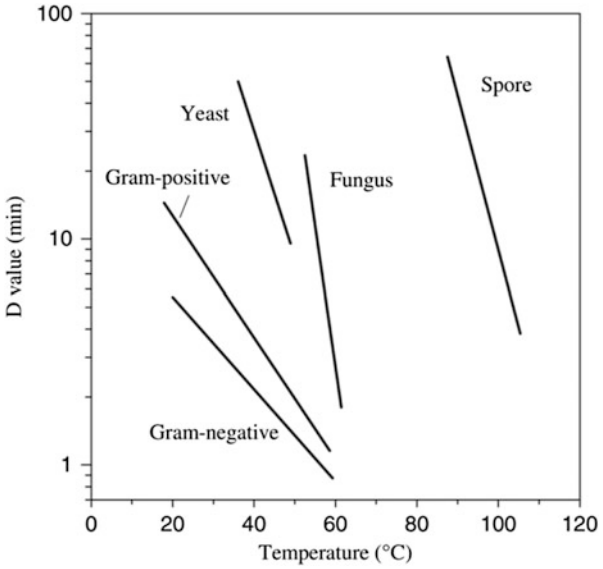

Fig. 22.7 D-values of bacteria with respect to temperature. Data for each bacterial group were collected from the literature (Feng 2011)

Fig. 22.8 An automatic oak wine barrel cleaning system using a 4 kW ultrasonic transducer (Cavitus Pty Ltd.)

22.4 Conclusion and Future Trends

Studies tapping into the power of high intensity ultrasound have shown effectiveness in inactivation of pathogenic microorganisms in different food systems. Most of the inactivation actions are attributed to the activities induced by acoustic cavitation in the presence of a liquid. In liquid food systems, ultrasound treatment at high cavitation intensity can result in a 5-log reduction of target pathogenic organisms in a reasonably short time (15–30 s) comparable to conventional thermal processing counterparts (normally 15 s). The treatment can be enhanced by a combination of heat (lethal temperatures) and low hydrostatic pressure in the treatment chamber, a process termed as mano-thermo-sonication (MTS). Such a high intensity and short time (HIST) ultrasonic treatment may provide a promising alternative to traditional thermal pasteurization processes to produce liquid products with improved quality. In surface decontamination applications with an ultrasound-assisted washing system, attention must be paid to the uniformity of the ultrasound field in the treatment chamber. The blockage of product pieces to the propagation of ultrasound wave in the treatment chamber has to be considered. For a well-designed washing system taking into consideration of the above two issues, an improved removal of attached pathogens and enhanced disinfection efficacy can be achieved in a continuous, commercial scale washing unit.

Future investigations should aim at generating more inactivation data for different microorganisms in different food systems. Studies on microbial inactivation with ultrasound have to report all the details in the experimental design, parameters used in the studies, and the developed experimental set-up so the work can be replicated and validated by other research groups. This will ensure the consistency in the inactivation data produced and that reliable kinetics datasets become available for process design. Investigations into the inactivation mechanism of different types of microorganisms in different food systems are also important for the design of future ultrasonic microbial inactivation units. More studies on the selection of design parameters for an ultrasonic microbial inactivation process are needed. Different from the thermal processing counterparts, the control of an ultrasonic process seems to be more demanding. For instance, in an MTS treatment, process temperature, system hydrostatic pressure, treatment time, and intensity and frequency of ultrasound are all important parameters to be controlled. One can also use cavitation intensity or acoustic power density as control parameters. Availability of pilot-scale and commercial scale ultrasonic equipment will be crucial for large-scale processing applications. Substantial effort should be made by the ultrasound equipment manufacturers to provide the hardware for future research and development and for commercialization.

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Chapter 23
Nonthermal Plasma Technology

Ximena V. Yepez, N. N. Misra, and Kevin M. Keener

23.1 Introduction

According to the United Nations, an estimated increment of 30% in the world population is expected by 2050, demanding doubling the current global food production (Tilman et al. 2011). The most important considerations to reach this goal include improving the food safety and minimizing the food losses. Food contamination is an important source of these losses and can be prevented with adequate food control systems in-place. The emerging nonthermal decontamination technologies can help to effectively reduce food contamination and extend the shelf-life of many food products. Cold plasma is an innovative processing technology used to reduce the microbial load in food products to safe levels. Some of the most noticeable advantages of this process are: (1) no chemical additives are required; (2) it is a low temperature treatment (20–50 °C); (3) no known chemical residues are generated during its application; and (4) it is widely accepted to be environment friendly.

The technology of cold plasma involves creation of highly reactive species by applying enough energy to a gas to cause partial ionization. The reactive chemical

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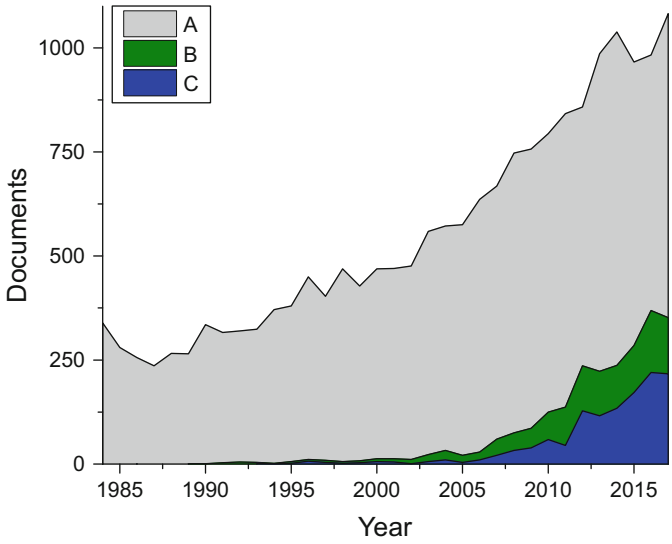

Fig. 23.1 Number of technical publications related to (**a**) cold plasma (**b**) cold plasma decontamination (**c**) cold plasma decontamination of food (Scopus 2018)

species generated in most plasmas with atmospheric gases are well-known bactericidal agents. According to several publications, cold plasma technology can reduce the microbial contamination in fruits, vegetables, spices, or other minimally processed foods to levels of 3–5 $\log_{10}$ reduction of aerobic bacteria (Niemira 2012; Misra et al. 2014a, c), suggesting that cold plasma can pasteurize and preserve 'fresh-like' properties of food products.

Development of cold plasma technology involves a combination of scientific disciplines such as plasma physics, chemistry, and several areas of engineering. Plasma physics and chemistry are required for understanding the processes of transferring energy into a gas state, to produce charged species and their chemical activity and physical interactions. Plasma engineering involves the design and development of plasma sources. Plasmas have been widely studied since the eighteenth century and the knowledge accumulated provides theoretical support for the underlying physics in the food decontamination process. The application of cold plasma for microbial decontamination dates back to the early 2000s, including applications in medicine. Since 2005, this technology has been studied extensively for the reduction of microorganisms in food, where a significant increase in scientific publications on the topic can be observed (Fig. 23.1).

This chapter provides a technical overview of the principles behind the plasma technologies applied to food decontamination. It introduces the reader with fundamental aspects, including plasma chemistry, and plasma sources. Subsequently, a discussion of the mechanisms of inactivation of food pathogens is provided. Finally, a review of applications in various foods systems including fruits, vegetables, meat, fish, seafood, dairy, nuts, and grains are included.

23.2 Fundamental Aspects

The arrangement of molecular and/or atomic energy in a system determines the state of matter as a solid, liquid, gas, or plasma. The difference between the states is the absolute total temperature or energy. In a plasma state, the molecules are not only distant from each other, but they break apart into their atomic elements, losing electrons and acquiring a higher energy state. Plasma is a partially ionized state of a gas, comprising of neutral and charged particles, collectively referred to as reactive species (RS).

The feature of having a higher energy state is useful in a large number of applications. Plasma technology is used in industrial processing for printing, automobile, plastics, wood processing, medicine, environmental, and packaging. Today much work has already been done in biological, physical, and chemical systems by the application of plasma reactive species (Adamovich et al. 2017). Plasma reactive species can be used for deposition or etching reactions. Current plasma deposition processes include thin film formation or coating and molecular modifications such as polymerization or crosslinking (Ostrikov et al. 2013; Neyts et al. 2015; Yepez and Keener 2016). Plasma etching processes include surface cleaning, sterilization, or decontamination (Van Durme et al. 2008; Vandenbroucke et al. 2011).

The energy of plasma is defined in terms of pressure, temperature, and the thermodynamic equilibrium between the particles. Pressure dictates particle density, meaning the number of ionized particles or free electrons per unit of volume. A system can work at low, atmospheric, or high pressure. Low and high-pressure systems increase the cost of equipment required to maintain specific conditions. Atmospheric pressure plasma has a higher particle density compared to low pressure plasma (Bárdos and Baránková 2010).

Thermal equilibrium is determined by the relationship between the temperature of electrons and heavy particles. The latter are formed by ions, radicals, neutrals, and excited species. Electrons gain energy faster than heavy particles, because of their low mass and high mobility. The temperature of the system is determined by the temperature of heavy particles. A ‘cold plasma’ is established when heavy particles have a much lower temperature than electron temperature, therefore they are in a non-equilibrium state. This difference is the key element to maintain a plasma treatment at room temperature. It is in contrast to a thermal plasma, which can reach temperatures of 1000–10,000 °C. The heavy particles can reach the electron temperature (equilibrium),

A plasma state is formed when a system gains enough energy to at least partially ionize a gas, forming a uniform glow discharge. The transition between a gas and a plasma state is marked by an ignition point that involves an increase in current because of a higher flow of electrons produced by collisions of atoms or molecules. At this point, atoms and molecules move into different energetic states and may emit photons (Fig. 23.2).

Increasing the intensity of the electric field can convert the uniform glow plasma, from a dielectric material into a conductive medium. Arc filaments or

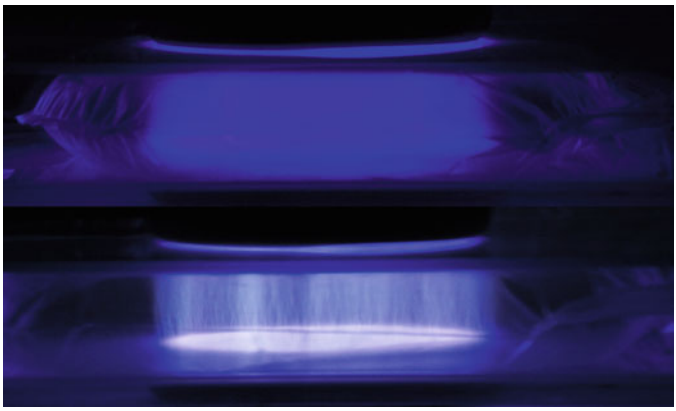

Fig. 23.2 Cold plasma generated with a dielectric barrier discharge system that shows a uniform glow (top) and streamers (bottom) discharge

streamers are formed in a conductive medium, where the system can reach a breakdown condition. In cold plasma treatment, it is preferred to maintain a uniform glow discharge. Gas composition, electrode configuration, dielectric properties, and operating pressure have influence on maintaining a uniform glow plasma (Bruggeman and Leys 2009). The type of gas used is an important factor; for example, helium requires less energy to form a uniform glow plasma compared to nitrogen. The differences between gases has been acknowledged by the value of 'Townsend breakdown', that determines the amount of energy that each gas needs to reach a system breakdown. As examples, the 'Townsend breakdown' values for helium, hydrogen, air, and nitrogen are 10, 20, 32, and 35 kV/cm, respectively (Fridman et al. 2005).

23.2.1 Plasma Chemistry

Plasma is a state in constant movement, capable of producing hundreds of reactions in minutes or seconds, generating unique reactive species (Bogaerts and Gijbels 2000; Liu et al. 2010). Modeling cold plasma treatment using helium and water described 577 reactions involving 46 species (Liu et al. 2010). As the number of gas species increases in the plasma field, it leads to a more complex chemical system. The generation of plasma species is initiated by 'electron collisions', including reactions such as:

Excitation	$A + e \rightarrow A* + e$
Ionization	$A + e \rightarrow A^+ + 2e$
Dissociation	$A_2 + e \rightarrow A + A + e$
Electron attachment	$A + e \rightarrow A^-$
Electron detachment	$A^- + M \rightarrow e + A + M$
Ion-Ion recombination	$A^- + B^+ + M \rightarrow e + A + B + M$

Atoms and molecules acquire different electronic states by electron collisions, forming excited species (A∗). In addition to electronic states, molecules also acquire rotational and vibrational excitation, increasing the complexity of the system. The main process of plasma generation is the formation of ions. Ionization occurs when an electron is knocked off an atom or molecule. Ionization is an inelastic collision, meaning that the internal energy state of the atom or molecules changes.

Reactive species in the plasma are formed not only from electron collisions, but also via reactions with each other, such as ion-ion, or ion-molecule reactions. The result is a hundred of reactions that occur in seconds, including recombination, neutralization, fragmentation, or polymerization. Reactive species in cold atmospheric plasma can reach energies of 0.1–20 eV.

A plasma begins to revert into the original gaseous state when the source of energy is disconnected. Electrons, ions, and excited species return to their original position. This is due to reactions such as electron detachment by negative ions or ion-ion recombination. A decay of charged particles is established when electrons return to a lower energy level.

23.2.1.1 Plasma Species

Gases commonly used to inactivate microorganisms include helium, argon, carbon dioxide, nitrogen, oxygen, air, or mixture of gases. However, atmospheric air is the preferred gas for food applications and is widely under exploration. Reactive species from air include reactive oxygen species (ROS), reactive nitrogen species (RNS), ultraviolet radiation, several other energetic ions, and charged species (von Woedtke et al. 2013). Table 23.1 summarizes some of the reactive species in plasma, including the ROS and RNS responsible for microbial inactivation.

ROS are identified as species with a high oxidation capacity. The main ROS include atomic oxygen, hydroxyl ion, hydrogen peroxide, and ozone. Molecular oxygen ($O(^3P)$) acquires excited states, $O(^1S)$ and $O(^1D)$, and dissociate into atomic oxygen. Hydroxyl radicals ($^{\bullet}OH$) are formed in the gas phase via electron impact dissociation of water molecules. Ozone generation reactions require an energy of

Table 23.1 Exemplary list of reactive species in plasma, some of which are responsible for microbial inactivation (Eliasson et al. 1987; Laroussi and Leipold 2004; Liu et al. 2010; Takamatsu et al. 2014)

Species type	Example	References
Positive ion	N_2O^+, N_2^+, H^+, H_2O^+, N^+	Newton and Sciamanna (1966) and Wakenne and Momigny (1971)
Negative ion	$ONOO^-$, NO_2^-, NO_3^-, O_2^- (superoxide), O_2^{2-}	Crow and Beckman (1995), Sies and Stahl (1995), and Kelm (1999)
Radical	NO (nitric oxide), NH, OH, NO_2 (nitrogen dioxide), OOH	Thomas et al. (1956), Sies and Stahl (1995), Wright and Winkler (1968), and Dhawan (2014)
Atom	H, N(^{2}D), O(^{1}D), O(^{1}S)	Tom G. Slanger and Copeland (2003) and Wright and Winkler (1968)
Molecule	O_3, $^1O_2$ (singlet oxygen), CO, H_2O_2	Meredith and Vale (1988), Sies and Stahl (1995), Klockow and Keener (2009), and Forkink et al. (2010)
Metastable	$O_2(^1\Delta g)$, $N_2(A^3\sum)$, $N_2(A^3\sum_u^-)$, $N_2O^*(^1\sum^+)$, O_2(a), $O_2(b^1\sum)$	Bills et al. (1962), Oldenberg (1971), and Barry (1980)

6–9 eV (Blanksby and Ellison 2003). Reactions of molecular and atomic oxygen lead to the formation of ozone, following a two-step reaction:

$$O_2 + e \rightarrow 2O + e$$

$$O + O_2 + M \rightarrow O_3 + M \quad (M = O, O_2, O_3)$$

RNS are formed from nitrogen, oxygen, and water (as humidity). Molecular nitrogen when dissociated, reacts with oxygen, forming nitrogen oxides (NO, NO_2, N_2O_3, N_2O_5). Nitrogen oxides react with water forming nitric and nitrous acids (HNO_3 and HNO_2), and then decomposed to nitrate and nitrite (NO^-_3 and NO^-_2). These RNS have been attributed to increase the nitrite content in water, plant extracts, and processed meat (Jung et al. 2017a, 2017b).

Plasma systems produce short and long lifetime species. The first type are species formed in the plasma volume within the electrodes of a dielectric barrier discharge (DBD, described later). These species play an important function in microbial inactivation. However, it is difficult to identify them because of their short lifetime. Optical emission spectroscopy (OES) has been used to identify the wavelength in the range of UV-VIS spectrum at which the transitions of these species occur. OES allows to identify excited species (A^*) that emit photons when they move to a lower energy state. For example, in atmospheric air, there are strong lines in the 300–425 nm wavelength range, identified as transitions of second positive system of N_2, first negative system of N_2^+, NO, and OH (Connolly et al. 2013).

The long lifetime species can be identified when the source of energy is disconnected. These species are frequently analyzed by optical absorption spectroscopy (OAS), mass spectroscopy (MS), or sampled for further analysis. The main long

lived species that have been studied are ozone, that can reach levels of 100–10,000 ppm by volume (Klockow and Keener 2009; Patil et al. 2014). High concentrations of ozone have been detected 24 h after plasma generation in sealed packages. Detailed examination of long lifetime plasma species showed the quantification of 11 chemical parameters by colorimetric methods (ammonia, ammonium, orthophosphates, nitrites, nitrates, and hydrogen peroxide), titration (carbonate ions, bicarbonate, carbonic acid), conductivity and pH measurement (Judée et al. 2018). The identification and quantification of plasma species responsible for microbial reduction is an area of research that needs further development and is under active investigation.

23.2.2 Plasma Sources

Plasma sources operating at atmospheric pressure is commonly used to decontaminate food-borne pathogens. A plasma process is required to be an efficient method to kill microbes, and yet gentle enough to maintain the physical and nutritional properties of food. There are different systems to partially ionize a gas, according to power characteristics and electrode geometries. Electricity is the common source of energy for most of the plasma systems though different voltages and frequencies are applied. Plasma sources are classified according to electrode configurations as: dielectric barrier discharge, plasma jet, corona discharge, and microwave plasma, as shown in Fig. 23.3.

23.2.2.1 Dielectric Barrier Discharge

Atmospheric cold plasma for food and biological applications is normally generated using dielectric barrier discharge (DBD) configurations. DBD generates a plasma glow between two electrodes separated by a given distance with a dielectric layer in between. The main electrode is connected to the energy source, and the lower electrode is grounded. The gas volume between barriers is subjected to an electrical field, forming a plasma state if the energy surpasses the ignition point. Voltage reported in literature range from 5 to 90 kV and frequency from line (50/60 Hz) to low radio frequency (1–60 kHz). Among this frequency range, electrons and ions follow the electric field oscillation (Tendero et al. 2006). Typical materials used for electrodes are copper or aluminum.

An important characteristic of DBD is the addition of a dielectric material. It covers one or both electrodes, which are separated by a few millimeters. Its function is to maintain a uniform plasma glow, by preventing arc formation and distributing streamers throughout the surface. Common materials used as dielectric barriers are teflon, plastic, glass, or ceramic.

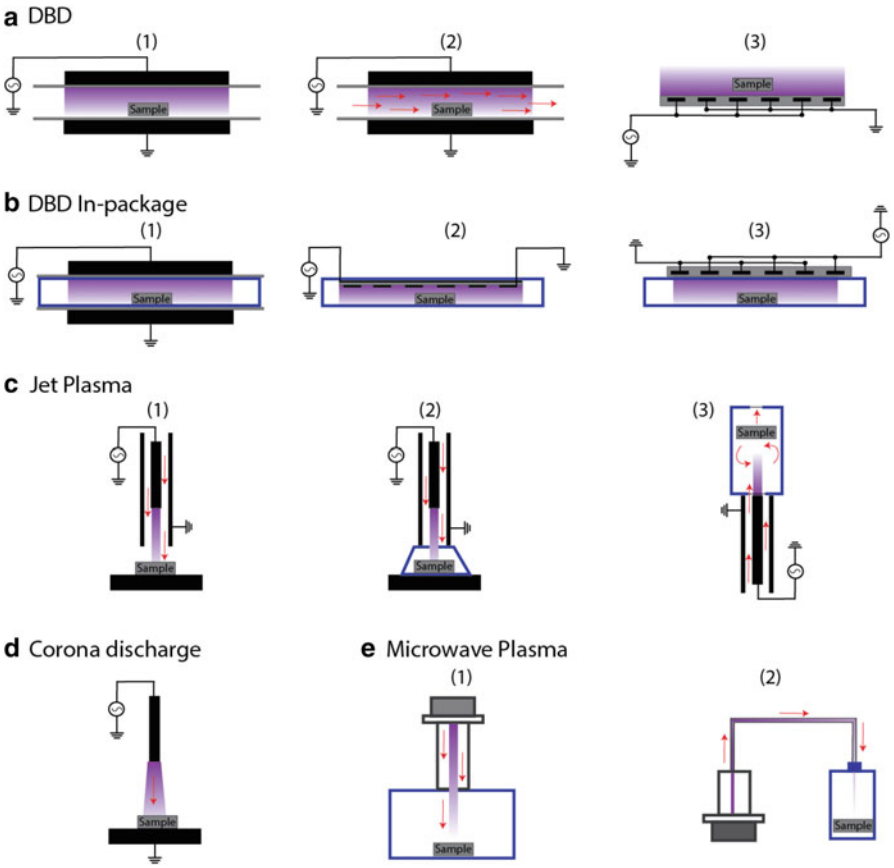

Fig. 23.3 Sources of cold plasma for food applications. Electrodes ■, dielectric barrier ■, power supply ⏲, plasma ■, plasma chamber/package □, microwave energy source ■, and red arrows indicate a gas flowing

In-package DBD involves the treatment of food in their final packaging materials. It includes three types of configurations, as shown in Fig. 23.3b. The first setup creates a plasma state between two external electrodes, that are at the top and bottom of the package. This geometric configuration provides a volumetric type of discharge. The second and third setup create a plasma through pairs of electrodes, placed across one side of the package providing a surface type of discharge. The electrodes in the second setup are embedded in the package. This is in contrast to the electrodes of the third setup that are outside the package (Misra et al. 2019).

23.2.2.2 Jet Plasma

A plasma jet configuration is characterized by the formation of a plasma through two concentric electrodes, with a gas flowing between them. Different setups have been applied in food research primarily by varying voltage and frequency. The frequencies typically employed fall in the range of 1 kHz – 30 MHz. Gases used in plasma jet include helium, argon, air, oxygen, and nitrogen. The gas flow rate controls the time of exposure of reactive species with the substrate and also provides a cooling effect. Plasma jet does not necessarily require a dielectric barrier as the flowing gas itself produces a stable and uniform plasma glow.

Different configurations of plasma jet have been reported for food applications (Fig. 23.3c). The first setup includes an open system, where the gas flows through the electrodes and the substrate is exposed to reactive species. The second setup involves the use of a closed container that keeps reactive species inside for a longer exposure duration with the substrate (Lacombe et al. 2015). The last setup is an inverted configuration, that inject the plasma reactive species in fluidized bed, where the sample is suspended (Dasan et al. 2016; Dasan et al. 2017). These configurations evolved to increase the surface exposure of the substrate to reactive species.

23.2.2.3 Corona Discharge

In a corona discharge configuration, the plasma state is formed between an upper electrode tip and a grounded electrode (Fig. 23.3d). It is called corona, because a glowing crown is formed around the tip. The volume of plasma formed depends on the space between electrodes and the geometry of the grounded electrode (Puligundla et al. 2017). This setup may not be stable and usually generates streamers and arcs. Therefore, a dielectric barrier discharge was developed to avoid the instability of a corona discharge.

23.2.2.4 Microwave Plasma

Microwave plasmas are characterized by a uniform glow produced from a high frequency discharge in the range of RF and microwaves (800 MHz – 3 GHz). This is an electrode-less system with a source of energy that generates the microwave field in a plasma treatment chamber at low or atmospheric pressure (Bárdos and Baránková 2010).

Figure 23.3e. describes two types of microwave plasmas that have been used in food applications: direct and remote exposure. In the first type, the substrate is exposed directly to plasma species produced by the microwaves (Won et al. 2017). The second type is a remote microwave system that generates plasma species at high temperature, which is subsequently transported through a tube to a treatment

chamber. Thus, the food remotely receives the plasma reactive species at room temperature (Hertwig et al. 2015a; Reineke et al. 2015).

23.2.3 In-Package Plasma

As described previously, an in-package plasma is a modified version of the DBD configuration. A food item is packaged and sealed in its primary packaging material. It can be filled with ambient air or modified gas compositions. Then, it is subjected to a strong electric field, creating a cold plasma inside for a short period of time. The reactive plasma species have antimicrobial properties. Under this scenario, the packaging material has the function of a dielectric barrier and a plasma chamber.

In-package plasma minimizes the risk of post process contamination. The plasma species produced inside the package reduce the microbial load of the food product as well as the internal surface of the package. After treatment, the product can be packaged with secondary materials and stored under refrigeration or room temperature conditions.

The in-package plasma technology allows to maintain a continued exposure of microbes with reactive species for an extended period of time. A study from Klockow and Keener (2009) reported the effect of long lifetime species in spinach inoculated with *Escherichia coli* O157:H7 (Klockow and Keener 2009). A 5 min in-package treatment with ambient air reduced the microbial load by 0.5 log CFU/leaf, and an extended exposure of 24 h had an additional reduction of 3.5 log CFU/leaf. Similar effect was reported with sliced cheese, where *Listeria monocytogenes* was reduced to no detectable limits after 1 week of storage at 10 °C (Song et al. 2009). This prolonged microbicidal effect may allow to increase the shelf-life of foods. Another study from Min et al. (2016) found that the type of gas and microorganisms affected the post-treatment storage (Min et al. 2016). They analyzed the effect of post-treatment storage of lettuce inoculated with *E. coli, Salmonella*, and *L. monocytogenes* and found a significant reduction only for the latter. Therefore, the effect of in-package continued exposure of plasma species during storage may extend the shelf-life of food products, depending on the system configuration, type of gas, microorganism, and food composition.

23.3 Microbial Inactivation Mechanisms by Plasma Agents

Cold plasma being a cocktail of reactive species is an effective antimicrobial agent capable of affecting the microbial cell structure and function. The antimicrobial action of plasma species is multimodal and ranges from physical to chemical effects. However, there is no known dominant mode of action. Additionally, the action of plasma species on a given microbial cell has been found to vary depending on the microbial class (bacteria, fungi, or virus), the operating gas, the type of plasma

source, the process parameters, the substrate (food or model media), the state of the substrate (solid or liquid), and the surface roughness. As an example, argon doped with oxygen and nitrogen emit four times more UV photons than pure argon (Hertwig et al. 2015b; Reineke et al. 2015); thus, the mode of action would considerably vary in the two cases. The interaction of plasma agents with food borne pathogenic bacterial cells can help us in understanding the underlying inactivation mechanisms.

The ROS in plasma adversely affects the bacterial cell functionality by causing changes in cellular biomolecules. The established interactions between plasma agents and cellular biomolecules include the adverse effects on nucleic acid chains, proteins, and enzyme systems. The ROS can disturb the stability and functionality of a bacterial membrane by acting on the constituent membrane lipids to result in the formation of unsaturated fatty acid peroxides and further oxidizing the amino acids constituting the proteins (Misra et al. 2016). Cold plasma could destroy the outer membrane of bacterium and this aspect has been confirmed through experiments (Sun et al. 2007), as well as computer simulations (Yusupov et al. 2012, 2013). The computer simulations have suggested that the reactive oxygen species break the C-O, C-N and C-C bonds of the peptidoglycan molecule of the Gram-positive bacterial cell wall. In the case of Gram-negative bacteria, it has been reported that the lipopolysaccharides are chemically modified on exposure to argon plasma (Bartis et al. 2013).

When bacterial cells are exposed to a strong electric field, they experience strong electrostatic tension due to the development of surface charge, which potentially results in cell membrane rupturing (Laroussi et al. 2003). Electron microscopy studies have revealed that cold plasma treatment causes change in morphology of bacterial cells resulting in cell lysis (Misra et al. 2013). The result of such a wide scale attack on the bacterium is the cell leakage and loss of functionality. A summary of the inactivation mechanisms of plasma agents against bacterial cells is presented in Fig. 23.4.

23.4 Applications to Food Systems

There is an increased demand for minimally processed food and a desire to eliminate chemical additives from foods. Non-thermal processing techniques fulfill these demands, by enabling a gentle treatment of heat sensitive foods without the use of chemical additives. Therefore, cold plasma has been used to reduce the microbial population including bacteria, mold, virus, and parasites in an array of food systems. A discussion of the application of cold plasma to decontaminate various food categories is presented in the following sub-sections.

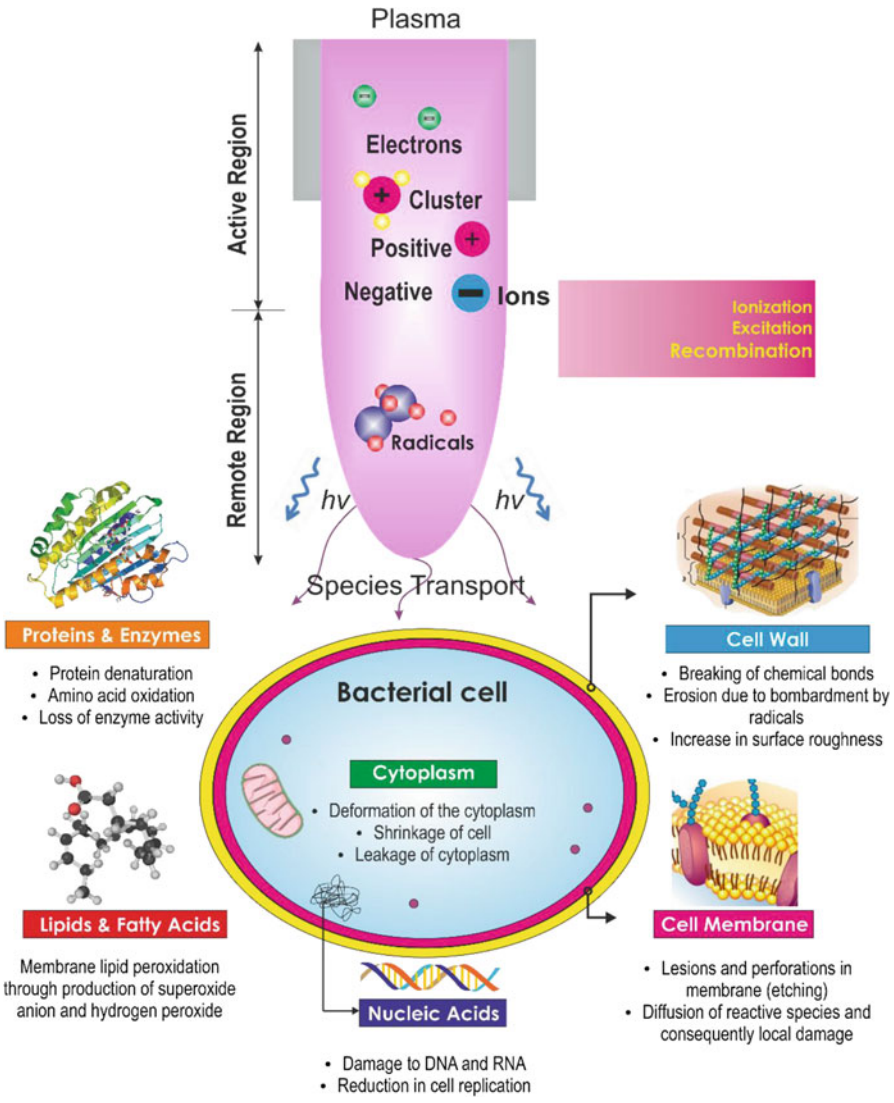

Fig. 23.4 A pictorial summary of the mechanism of cold plasma induced bacterial cell damage (Misra and Jo 2017)

23.4.1 Fruits and Vegetables

Dietary guidelines recommend the consumption of fresh fruits and vegetables to obtain sufficient vitamins and nutrients for a healthy diet. Fruits and vegetables are often minimally processed and have a high risk of biological contamination. They are mainly associated with human pathogens such as *E. coli* O157:H7, *Salmonella*

enterica, *Clostridium perfringens*, *Clostridium botulinum*, or *L. monocytogenes*. Fruits and vegetables are implicated with an increased number of foodborne illness and linked to major food outbreaks. Raw agricultural products have their maximum microbial load on the surface and negligible microbial load on the inside, if the surface is intact. Pathogens in fresh produce adhere to the surface, trapped in stomata, and partially buried in cracks that are protected against surface disinfectants (Bhunia 2018). Cold plasma processing reduces the microbial load of the surface without affecting the bulk properties of the product. Also, this technology retains the natural flavor and extends the shelf life of food products. For example, cold plasma has been shown as a highly effective means to remove pathogens in tomatoes, without major changes in quality parameters such as color, pH, or firmness (Misra et al. 2014a, b, c; Prasad et al. 2017). A microwave plasma system was used to reduce 3.5 $\log_{10}$ cfu/tomato of *Salmonella*, with a 10 min treatment using a power input of 900 W (Kim and Min 2017). An in-package DBD plasma treatment of tomatoes inoculated with *E. coli*, *Salmonella enterica* serovar Typhimurium, and *L. monocytogenes* reduced the population to undetectable levels with treatments of 2–5 min (Ziuzina et al. 2014). Furthermore, an industrial scale in-package system was tested with tomatoes packed and treated with cold plasma in a continuous mode (Ziuzina et al. 2016). The product was treated in a conveyor belt and the treatment time was determined by its speed. This system achieved a 5-$\log_{10}$ cfu/sample reduction in *E. coli* population in 150 s. Other foods had been tested successfully with cold plasma treatment, including apples (Niemira and Sites 2008) and melon (Tappi et al. 2016).

Berries naturally have a high quantity of yeast and mold and have a potential tendency for harboring pathogens. They require a gentle treatment to maintain the quality and increase shelf-life. Conventional washing and sanitizing treatments are costly and ineffective. Blueberries treated with a plasma jet system for less than 1 min (549 W) achieved a reduction of 2 $\log_{10}$ cfu/g for total aerobic, yeast and mold (Lacombe et al. 2015). This treatment didn't show significant changes in firmness, color, and anthocyanins content. Moreover, cold plasma has been used to effectively reduce the microbial load on strawberries with a DBD plasma system and the effect of treatment in respiration rate was also studied (Misra et al. 2014a, b, c; Ziuzina et al. 2014; Misra et al. 2015). Respiration rate of fresh produce depend on gas composition (especially oxygen and CO_2). No differences in respiration rate were observed with a 5 min in-package cold plasma treatment of strawberries packed in air (Misra et al. 2014a, b, c).

Fresh cut leaves such as spinach or lettuce require a postharvest sanitation to meet food safety standards. Plasma jet system was tested to treat fresh corn salad leaves. A 15-s treatment reduced *E. coli* population by 3.6 $\log_{10}$, with an initial concentration of 10^4 cfu/cm^2 (Baier et al. 2013). This short treatment time is important for scalability. The application of a DBD cold plasma to sanitize spinach inoculated with *E. coli* O157:H7 was studied. A 3–5 $\log_{10}$ cfu/leaf reduction was achieved after a 5-min treatment and 24-h of storage (Klockow and Keener 2009). However, changes in color were observed with longer treatment times. The efficacy of the treatment is reduced with products that have a complex surface topology, such as

alfalfa or sprout seeds (Butscher et al. 2016). Limitations of cold plasma technology for fruit and vegetable decontamination may include long treatment times, limited surface exposure, and changes in physical properties.

Liquid food products such as orange juice or coconut water were also decontaminated with cold plasma. In orange juice, a DBD treatment of 3 min reduced the population of *Salmonella enterica* serovar Typhimurium by 3.8 $\log_{10}$ cfu/ml using a modified atmosphere with 65% oxygen, 30% nitrogen and 5% carbon dioxide. A low reduction (22%) of vitamin C was observed with this treatment, in contrast to traditional pasteurization that reduces vitamin C by 50%. Furthermore, microwave jet plasma (650 W) effectively reduced the population of *E. coli*, *Salmonella* spp. and *L. monocytogenes* in coconut water at processing temperatures of less than 30 °C. A 3 $\log_{10}$ cfu/ml reduction was observed for *E. coli* and *Salmonella,* and a 5 $\log_{10}$ cfu/ml reduction for *L. monocytogenes* with a 15 min treatment (Gabriel et al. 2016).

23.4.2 Meat and Poultry

Meat and poultry products have a natural microflora, commonly psychrotrophic bacteria that grow under aerobic conditions. They are easily contaminated with *E. coli*, since it is a natural inhabitant of the intestines. *Salmonella enterica* serovar Typhimurium and *Staphylococcus aureus* may come from the skin. Adequate hygienic practices during processing and storage help in reducing the microbial contamination and proliferation. However, these temperature sensitive products are sold as uncooked products. A non-thermal processing technology could therefore be an appropriate option to reduce the biological risks and ensure food safety.

In one of the notable studies, beef loin inoculated with *E. coli, Salmonella enterica* serovar Typhimurium*,* and *L. monocytogenes* with an initial population of 10^8–10^9 cfu/g was treated for 10 min with a plasma jet using nitrogen/oxygen gas. The treatment resulted in 2–3 $\log_{10}$ cfu/g reduction (Jayasena et al. 2015). A DBD plasma treatment of beef reduced 1.3–1.4 $\log_{10}$ cfu/cm^2 of psychrotrophs, using argon and helium, at low pressure (Ulbin-Figlewicz et al. 2015). Plasma conditions can be improved to reach a higher decontamination efficacy; however, a more intense treatment may affect quality attributes of the product. For example, the color of beef products changes when they are exposed to a high concentration of plasma species. Myoglobin may react with nitrogen reactive species, producing nitrimyoglobin associated with a green color (Yong et al. 2018). Use of reducing agents such as sodium dithionite can potentially prevent color changes in meat treated with cold plasma (Yong et al. 2018). The reducing agents prevent the green discoloration in beef, maintaining a red color and minimizing undesirable quality alterations.

Due to the difference in surface characteristics, meat and poultry products treated under the same conditions (plasma source, gas, treatment time, and microbial load) can result in different bacterial inactivation rates. For instance, ham and chicken breast inoculated with *L. monocytogenes* showed a 6.5 and 4.7 $\log_{10}$ cfu/g reduction, respectively. It has been suggested that factors such as surface diffusion, adsorption, or sputtering can influence surface decontamination (Lee et al. 2011).

Cold plasma can also be used to sanitize food processing equipment. Leipold et al. (2010) used cold plasma for sanitizing a deli meat cutting machine. Knifes used in meat industry are a critical point for biological contamination, especially with *L. monocytogenes*. The knife itself was used as the ground electrode in the DBD setup developed by Leipold et al. (2010). This system was able to reduce 5-log cfu/ml of *L. monocytogenes* from the surface of an industrial rotating cutting tool during operation. This is a noteworthy application of cold plasma decontamination for a continuous process.

23.4.3 Fish and Fish Products

Products such as fresh fish fillets, squid, or sushi have been treated with cold plasma to reduce the microbial contamination. The cold plasma treatment of semi-dried squid reduced aerobic bacteria, yeasts, and molds by 1.5–2.1 $\log_{10}$ cfu/g reduction, using a corona discharge system (Choi et al. 2017). Sensory characteristics were not significantly affected by the treatment. Chiper et al. (2011) reported the use of a DBD plasma system to treat salmon (Chiper et al. 2011). A 2-min treatment reduced the population of *Photobacterium phosphoreum* by 3 $\log_{10}$ cfu/g; however, *L. monocytogenes* was not affected. Ready to eat sushi was treated with an in-package DBD system, where total aerobic counts were reduced by 1–1.5 $\log_{10}$ cfu/g, allowing an extension of the shelf life by 4 days at refrigerated condition (Kulawik et al. 2018).

The decontamination of fish and fish products is influenced by changes in quality, mostly related to lipid oxidation. The high polyunsaturated fatty acids content makes these products highly susceptible to lipid oxidation mainly due to the effect of ROS. Oxidation reaction products impart odd flavors and odors, reducing the product quality. Fresh filets of mackerel and herring treated with in-package plasma have shown significant results in microbial reduction (Albertos et al. 2017a, b). However, primary oxidation products measured as peroxide value and conjugated dienes have been reported to increase with longer treatment times. Peroxide value reaches 37 meq/g, with an 80 kV/5 min treatment using an in-package DBD system, and this value exceeds the limit for quality acceptance. However, aldehyde content measured as malonaldehyde (using TBARS assay) has been found to show no significant differences after treatment though their concentrations may increase during storage as oxidation reactions continue.

23.4.4 Dairy Products and Eggs

The use of cold plasma to decontaminate milk while preserving its nutritional characteristics has been studied. *E. coli* is one of the main biological hazards associated with liquid milk and cold plasma can effectively inactivate this microorganism. A DBD in-package configuration operating at a frequency of 15 kHz (250 W) was used to treat liquid milk yielding a 2.4 $\log_{10}$ cfu/ml reduction of with a 10 min treatment (Kim et al. 2015). In the same way, a corona discharge source operating at 9 kV was effective in reducing the *E. coli* population by 3.6 $\log_{10}$ cfu/ml in liquid milk after a 20 min treatment (Gurol et al. 2012). Both studies observed minimal changes in the physicochemical properties of liquid milk. Similar reductions in the populations of *E. coli*, *L. monocytogenes*, and *Salmonella enterica* serovar Typhimurium were observed in cheese as well. Post-treatment exposure refers to the amount of time that the product is exposed to reactive species after treatment. Meaning that the energy source is disconnected but the product is maintained inside the sealed package for a specific amount of time. The effect of post-treatment exposure on the microbial reduction for in-package configuration was analyzed in sliced cheese, where an additional 1.6–2 $\log_{10}$ cfu/g reduction was achieved when the product was maintained inside the package for 5 min (Yong et al. 2015).

S. Enteritis is a major biological risk in chicken eggs and studies have shown a significant reduction in the range of 5–8 $\log_{10}$ cfu/egg in inoculated egg shells (Georgescu et al. 2017; Wan et al. 2017; Dasan et al. 2018). Treatments to decontaminate egg shells have several challenges, for example the high mineral content of shells create hotspots and sparks during the treatment. The size and shape egg also require additional modification in common cold plasma sources. For instance, a DBD arrangement requires a large gap between electrodes. In a plasma jet configuration, the distance between the product and the electrodes determine its effectivity as well as the flow rate determines temperature fluctuations.

23.4.5 Nuts and Grains

Low moisture foods such as nuts and grains are considered as low risk foods in the context of microbial safety. However, almonds, pistachios, and other products have frequently been recalled due to contamination with pathogens in recent years. *Salmonella* has been the main bacteria associated with outbreaks related to nuts. Post-harvest treatments used to inactivate pathogens in nuts include chemical treatment such as propylene oxide fumigation, hot water blanching, oil roasting, and irradiation. These treatments adversely affect the quality and nutrition. In addition, these technologies are costly and have limited efficacy (Deng et al. 2007).

Cold plasma has been used as a low temperature treatment to treat almonds. Almonds inoculated with *E. coli* were treated with a DBD system using air and a

voltage/frequency of 25 kV/2 kHz. A 5 $\log_{10}$ cfu/g reduction was obtained with a 30 s treatment (Deng et al. 2007). Another study showed similar reduction in the population of *Salmonella*, with a 15 min treatment in a barrier discharge system operating at 20 kV/15 kHz (Hertwig et al. 2017). The microbial reduction was attributed to production of high concentrations of ozone, nitrous gases, and UV photons.

The presence of mycotoxins is an important microbial risk in nuts and grains. Fungi contamination in the field produce aflatoxins during storage under hot and humid conditions. The allowable limit of mycotoxins in nuts is below 20 ppb (Bhunia 2018). Cold plasma can potentially be used for mycotoxin inactivation. For instance, complete inactivation of a standard solution of aflatoxin B1 was achieved with a 12 min cold plasma treatment at 400 W (Siciliano et al. 2016). Similarly, the aflatoxin content on the surface of hazelnut was reduced by 54.3% under the same treatment conditions. A further treatment at 1150 W reduced the aflatoxin B1 content to 29.1% (Siciliano et al. 2016). The inactivation of aflatoxin was more effective when nitrogen gas was used rather than oxygen. The inactivation mechanism of mycotoxins involves the attack of plasma species on unsaturated bonds of aflatoxins. Therefore, aflatoxin B1 and G1 are more sensitive to cold plasma than B2 and G2, because they have more double bonds in their structure. Breakdown products of mycotoxins are considered as non-toxic or less toxic.

Cold plasma has also been used to inactivate spores. Hazelnuts inoculated with an initial load of 6 $\log_{10}$ cfu/g of *Aspergillus flavus* spores were treated with plasma jet with nitrogen gas. A 4.5 $\log_{10}$ cfu/g reduction was achieved with treatment at 655 W power and 25 kHz frequency, and the spores were broken and adhered to one another (Dasan et al. 2016). Cold plasma treatment of *Bacillus subtilis* spores on the surface of glass beads, Petri dish, and peppercorns, resulted in 4.6, 2.8, and 1 $\log_{10}$ cfu/g reductions, respectively (Hertwig et al. 2015b). The complex surface structure of peppercorns limited the microbial reduction as microorganisms can hide under uneven surfaces where plasma species have a limited penetration.

23.5 Conclusions and Future Trends

Cold plasma, the partially ionized state of a gas, is essentially a cocktail of reactive chemical species. These reactive species can induce physical and chemical changes in a range of biological systems, including bacteria, fungi, spores, viruses, and mammalian cells, and therefore can be used for decontamination of these biological systems. In addition, the reactive species can also attack chemical contaminants such as pesticides and mycotoxins. Suitable plasma chemistry for a given application can be achieved by carefully selecting a plasma source and tuning the plasma process parameters. Examples of plasma sources commonly employed in food and bio-decontamination include dielectric barrier discharges, plasma jets, and microwave plasma. Cold plasma has been applied for decontamination of fresh fruits and vegetables, meat and meat products, cereals, nuts, egg, and dairy foods. Majority of

the studies reported in literature have focused on lab-scale evaluation of the technology. Only a few recent studies have focused on industrial scale systems. The use of water activated using plasma technology, referred to as "plasma activated water" (PAW) is gaining popularity for various food processing applications, including for washing of fresh fruits and vegetables. However, much research remains to be done with regards to a good understanding of the chemistry of PAW.

The long processing times, together with the non-availability of suitable equipment and regulatory approval remains the limiting factor for adoption of the plasma technology at this point. The plasma community (manufacturers, distributors, scientists, engineers, consumer groups, etc.), should come together to develop guidance documents that can aid atmospheric plasma technology manufacturers in obtaining regulatory approval (Misra and Keener 2014).

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Chapter 24
Selected Novel Food Processing Technologies Used as Hurdles

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24.1 Introduction

Microbial food safety is a major concern for consumers, food industries, as well as regulatory agencies worldwide. Many conventional food preservation methods such as freezing, chilling, acidification, fermentation, use of chemical or biological antimicrobials, and heat treatment have been commonly used to control the microbial quality of food products (Khan et al. 2017). However, some of these applications can adversely affect the food quality and reduce the consumer acceptability. For instance, thermal processing is the most common preservation technique that has been extensively used in the food industry due to its effect on microbial and enzymatic inactivation and convenience in producing shelf-stable and safe products. However, conventional thermal treatments may promote chemical and physical reactions in foods resulting in degradation of food quality due to the long heating

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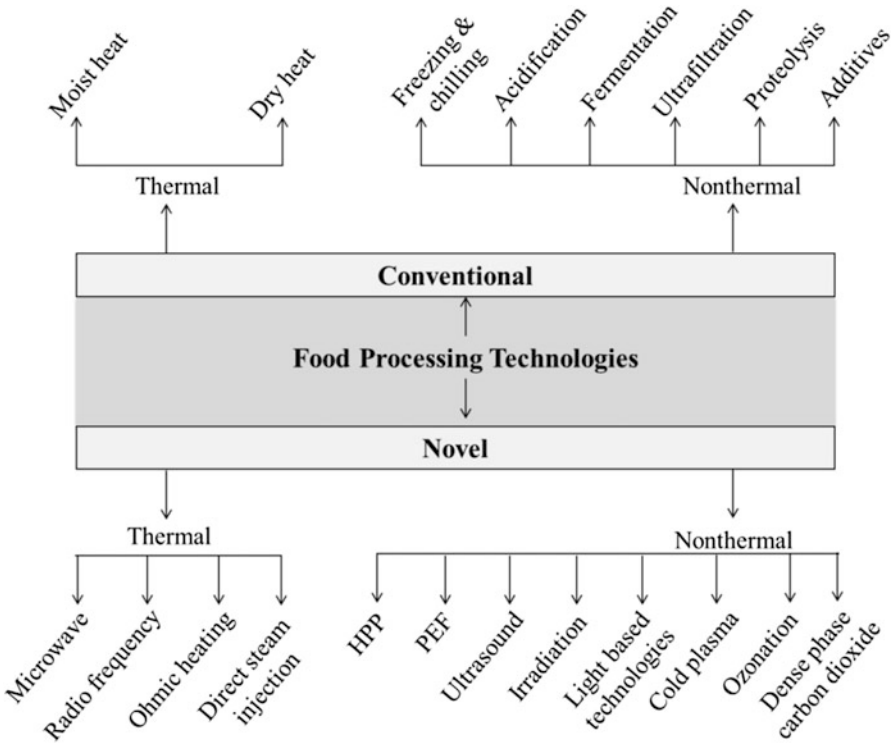

Fig. 24.1 Conventional and novel food processing technologies. (Adapted from Barbosa-Cánovas and Bermúdez-Aguirre (2010), Khan et al. (2017))

times at high temperatures (Fellows 2009). In this regard, recent consumer demands for safe and minimally processed foods led the food industry and scientist to investigate novel food processing technologies and food preservatives that can provide high quality food products with required safety, extended shelf life, and desirable nutritional and organoleptic attributes (Tiwari 2014). Figure 24.1 summarizes the novel thermal and nonthermal food processing technologies. Although nonthermal preservation processes are highly effective on vegetative bacteria, yeast, and mold inactivation, they are not sufficient to inactivate bacterial spores and reduce the activity of most enzymes (Raso and Barbosa-Cánovas 2003). Depending on the type of food product and the microorganism of concern, nonthermal food processing technologies including high pressure processing (HPP), pulsed electric fields (PEF), ultrasound (US), and light-based technologies (UV-C light, pulsed-UV light) can be used in combination with each other, heat treatment, and antimicrobials in order to enhance the lethal effect of the process and to reduce the treatment intensity compared to individual processes (Fig. 24.2). This is known as the "hurdle technology approach (or concept)" and defined by Leistner (2000) as the combination of different preservation factors or techniques to improve the shelf life of foods by inactivating spoilage and pathogenic microorganisms without changing

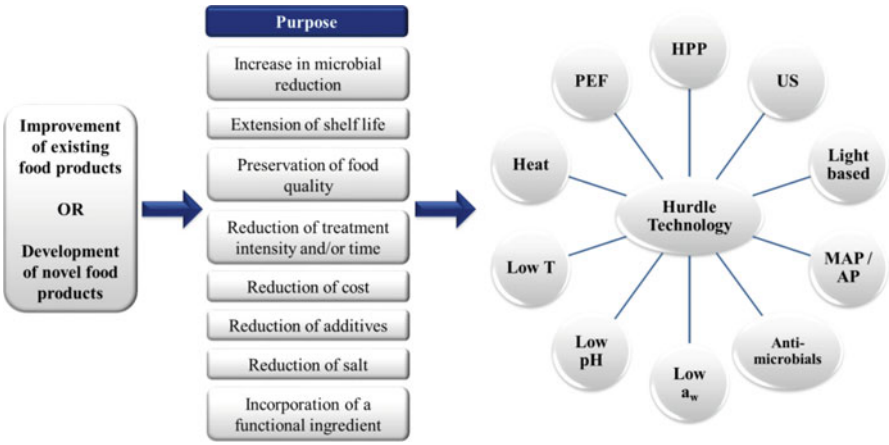

Fig. 24.2 The concept of hurdle technology including nonthermal food processing technologies. (Adapted from Horita et al. 2018). (PEF: pulsed electric fields, HPP: high pressure processing, US: ultrasound, Light-based technologies such as UV-C light and pulsed light, MAP/AP: modified atmosphere packaging/active packaging, a_w: water activity, T: temperature)

their organoleptic and nutritional characteristics. Thus, hurdle technology is a promising approach to optimize traditional foods and develop novel products (Leistner and Gould 2002). Several preservation stress factors (called hurdles) can be used when they are applicable for the treatment medium (Table 24.1). Utilization of intelligent combinations of hurdles provides not only the microbial stability and safety, but also nutritional, sensorial, and economic benefits for a food product (Leistner and Gould 2002). Application of different hurdles including temperature, water activity, acidity, redox potential, preservatives, and competitive microorganisms may improve the total food quality; however, depending on hurdle intensity, may also cause detrimental effect unless they are kept in an optimal range (Leistner 1994). Regarding the microbial population, the applied hurdles must either inhibit the microbial growth or achieve satisfactory inactivation levels in order to be effective for food preservation (Leistner and Gould 2002). For instance, an alternative pasteurization treatment based on either combined with hurdles/technologies or individual treatments must accomplish at least 5-log reduction of the pathogen of interest in the food product (US FDA 2001). Selected combination of hurdles can facilitate gentle and efficient food preservation in terms of safety and quality.

The effect of hurdle technology for microbial inhibition in food products is based on homeostasis, metabolic exhaustion, and stress reactions of microorganisms (Leistner 2000). Homeostasis is a constant tendency of microorganisms to remain stable in their internal environment by adjusting conditions for optimal survival. Use of hurdles can disrupt the homeostasis of microorganisms; thereby, proliferation of microorganisms can be prevented either by keeping them at the lag phase or causing the microbial death before homeostasis is reestablished (Leistner 2000). Metabolic exhaustion occurs when microorganisms use up their energy to overcome

Table 24.1 Selected hurdles effective in food preservation

Factors	Application/mechanism
Reduced water activity	Drying, osmotic dehydration
Increased acidity	Acid addition or formation
Reduced redox potential	Removal of oxygen or addition of ascorbate etc.
Chemical preservatives	Sorbate, sulfide, nitrite etc.
Antimicrobials/ additives	Bacteriocins, essential oils, organic acids etc.
Competitive flora	Microbial fermentations
Low temperature	Chilling, freezing
Mild or high temperature	Microbial inactivation by heating
High pressure	Microbial inactivation by high pressure processing (HPP)
Pulsed electric fields	Microbial inactivation due to the electroporation effect of pulsed electric fields (PEF)
Ultrasound	Microbial inactivation due to the cavitation effect of ultrasonication
Light-based technologies	Damage in DNA of microorganism by UV-C light, light emitting diodes (LEDs), pulse light
Ionizing radiation	Damage in DNA of microorganism by β, γ, and χ radiation

Adapted from Leistner and Gould (2002), Leistner and Gorris (1995)

homeostasis and then die, which is called autosterilization of the food product (Leistner 2000). Some bacteria become resistant under stress by synthesizing protective stress shock proteins. Stress reactions may cause microorganisms to be more tolerant to various stresses, however, application of multi-target food preservation, which is exposure of microorganisms to different stresses, may prevent generation of stress shock proteins because microorganisms become metabolically exhausted during this energy consuming process and are not able to synthesize (Leistner and Gould 2002).

The hurdle technology concept has been widely studied to increase the use of nonthermal processing technologies (Leistner and Gorris 1995). Even though the total preservation effect of combined treatments can be merely additive, which means the addition of the effect of individual methods is equal to that of the combined preservation method, the purpose is to attain synergistic lethal effects that occur when the effectiveness of the treatment is greater than the effect of each method applied individually, and thereby, to reduce the severity of the treatment (Raso and Barbosa-Cánovas 2003). Under some circumstances, antagonistic effect may result when the effect of the individual methods is greater than that of the combined preservation methods (Raso and Barbosa-Cánovas 2003).

Therefore, nonthermal food processing technologies in combination with another preservation (or stress) factor might result in relevant benefits including increased microbial reduction, reduced treatment time and intensity, reduced concentration of

antimicrobials and other preservatives (Fig. 24.2). In this respect, the current applications and future directions of the utilization of different hurdles in combination with the most common nonthermal food processing technologies such as high pressure processing (HPP), pulsed electric fields (PEF), ultrasound (US), and light-based technologies (UV-C light and pulsed-UV light) are reviewed in this chapter.

24.2 Combinations with High Pressure Processing (HPP)

As explained in more details in another chapter of the book, HPP is a commercially viable and convenient technology to extend the shelf life of food products with minimal or no effect on sensorial quality because the overall treatment is less intense than most thermal processes. In HPP, either solid or liquid food products are firstly placed into a suitable packaging container and then subjected to varying pressure levels between 100 and 700 MPa while submerged in a vessel with water (or other appropriate fluid) (Betoret et al. 2015). HPP is effective on inactivating vegetative cells, yeasts, and molds; however, this treatment alone is not sufficient to inactivate spores (Hendrickx et al. 1998; Smelt 1998). This is because bacterial spores are more baroresistant compared to the vegetative bacteria, and therefore, bacterial spores survive at elevated pressures (Cheftel 1992). Combining HPP with moderate heat was effective on the inactivation of bacterial spores (Furukawa and Hayakawa 2001; Furukawa et al. 2001) and also increased inactivation level of vegetative bacteria (Patterson and Kilpatrick 1998), indicating synergism between pressure and temperature. Several studies that applied HPP in combination with other hurdles are summarized in Table 24.2. Different microorganisms such as *Bacillus* spp., *Listeria monocytogenes, Escherichia coli* O157:H7, *Salmonella* Enteritidis, *and S.* Typhimurium in juices and organic acid liquids were reduced by 8 log with a treatment of 345 MPa, 50 °C for 5 min (Alpas et al. 2000). A 6-log reduction of *E. coli* O157:H7 was obtained in orange juice after HPP at 550 MPa, 30 °C for 5 min (Linton et al. 1999). Studies showed that some spores could be inactivated with long time pressurization and moderate heat. Aouadhi et al. (2013) reported 5 log reduction of *B. sporothermodurans* in water and milk with treatment conditions of 477 MPa and 48 °C for 26 min and 495 MPa and 49 °C for 30 min, respectively. High pressure processing at 576 MPa and 87 °C for 13 min resulted in 6 log reduction of *B. subtilis* spores (Gao et al. 2006). In other words, HPP can be limited to pasteurization of food products in the absence of heat (Wimalaratne and Farid 2008) due to the pressure resistant spores (Patterson 2005). For sterilization processes, pressure assisted thermal sterilization (PATS) technology, which employs pressure in combination with heat, is one of the emerging methods to increase the product shelf life and making them shelf stable (Barbosa-Cánovas et al. 2014). PATS process could reduce the decimal reduction time and the required sterilization temperature (Wimalaratne and Farid 2008). This is very promising for heat-sensitive food products. Moreover, PATS can reduce undesired formation of food process contaminants by decreasing processing time (Sevenich et al. 2013).

Table 24.2 Microbial inactivation using selected nonthermal processing technologies in combination with other stress factors

Medium	Microorganism	Treatment conditions for the combined processes							Log reductions	Reference
		HPP	PEF	US	T (°C)	Light based	Antimicrobial agent	MAP/AP		
Apple cider	*L. monocytogenes*	–	–	20 kHz	50–60	–	–	–	5	Baumann et al. (2005)
Apple cider	*E. coli* K12	400 kPa	–	MTS, 1.4 min	59	–	–	–	5	Lee et al. (2013)
Apple juice	*N. fischeri ascospores*	600 MPa, 10 min	–	–	75	–	–	–	3.3	Evelyn Kim and Silva (2016)
Apple juice	*S. cerevisiae*	–	–	20 kHz, 95.2 μm, 30 min	44	PL, 71.4 J/cm^2	–	–	6.4 (commercial juice) 5.8 (fresh squeezed juice)	Ferrario et al. (2015)
Apple juice	*E. coli*	–	–	–	55	UV-C, 27.10 J/mL, 3.58 min	–	–	5	Gayán et al. (2013)
Apple juice	Aerobic bacteria	–	–	40 min	52	–	100 ppm of nisin	–	3.55	Liao et al. (2018a)
Apple juice	*A. acidoterrestris*	–	–	35 kHz, 5 min in water bath	–	UV-C, 13.44 W/m^2.	–	–	~5	Tremarin et al. (2017)
Apple juice	*A. Acidoterrestris*	200 MPa, 45 min	–	–	–	–	250 IU/mL of nisin	–	6	Sokołowska et al. (2012)
Apple carrot blended juice	*E. coli*	100 kPa	–	MTS, 30 s	60	–	–	–	–	Kahraman et al. (2017)
Blueberry juice	*E. coli*	350 MPa	–	MTS,40 °C, 5 min	40	–	–	–	5.85	Zhu et al. (2017)

Broth (BHI, pH 4.5)	*S. flexneri*	–	–	20 kHz, 18–20 min	≤ 52	–	240–250 μM of nisin	–	4	de Freitas et al. (2019)
Buffer system	*E. coli* (pH 4.0) *L. innocua* (pH 7.0)	–	24 kV/cm, 18 Hz, 1 μs Of pulse width	–	–	HILP, 3.3 J/cm^2	–	–	6.6 4.1	Muñoz et al. (2012)
Carrot juice	*L. innocua* *E. coli*	500 MPa, 2 min	–	–	20	–	25-ppm nisin	–	7	Pokhrel et al. (2019)
Carrot juice	*E.coli*	–	–	24 kHz, 120 μm	54 58	–	–	–	5	Pokhrel et al. (2017)
Cranberry, grape, pineapple juice	*S. cerevisiae*	–	–	24 kHz, 117 mm, 6 min	60	–	–	–	5	Bermúdez-Aguirre and Barbosa-Cánovas (2012)
Dry-cured ham	*L. monocytogenes*	450 MPa, 10 min	–	–	–	–	1054 AU/g of enterocins	–	4.4	Perez-Baltar et al. (2019)
Fruit juices	*S. aureus* *L. monocytogenes*	–	30 kV/cm, 99 μs	–	50	–	200 μs/mL of nisin	–	4.5 5.5	Saldaña et al. (2011)
Goat meat	*E. coli*	–	–	–	22	UV-C, 200 μW/cm^2, 2 min	1% of lemon-grass oil	–	6.66	Degala et al. (2018)
Grape tomato	*Salmonella*	–	–	–	30	PL, 0.150 J/cm^2 per pulse, 2 min	1% of H_2O_2	–	5.69 (spot inoculation) 3.05 (dip inoculation)	Huang and Chen (2019)

(continued)

Table 24.2 (continued)

Medium	Microorganism	Treatment conditions for the combined processes							Log reductions	Reference
		HPP	PEF	US	T (°C)	Light based	Antimicrobial agent	MAP/ AP		
Juice and organic liquids	*Bacillus* spp., *L. monocytogenes, E. coli* O157:H7 *S. enteritidis S. typhimurium*	345 MPa, 5 min	–	–	50	–	–	–	8	Alpas et al. (2000)
Liquid whole egg	*E. coli* *L. innocua*	450 MPa	–	–	–	–	1.25 mg/L of nisin 5 mg/L of nisin	–	5 6	Ponce et al. (1998)
Liquid whole egg	*E. coli* O157:H7	–	11 kV/ cm	–	60	–	–	–	4	Bazhal et al. (2006)
Liquid whole egg	*L. innocua*	–	50 kV/ cm, 33 pulses	–	36	–	–	–	3.5	Calderón-Miranda et al. (1999)
Liquid whole egg	*L. innocua*	–	50 kV/ cm, 33 pulses	–	36	–	100 IU/mL of nisin	–	5.5	Calderón-Miranda et al. (1999)
Milk	*Bacillus sporothermodurans*	495 MPa, 30 min	–	–	49	–	–	–	5	Aouadhi et al. (2013)
Milk buffer	*B. subtilis* spores	576 MPa, 13 min	–	–	87	–	–	–	6	Gao et al. (2006)
Orange juice	*E. coli* O157:H7	550 MPa, 5 min	–	–	30	–	–	–	6	Linton et al. (1999)
Orange juice	*L. monocytogenes*	–	–	20 kHz, 95 μm, 600 W	45	–	1000 ppm of vanillin, 75 ppm of citral	–	<5	Ferrante et al. (2007)
Sabouraud broth	*S. cerevisiae*	–	–	20 kHz, 95 μm, 600 W	45	–	1000 ppm of chitosan	–	3	Guerrero et al. (2005)

Skim milk	Incubated natural microbiota	–	80 kV/cm, 50 pulses	–	52	–	38 IU/mL of nisin and 1638 IU/mL of lysozyme	–	7	Smith et al. (2002)
Sea bream fillets	Aerobic mesophiles	300 MPa, 5 min	–	–	Ambient	–	–	AP with thymol and enterocin	4	Ortega Blázquez et al. (2018)
Strawberry puree	*B. nivea ascospores*	600 MPa, 10 min	–	–	75	–	–	–	1.4	Evelyn Kim and Silva (2015)
Verjuice	*S. cerevisiae*	–	–	–	51	0.25 J/cm^2	–	–	5.16	Kaya and Unluturk (2019)
Water	*B. sporothermodurans*	477 MPa, 26 min	–	–	48	–	–	–	5	Aouadhi et al. (2013)

AP active packaging, *HILP* high intensity light pulses, *MAP* modified atmosphere packaging, *MTS* manothermosonication

In addition to heat, HPP has been used in combination with several other hurdles such as antimicrobials and osmotic dehydration in order to increase the synergistic lethal effect in different types of food products (Andreou et al. 2018; de Oliveira et al. 2015; O'Neill et al. 2018; Misiou et al. 2018; Perez-Baltar et al. 2019; Pokhrel et al. 2019). Combining HPP with antimicrobials is a very effective approach to deliver safe food products of excellent quality (Raso and Barbosa-Cánovas 2003). Since bacteriocins (i.e. nisin, lysozyme) are safe and natural antimicrobials, they are preferred over antimicrobials such as chemicals to reduce the growth of microorganisms. HPP at 450 MPa with nisin concentrations of 1.25 and 5 mg/L reduced *E. coli* and *L. innocua* in liquid whole egg by 5 and 6 log, respectively (Ponce et al. 1998). A recent study demonstrated synergistic effect on the inactivation of *L. innocua* and *E. coli* in carrot juice subjected to HPP (200–500 MPa) in combination with a mild thermal treatment (35 and 50 °C) and nisin (25 and 50 ppm), suggesting that relevant combinations of HPP, mild temperatures, and nisin can result in less intense pressures, more energy efficient and cost effective processes as well as high quality food products (Pokhrel et al. 2019). Therefore, it is important to make judicious hurdle combinations in order to achieve selected benchmarks. For instance, Alcántara-Zavala et al. (2018) suggested that HPP and an in-situ biosynthesized nisin combination can be used against *L. innocua* for the pasteurization of milk at lower pressure levels and/or shorter pressure holding times than using just high pressure. Likewise, application of endolysins prior to HPP allows pathogen elimination at reduced pressure levels which could be considered as a promising approach for the pressure-sensitive foods (Misiou et al. 2018). Pokhrel et al. (2019) also reported that HPP at 500 MPa and 20 °C for 2 min without nisin resulted in 4- and 5-log CFU/mL reduction of *L. innocua* and *E. coli*, respectively, while incorporation of 25-ppm nisin achieved 7-log reduction in carrot juice at the same pressure and temperature. Lee and Kaletunc (2010) reported that individual application of HPP up to 200 MPa (10 min at 25 °C) and nisin alone treatments (200 IU/mL) were not effective on the inactivation of two different strains of *S.* Enteritidis in culture broth. Increasing pressure level up to 450–500 MPa reduced both strains about 8 log. Similar result was also obtained at lower pressure levels, 350–400 MPa in the presence of nisin (200 IU/mL) due to the pressure enhanced penetration of nisin into the bacterial cell. This finding allows development of a promising treatment to reduce HPP cost. Regarding the use of lysozyme as a hurdle, Sokolowska et al. (Sokołowska et al. 2012) reported that lysozyme was not as effective as nisin when it is combined with HPP for the inactivation of *Alicyclobacillus acidoterrestris* in apple juice. By the opposite, combining lysozyme with HPP has been reported to enhance inactivation of some *E. coli* strains in phosphate buffer solution at pH 7 (Masschalck et al. 2000).

Efficacy of antimicrobials is not only dependent on stress factors but also the composition of the food matrix and storage period (Alzamora et al. 2003). Earlier studies have reported that the use of bacteriocin producing lactic acid bacteria combined with HPP treatments show a synergistic lethal effect on *L. monocytogenes* inactivation in different types of foods such as cheese (Arqués et al. 2005), fermented sausages (Ananou et al. 2010), and sliced cooked ham (Liu

et al. 2012). In another study, Perez-Baltar et al. (2019) demonstrated that low intensity pressure treatment at 450 MPa for 10 min reduced *L. monocytogenes* cells by less than 1.0 log unit due to the relatively low water activity (0.873) of the dry-cured ham, while HPP combined with 1054 AU/g of enterocins at the same pressure level achieved approximately 4.4 log reduction. The authors also found that combination of enterocins and HPP resulted in a synergistic bactericidal effect during 30 days of storage at 4 and 12 °C and delayed the spoilage of dry-cured ham by inhibiting the growth of total viable counts (Perez-Baltar et al. 2019). Thus, HPP in combination with antimicrobials can be taken into consideration to synergistically increase the lethal effect of the treatment for food products with low water content.

As demonstrated by Andreou et al. (2018), HPP (25 °C, 600 MPa, 5 min) in combination with osmotic dehydration (15 °C, 45 min in osmotic solution of 60% maltodextrin and 5% NaCl) rendered shelf life extension of chicken breast fillets over 3 weeks at 5 °C in terms of microbial quality and organoleptic properties. The authors indicated that the combined HPP and osmotic dehydration treatment synergistically enhances the microbial quality as well as the color, texture and the sensorial characteristics of the product compared to the individual treatments. Thus, combining HPP treatment with other preservation methods at moderate intensity is considered as a promising strategy to adequately control microorganisms while reducing the loss of food quality (Khan et al. 2017; de Oliveira et al. 2015). Another benefit of using HPP in a hurdle system can be associated with the reduced salt content of a number of food products. As O'Neill et al. (2018) reported, HPP combined with a mix of organic acids and salt replacer extended the shelf life of low-salt frankfurters by 51% (at 580 MPa) and low-salt cooked ham by 97% (at 535 MPa) without changing their sensorial acceptability. Rodrigues et al. (2016) investigated the effects of high pressure (300, 450 or 600 MPa) treatment on the inactivation of *L. innocua* and *Enterococcus faecium* in marinated beef with different concentrations of NaCl (1 or 2%) and citric acid (1 or 2%). Samples treated at 600 MPa were subjected to a 14-day refrigerated storage and evaluated in terms of physicochemical properties. The solution with high salt (%2) + high citric acid (%2) concentrations was the most effective one resulting in 4 or 6 log cycles reduction in *E. faecium* count at 450 and 600 MPa applications, respectively, and 6 log cycles reduction in *L. innocua* count for all pressures applied.

Moreover, synergistic microbial inactivation effect has been utilized for water disinfection using pressurized CO_2 (Zhang et al. 2016). It is well known that CO_2 has an inhibition effect on several microorganisms (Damar and Balaban 2006; Kobayashi et al. 2009). A greater inactivation effect could be achieved in case of a high pressure carbon dioxide (HPCD) treatment. This can be attributed to the rapid release of pressurized CO_2 (Fraser 1951) and reduction of extracellular pH (Zhang et al. 2016). Applied pressure increases the solubilization rate of CO_2 which further causes reduction of the intracellular pH and results in enhanced lethal bactericidal effect. Combined use of CO_2 with 0.7 MPa at room temperature for 10 min was reported to render the best inactivation performance for water disinfection (Zhang et al. 2016). Acidification also enhances the effect of HPP on inactivation of

microorganisms. Decreasing pH of the treatment buffer from 7 to 3 increased additional 2.5 log of *B. subtilis* inactivation when combined with HPP (Balasubramaniam et al. 2001).

Another advantage of HPP is to avoid any post-contamination risk since HPP is applied, in most cases, in packed foods (Morris et al. 2007). The contribution of modified atmosphere packaging (MAP) (50% CO_2–50% N_2) to the shelf life extension of high pressure treated (500 MPa, 5 min) raw poultry sausages was investigated by Lerasle et al. (2014). The authors reported that microbiological quality of the product in terms of aerobic mesophilic counts and lactic acid bacteria was ensured by an HPP treatment while MAP was only successful to limit the lipid oxidation during refrigerated storage. On the other hand, active packaging combined with high pressure has been reported to achieve more remarkable microbial reductions. The application of 400 MPa HPP treatment and the use of interleavers containing nisin resulted in absence of *Salmonella* in 25 g of cooked ham 24 h after pressurization (Jofré et al. 2008). Similarly, Stratakos et al. (2015) found that HPP in conjunction with an essential oil-based active packaging on the surface of ready-to-eat (RTE) chicken breast exhibited a synergistic effect and reduced the *L. monocytogenes* counts below detection limits throughout 60 days of storage at 4 °C. The lethal effect was reported to be synergistic because the reduction was higher than the sum of the individual reductions. Therefore, the authors suggested to use active packaging followed by a pressure treatment to decrease the risk of *L. monocytogenes* in cooked chicken without diminishing its quality. Regarding the seafood products, it is also possible to obtain promising results with the combined application of HPP and active packaging. For instance, Ortega Blázquez et al. (2018) demonstrated for sea bream fillets that HPP alone, and activated plastic film with thymol plus enterocin treatments, alone as well, decreased the aerophilic mesophiles viable count by 1.46 and 2.36 log, respectively; whereas, application of both HPP and activated plastic film synergistically achieved 4.13 log reduction of aerophilic mesophiles.

The food industry has been increasingly utilizing HPP technology worldwide: North America (54%), Europe (25%), Asia (12%) for different types of food products including meat, vegetable products, seafood, juices, and beverages (Huang et al. 2017). It is recognized that HPP technology provides clean label food products with extended shelf life, preserves nutritional and organoleptic attributes, and reduces the use of additives. However, there are some drawbacks such as refrigeration requirement after pressurization and limited applications for low moisture or bubble containing food products which can reduce the efficacy of HPP treatments. Therefore, the use of appropriate hurdles is beneficial to overcome those disadvantages and facilitate developing high quality food products.

24.3 Combinations with Pulsed Electric Fields

In a pulsed electric fields (PEF) treatment, pumpable food products are passed through a couple of electrodes inside a processing chamber where the generated electric fields vary between 10 to 80 kV/cm if microbial inactivation is the target. These electric fields are pulsed where the pulse duration typically ranges from 1 to 4 microseconds. These chambers could have multiple pair of electrodes (Barbosa-Cánovas et al. 2000). More details for PEF can be found in another chapter of the book. This nonthermal technology has been applied to different type of products such as apple juice, orange juice, milk, and eggs, and does not negatively affect the sensory and physicochemical quality of the products (Vega-Mercado et al. 1997; Zhang et al. 1997; Qin et al. 1995; Ma et al. 1997). However, PEF is not suitable for food products with air bubbles, high electric conductivity, and particle size larger than the gap of the treatment region, i.e. distance between electrodes (Barbosa-Cánovas et al. 2000).

The lethal effect of PEF relates to the pore formation and physical damage of the microbe cell membrane. The damage on the microbial cells can be enhanced by applying additional preservation factors such as pH, temperature, antimicrobial agents, and other nonthermal processing technologies (Vega-Mercado et al. 1996). As shown in Table 24.2, most of the studies indicate that there is an increased lethal effect by combining PEF with heat. For instance, combining a PEF treatment (11 kV/cm) with heat (60 °C) resulted in 4 log of *E. coli* O157:H7 reduction in liquid whole eggs while thermal treatment alone at 60 °C for 4 min reduced it only 2 log (Bazhal et al. 2006). Moreover, combination of PEF with moderate heat and natural antimicrobials such as nisin and lysozyme has been found quite effective by reducing *E. coli* O157:H7 in apple juice (Iu et al. 2001). Similar results were observed in fresh orange juice (Hodgins et al. 2002). Calderón-Miranda et al. (1999) reported that liquid whole egg exposed to 10 and 100 IU nisin/mL after PEF treatment with an electric field intensity of 50 kV/cm and 32 pulses achieved 4.1 and 5.5 log reductions of *L. innocua*, respectively, while a PEF only treatment reduced it by 3.5 log. Likewise, inactivation of 4.5 log of *S. aureus* and 5.5 log of *L. monocytogenes* in fruit juices were attained when PEF was applied at 50 °C in the presence of 200 μg/mL of nisin (Saldaña et al. 2011). Combination of PEF treatment (80 kV/cm and 50 pulses) with mild heat (52 °C), nisin (38 IU/mL) and lysozyme (1638 IU/mL) resulted in 7 log reduction of incubated natural microbiota in raw skim milk (Smith et al. 2002). The PEF and heat combination reduces the specific energy input for the microbial inactivation due to the synergistic effect of temperature and PEF compared to the PEF treatment alone. Thus, reduction of thermal load to moderate temperatures decreases the detrimental effect of thermal process on food quality (Toepfl et al. 2007). Other advantages of using PEF combined with other stress factors include lowering PEF treatment intensity (up to 5 kV/cm) and lowering

doses of antimicrobial agents (up to 4 times) that can still guarantee the microbial safety of food products (de Carvalho et al. 2018). PEF at moderate temperatures with antimicrobials is a promising hurdle combination. However, it is worth mentioning that the effect of antimicrobials on inactivation of microorganisms may change with the application sequence. Addition of nisin before PEF treatment resulted in an additive effect on *L. innocua*, but antagonistic when applied after PEF treatment (Gallo et al. 2007). The required electric field strength to obtain the desired level of microbial inactivation varies depending on the type of microorganism. Toepfl et al. (2007) reported that an electric field strength of 15 kV/cm could be sufficient to inactivate *E. coli* cells in a suspension while more than 35 kV/cm is required for the complete inactivation of *L. innocua*.

Other hurdles that have been studied in combination with PEF are reported in Table 24.3. A clear synergistic effect has been identified for PEF and high pressure carbon dioxide processing for inactivation of *E. coli* cells (Pataro et al. 2014). Muñoz et al. (2012) demonstrated that the combination of PEF with high intensity light pulses resulted in sufficient inactivation of *E. coli* without antimicrobial addition; whereas no significant reductions for *E. coli* in case of PEF alone treatment at 24 kV/cm. Regarding PEF and HPP combination, Pyatkovskyy et al. (2018) demonstrated that sequential application of HPP-PEF and PEF-HPP processes exhibited mostly additive effects while simultaneous PEF and HPP treatment offered a synergistic lethal effect.

24.4 Combinations with Ultrasound

Ultrasound converts electrical energy to sound energy by mechanically vibrating a given fluid promoting the so-called acoustic cavitation, i.e. formation, growth and rapid collapse of microscopic bubbles that cause damage on the surface of the microorganisms (Raso et al. 1998a). This type of ultrasound application in food processing is called high intensity, low frequency (10–100 kHz) or power ultrasound. Low intensity ultrasound, on the other hand, is referred as high frequency (1–10 MHz) or non-destructive ultrasound which is used in quality assurance (Bermúdez-Aguirre et al. 2011). Although power ultrasound is successful for microbial inactivation to pasteurize high acid products, it is less effective for low acid products; therefore, combination with hurdles such as heat, pressure, etc. is necessary to increase effectiveness of the treatment (Raso and Barbosa-Cánovas 2003). More details of ultrasound can be found in another chapter of the book.

Combining ultrasound with heat (thermosonication) at lethal temperatures has shown synergistic effects on microbial inactivation (Piyasena et al. 2003; Feng et al. 2008) and reduced processing time (Lee et al. 2009) (Table 24.3). Combined lethal effect of ultrasound and mild heat increases the microbial inactivation rates at low temperature and reduces the severe processing impact on nutritional and overall quality of food products (Abid et al. 2014; Anaya-Esparza et al. 2017). Therefore, thermosonication can be utilized to replace conventional thermal processes. So far,

Table 24.3 Selection of ideal hurdles for food preservation

Hurdles	HPP	PEF	US	Light based	Heat	CO_2	Organic acids & low pH	Anti-microbials	Low a_w	MAP / AP
HPP	△	?	? ≠		? ≠	? ≠ ◉	? ≠ ◉	? ≠ ◉	? ≠ ◉	? ◉
PEF	Pyatkovskyy et al. (2018)	△	? ≠	? ≠	? ≠	? ≠ ◉	? ◉	? ≠ ◉		
US	Zhu et al. (2017) Kahraman et al. (2017)	Lyu et al. (2016) Muñoz et al. (2012)	△	? ≠	? ≠		? ◉	? ≠ ◉		
Light based		Caminiti et al. (2011) Muñoz et al. (2012)	Muñoz et al. (2012) Ferrario et al. (2015)	△	? ≠			? ≠ ◉		
Heat	Pokhrel et al. (2019)	Monfort et al. (2011)	Li et al. (2017) Fan et al. (2019b) Yildiz et al. (2019)	Gayán et al. (2013) Gouma et al. (2015) Kaya and Unluturk (2019)	△					
CO_2	Zhang et al. (2016)	Pataro et al. (2014)				◎				
Organic acids & low pH	O'Neill et al. (2018)	Fernández-Molina et al. (2015)	Muñoz et al. (2012)				◎			
Anti-microbials	Alcántara-Zavala et al. (2018); Misiou et al. (2018); Perez-Baltar et al. (2019); Pokhrel et al. (2019)	Muñoz et al. (2012) Smith et al. (2002)	Muñoz et al. (2012)	Degala et al. (2018)				◎		
Low a_w	Andreou et al. (2018); Perez-Baltar et al. (2019)								◎	
MAP / AP	Stratakos et al. (2015) Ortega-Blázquez et al. (2018)									◎

△: lethality, ?: increased lethality, ≠: reduced treatment intensity and/or time , ◎: microbial growth inhibition, ◉: increased microbial growth inhibition

△: Lethality, ?: Increased lethality, ≠: Reduced treatment intensity and/or time, ◎: Microbial growth inhibition, ◉: Increased microbial growth inhibition

several studies contributed to the ultrasonic processing of different types of food products such as skim milk, fresh-cut products, fruit and vegetable juices (Anaya-Esparza et al. 2017; Evelyn Kim and Silva 2015; Fan et al. 2019a). Thermosonication (20 kHz, 0.3 W/cm^3) at 60 °C for 5 or 10 min reduced the total plate count and yeast-mold counts to below detection limits in apple juice (Abid et al. 2014). In addition to natural microbiota, it is of great interest to develop inactivation strategies for a target microorganism of concern. A recent study demonstrated that thermosonication synergistically inactivated *Bacillus subtilis* spores by 2.4 log (Fan et al. 2019b). Likewise, Pokhrel et al. (2017) were able to achieve up to 5 log and higher than 5 log reduction of *E. coli* in carrot juice by using an ultrasound treatment at 54 °C and 58 °C after 2 min, respectively. The required time and temperature to obtain the desired microbial inactivation levels are lower for thermosonication treatments compared to the individual thermal treatments. For instance, thermosonication reduced the time required for heat treatment by half for the same log cycles reduction of *L. monocytogenes* at 65 °C (Franco-Vega et al. 2015). In another study, application of ultrasound (114 μm, 1.1 W/mL, 5 min) as a pretreatment of whole milk reduced D-value of *B. subtilis* by 35% compared to thermal only treatment at 100 °C (Ansari et al. 2017).

Mild heat treatment at 55 °C for 15 min resulted in shrinkage of the cell and partial collapse of the cell wall. On the other hand, thermosonication promoted complete collapse of the cells, increased leakage of the cell contents and the disintegration of the cell walls and plasma membranes as shown by scanning electron microscopy (SEM) images (Fig. 24.3) (Li et al. 2017). Furthermore, transmission electron microscopy (TEM) analysis also proves that thermosonication causes greater damage on cell wall, cell membrane and inner structure compared to individual ultrasound and heat treatments as shown in Fig. 24.4. Similarly, inner cell components of *E. coli* exhibit partial damage and leakage due to the broken cells and membranes in response to thermosonication (Zhu et al. 2017). Thus, it is noticeable that increased release of the cell content and disintegration of the cell may occur with the synergistic combination of ultrasound and mild heat (Li et al. 2017). On the other hand, Baumann et al. (2005) reported that the effect of combining ultrasound (20 kHz, 750 W) with heat at lethal temperatures (50–60 °C) on inactivation of ultrasound resistant strain of *L. monocytogenes* 10403S in apple cider was additive. In another scenerio, temperatures higher than 58 °C resulted in antagonistic effect because ultrasound at high temperatures promotes a cushioning effect on the bubbles because of the generated vapor (Raso et al. 1998a).

Combining ultrasound with heat under pressure (manothermosonication) may lead to higher microbial inactivation due to increased cavitation implosion power and shorter implosion time when a hydrostatic pressure is incorporated in an ultrasound treatment (Lee et al. 2013). The manothermosonication treatment results in higher lethality than heat and ultrasound under pressure between 45 °C and 64 °C, indicating a synergistic effect (Arroyo et al. 2012). Treatment with 20 kHz, 300 kPa, 70 °C, 12 min at 90 μm and 150 μm resulted in 75% and 99.9% reduction of *B. subtilis* spore population, respectively (Raso et al. 1998b). In another study,

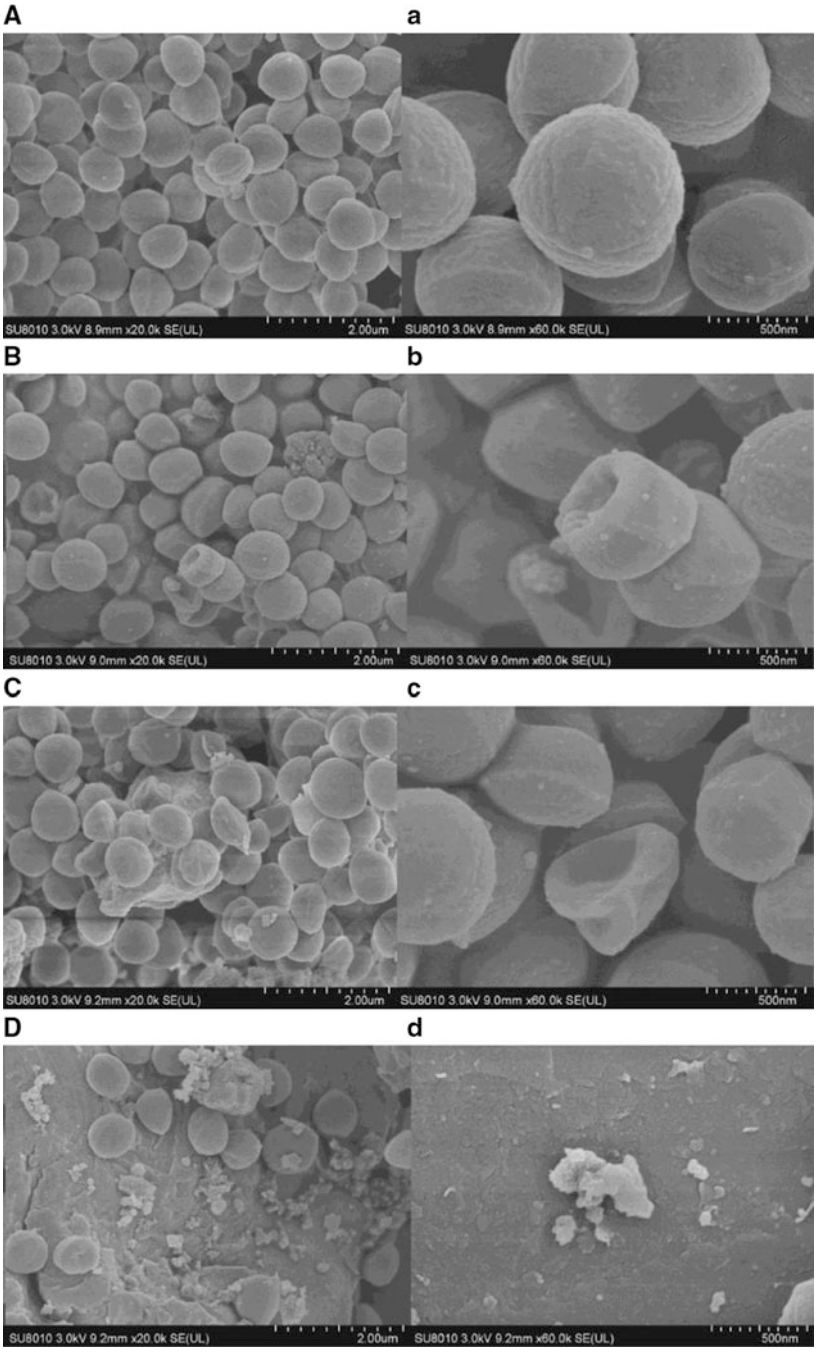

Fig. 24.3 SEM photographs of sublethal injury of *S. aureus* cells. Untreated bacteria (**A-a**). Bacteria treated with ultrasound for 15 min (**B-b**). Bacteria treated with mild heat for 15 min (**C-c**). Bacteria treated with thermo-sonication for 15 min (**D-d**) (Li et al. 2017 with permission)

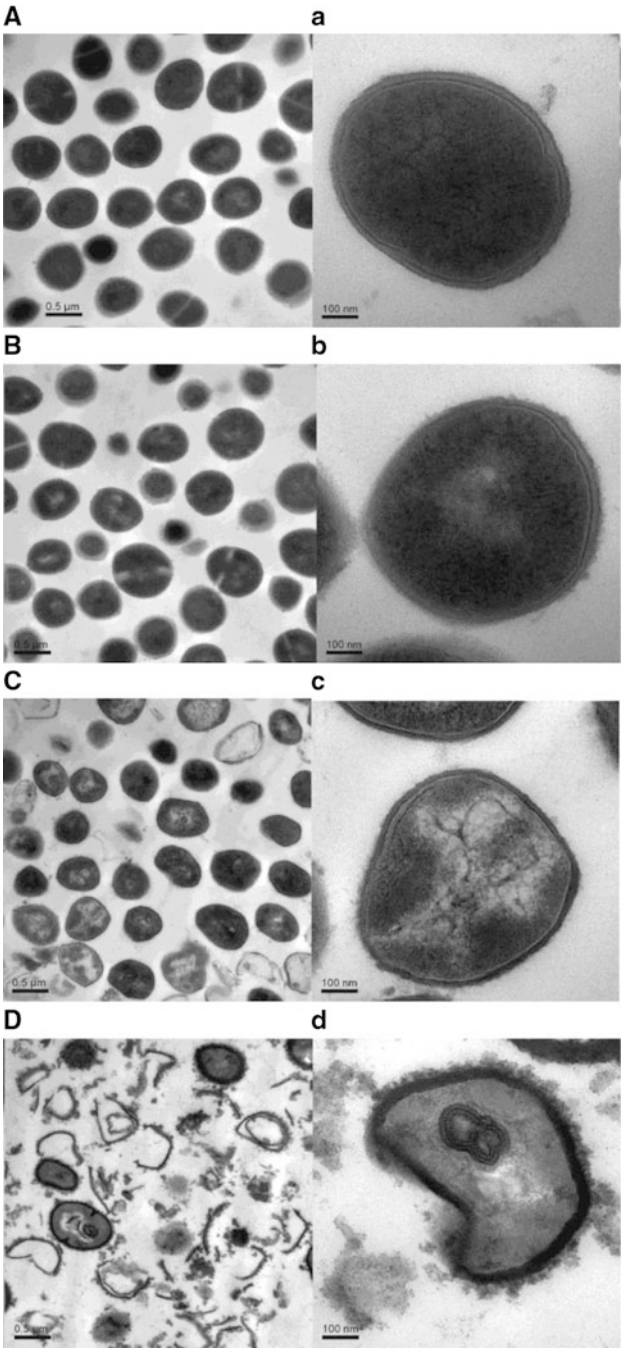

Fig. 24.4 TEM photographs of sublethal injury of *S. aureus* cells. Untreated bacteria (**A-a**). Bacteria treated with ultrasound for 15 min (**B-b**). Bacteria treated with mild heat for 15 min (**C-c**). Bacteria treated with thermo-sonication for 15 min (**D-d**) (Li et al. 2017 with permission)

higher than 5 log *E. coli* O157:H7 reduction was attained in apple-carrot blended juice by using continuous-flow manothermosonication at 100 kPa, 30 s, 60 °C (Kahraman et al. 2017). Zhu et al. (2017) subjected blueberry juice to sonication at 40 °C for 5 min, and subsequently exposed it to high pressure (350 MPa) for 5–20 min. The authors were able to achieve 5.85 log reduction of *E. coli* cells after manothermosonication. The hurdle lethal effect of manothermosonication causes shrinkage, deformation, and partial breakage of cell leading to release of cytoplasmic content while untreated cells have equally distributed intracellular content (Zhu et al. 2017). Moreover, manothermosonication of lemon juice and strawberry juice maintained cloud stability, color, pH, and conductivity which demonstrated the potential of this combination in juice processing (Kuldiloke 2002).

Ultrasound can also be used in combination with antimicrobials. Several studies have been conducted for the evaluation of lethal effect of ultrasound and antimicrobial combinations (de Freitas et al. 2019; Liao et al. 2018a; Muñoz et al. 2012). Gram negative bacteria exhibit slightly higher resistance to antimicrobials due to the presence of outer membrane that shows protective effect for the cell compared to Gram positive bacteria (Zou et al. 2013). Bacterial inactivation can be enhanced by combining ultrasound and antimicrobials due to damage to the outer membrane; ultrasound facilitates penetration of antimicrobial compounds into the cell (de São José and Vanetti 2015; Li et al. 2016; Runyan et al. 2006). Thus, combination of ultrasound with antimicrobials increases the efficacy on microorganisms by weakening the cell wall. Nisin is one of the commonly used antimicrobials that has been studied in combination with ultrasound. A recent study investigated the combination of ultrasound (20–25 kHz and 950 W) with nisin (100 ppm) in fresh apple juice at different temperatures (37, 42, 47, and 52 °C) for 5–40 min. The results showed that reduction in the number of aerobic bacteria was 3.55 log (CFU/mL) at 52 °C for 40 min (Liao et al. 2018a). Addition of 1000 ppm vanillin and 75 ppm citral followed by sonication (600 W, 20 kHz, 95 μm, 45 °C) resulted in >5 log reduction of *L. monocytogenes* in orange juice (Ferrante et al. 2007). Potassium sorbate, sodium benzoate, and eugenol incorporated with recovery media reduced the intensity and processing time of ultrasound treatment on the inactivation of *Z. rouxii* (Arce-García et al. 2002). However, combination of same ultrasound treatment conditions with 1000 ppm chitosan reduced up to 3 log of *S. cerevisiae* in Sabouraud broth (Guerrero et al. 2005).

The lethal effect of ultrasound at low pH depends on the type of microorganism. The resistance of *S. cerevisiae* to ultrasound treatments at 20 kHz, amplitude of 71–110 μm, 35–55 °C, and pH 3 and 5.6 was investigated by Guerrero et al. (2001). The authors showed that lowering pH did not significantly affect the sensitivity of yeast to the ultrasound processes. On the other hand, combining ultrasound with low pH decreased the sensitivity of *E. coli* to ultrasound (Salleh-Mack and Roberts 2007) due to the effectiveness of ultrasound treatment on gram-negative bacteria.

It is also relevant to evaluate the use of ultrasound in combination with other nonthermal processing technologies such as PEF, plasma, and light-based technologies. For instance, ultrasound alone (35 °C, 750 W, 120 min) and PEF alone (35 °C, 12 kV/cm, 120 μs) treatments reduced *S. cerevisiae* cells by 0.76 and 2.88 log

CFU/mL respectively in Chinese rice wine while their combination resulted in an additive lethal effect in either sonication-PEF or PEF-sonication sequence (Lyu et al. 2016). At this point, it is important to state that application of less sonication times, i.e. less than 120 min., is highly recommended for industrial applications. In another study, the sequence of nonthermal plasma (NTP) followed by an ultrasound treatment was reported to exhibit higher inactivation rate of *S. aureus* than the ultrasound-NTP sequence. The lower inactivation levels obtained after ultrasound-NTP sequence can be attributed to the effect of ultrasound on increasing oxidative response that further allows *S. aureus* to show resistance to the following NTP treatment (Liao et al. 2018b). Thus, the sequence of the hurdles should also be taken into consideration if they are not applied at the same time.

24.5 Combinations with Light-Based Technologies

Light-based technologies including UV-C light and pulsed UV light (PUV) in combination with other hurdles are evaluated in this section. UV-C light has a germicidal effect on microorganisms especially at 253.7 nm; therefore, prevents transcription and reproduction of the cells by forming dimers on thymine structure in their DNA (Bintsis et al. 2000). UV-C irradiation has been used for water and air disinfection, surface decontamination of meat, poultry, and seafood products, and pasteurization of liquid food products (Bintsis et al. 2000; Koutchma 2008; Shah et al. 2016; Gayán et al. 2014). Pulsed light technology, on the other hand, applies short time pulses (100–400 μs at a broad spectrum between 100 and 1100 nm) causing the formation of pyrimidine dimmers which impairs the process of cell replication (Gómez-López et al. 2007). More details for light-based technologies can be found in another chapter of the book. Although both UV-C irradiation and pulsed UV light technologies have been utilized for microbial inactivation studies for different types of food products, the efficacy of such technologies highly depends on the food matrix. For instance, Ferrario et al. (2015) found out that PUV exposure caused reduction of *A. acidoterrestris* spores and *S. cerevisiae* cells by 3.0 and 4.4 log, respectively, in a commercial apple juice; whereas 1.5 and 2 log reductions were achieved for *A. acidoterrestris* spores and *S. cerevisiae*, respectively, in a natural squeezed juice due to the differences in juice absorptivity. In this respect, combination of other thermal and nonthermal preservation factors with light-based technologies can enhance their potential applications for a wide range of food products and target microorganisms by increasing the lethal effect. Ferrario et al. (2015) demonstrated that individual application of ultrasound at 44 °C for 30 min reduced the yeast content by approximately 2.5 log cycles while the maximum yeast reduction by single PUV treatment (71.4 J/cm^2, 44 °C, 30 min) was 3.7 log in apple juice. On the other hand, application of PUV with a prior sonication treatment enhanced the lethal effect and resulted in 6.4 and 5.8 log reductions of *S. cerevisiae* in commercial and natural apple juice, respectively. By the opposite, combined PUV and ultrasound was reported not to improve inactivation of *A. acidoterrestris* spores in the same

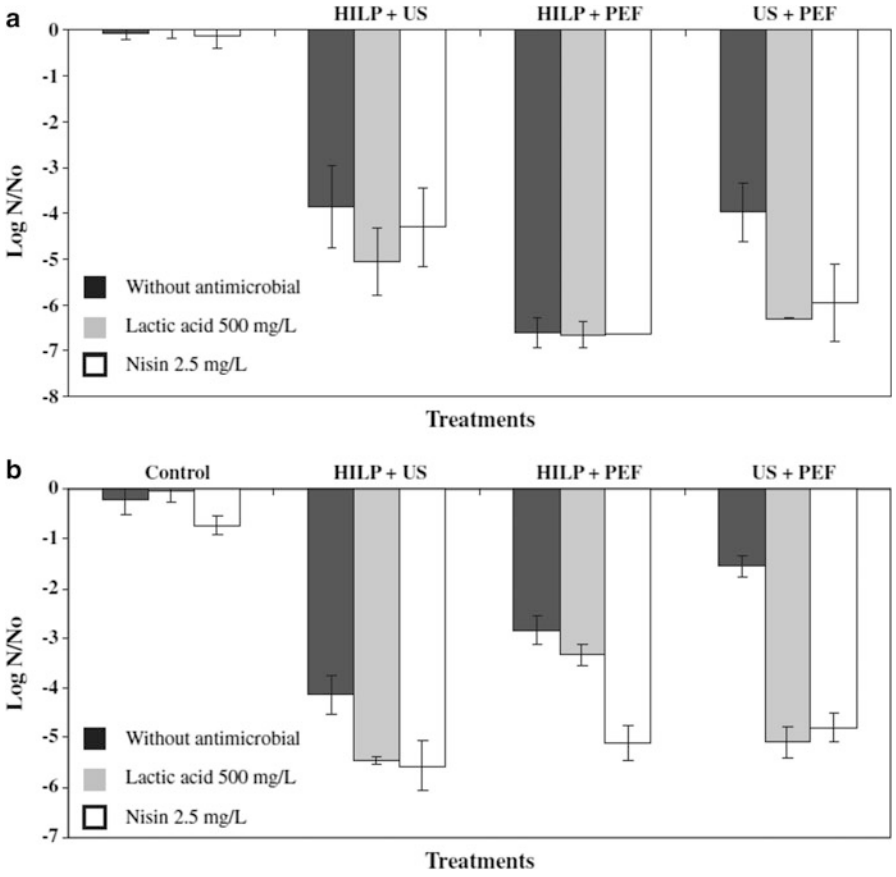

Fig. 24.5 Combined effect of nonthermal processing technologies and antimicrobials for the inactivation of *E. coli* K12 (**a**) and *L. innocua* (**b**) (Muñoz et al. 2012 with permission). (HILP: high intensity light pulses, US: ultrasound, PEF: pulsed electric fields)

food matrix. In another study, the individual application of high intensity light pulses (HILP) reduced *E. coli* K12 and *L. innocua* cells in a buffer system by 3.6 and 2.7 log, respectively. Although its combination with ultrasound (500 W, 40 °C, 126 s) did not result in significant reduction for *E. coli* presenting neither synergistic nor additive effect, significantly greater inactivation was achieved for *L. innocua* cells (4.1 log CFU/mL) when HIPL was combined with PEF (24 kV/cm, 18 Hz and 1 μs of pulse width). Significant reduction (6.6 log) was obtained for *E. coli* K12 when combining the use of HILP with PEF (Muñoz et al. 2012). The authors also reported that the lethal effect even increased more with the incorporation of nisin or lactic acid as antimicrobial agents (Fig. 24.5). Another application of pulse light is its incorporation in washing processes for decontamination purposes. Pulse light exposure can be used as an alternative to chlorine washing when it is combined with hydrogen peroxide (H_2O_2) in fruits and vegetables (Huang and Chen 2019). Likewise, UV

washing combined with peroxyacetic acid (PAA) was able to significantly reduce *Salmonella* cells on fresh produce and kept the *Salmonella* level in washing water below detection limits (Huang et al. 2018).

Regarding UV-C irradiation, combination of UV-C light and heat is one of the widely studied combinations in order to increase the germicidal effect of the treatment. UV-C irradiation combined with heat treatments has been reported to result in a synergistic inactivation of *E. coli* in fruit juices (Gayán et al. 2012). Moreover, UV-C light at mild temperatures can ensure 5-log reductions of the *E. coli* strains in fruit juices without affecting its physicochemical characteristics (Gayán et al. 2013). Yeasts have been reported to show greater resistance to UV-C irradiation than the Gram negative bacteria (Carrillo et al. 2017; Keyser et al. 2008). A recent study reported that an individual UV-C irradiation (2.30 J/mL) can only achieve 0.54 log CFU/mL reduction in *S. cerevisiae* population; whereas UV-C irradiation in combination with a heat treatment reduced the yeast cells by 5.16 log CFU/mL in verjuice at 1.01 J/mL and 51 °C (Kaya and Unluturk 2019). Besides heat as a hurdle, significant inactivation levels can be also achieved by utilizing UV-C light in combination with sonication (Gabriel 2015). Tremarin et al. (2017) reported that sonication for 5 min followed by UV-C irradiation for 25 min resulted in higher inactivation of *A. acidoterrestris* spores (~5 log) in apple juice compared to 5 min of UV-C light followed by 25 min of sonication treatment (~4 log). It is worth to indicate that the authors used an ultrasonic water bath rather than a probe system. Although the inactivation levels obtained from the combined treatments were significantly higher from the individual UV-C and ultrasound applications, the lethal effect of this hurdle combination was not reported as synergistic. On the other hand, Degala et al. (2018) demonstrated a 6.66-log CFU/mL reduction of *E. coli* K12 on goat meat surface after application of 1% of lemongrass oil together with UV-C irradiation; and called the lethal effect of this combined treatment as synergistic since it was greater than the sum of inactivation levels of the individual treatments.

24.6 Final Remarks and Future Trends

The relevance of wisely combining classic microbial stress factors with new food processing technologies as hurdles is shown throughout this chapter. The search for synergistic effects is the main goal for these combinations because it reduces the intensity of the overall treatment, resulting in food products of better quality compared to those processed using only one stress factor. Additive effects are also relevant for similar reasons even though their impact on the overall process is not as significant. The utilization of hurdle technologies including nonthermal treatments offers several benefits such as

1. better preservation of physical, nutritional and sensorial quality of food products,
2. development of viable, energy efficient, cost effective, and environment friendly processes once the appropriate optimization studies are applied for each type of food product (the optimum combination of hurdles is product dependent),

3. facilitating the development of new food products,
4. improvement of the overall quality of food products in comparison to those manufactured by conventional methods,
5. enhancement of microbial lethality by contributing to synergistic effects in a very effective way,
6. reduction of treatment intensity, time and cost compared to individual treatments.

Even though significant number of studies have been conducted in combining nonthermal food processing technologies with other stress factors, much more needs to be done including the development of specific mathematical models to predict what to expect from certain combinations in terms of safety, shelf life, overall quality, and associated costs, among other essential factors to determine the appropriateness of a given combination. Additional studies should take place to better understand what happens at the molecular level that promotes (or not) synergistic effects. Regardless of what needs to be done, the suitability of combining nonthermal technologies with other microbial stress factors is a reality and it is rendering benefits to users at different levels, laboratory and pilot plant studies, food industry, and equipment manufacturers.

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Part VI
Aseptic Processing and Post-packaging Technologies

Chapter 25
Aseptic Processing and Packaging

Nathan M. Anderson, Patnarin Benyathiar, and Dharmendra K. Mishra

25.1 Introduction

Aseptic processing and packaging are a method of preservation in which a liquid food (or pharmaceutical) product is commercially sterilized, typically by heating and holding at an elevated temperature followed by cooling, then filled into a sterilized package and hermetically sealed with a sterilized closure in a commercially sterile environment. Commercial sterility is the condition achieved that renders the food free of viable microorganisms of public health significance, as well as microorganisms of non-health significance, capable of reproducing under normal non-refrigerated conditions of storage and distribution (FDA 2011). Therefore, aseptic processing is commonly employed to produce shelf-stable, low-acid (pH > 4.6) foods such as milk, juice drinks, soup, and vegetable purees. Aseptic processing may also be employed to produce acid and acidified foods with pH $\leq$ 4.6. Over a century ago, milk was the first product to be aseptically processed and packaged into metal cans (Orla-Jensen 1913).

In the 1950's, the first commercial aseptic operation was established with the Dole Aseptic Canning System (White 1993). In this process, product is heated in tubular heat exchangers, held at temperature and then cooled. Metal cans and lids are sterilized by superheated steam. Sterile cans are then aseptically filled with cool sterile product and sealed in a superheated steam environment.

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Significant advancements have since been made in aseptic processing. Arguably the most important step in the global adoption of aseptic processing came in the 1980's when the US Food and Drug Administration (FDA) accepted the use of hydrogen-peroxide (H_2O_2) to sterilize consumer packages, which opened the door to significant growth in the consumer market (Davis and Dignan 1983). Another significant advancement came in the 1990's when the National Center for Food Safety and Technology (NCFST) and the Center for Advanced Processing and Packaging Studies (CAPPS) held a two Aseptic Processing of Multiphase Foods Workshops in 1995 and 1996. It was during these workshops that scientific consensus was established between stakeholders such as academia, regulatory authorities, and industry on the issues and considerations for aseptic processing of multiphase foods (Lechowich and Swartzel 1996). Though products containing discrete particles can be found in the marketplace, growth of this segment of the aseptic market has been slow, in part because validation is complex and costly, but also because large particles are often physically damaged by pumps, heating, and piping (Nelson 2010). Recent technological advancements, largely aimed at controlling or measuring residence time of discrete particles, have been developed to give the industry the tools necessary to validate these complex systems (Jasrotia et al. 2008; Kumar et al. 2007). Novel processing systems aimed at processing foods with large particulates in the absence of a liquid phase or with only minimal volume in the liquid phase have also been developed (Anderson and Walker 2011).

On the other hand, significant advancements have also been made in aseptic packaging and package sterilization, since the metal can was used in the Dole aseptic system. Bag-in-box technology quickly gained acceptance in the 1990's as the system of choice for bulk packaging of dairy products and fruit and vegetable purees. Aseptic packages now include portion cups, paperboard cartons, pouches, and plastic bottles. Since aseptic processing offers extended shelf life without the need for preservatives, the technology offers "clean labeling" of products. This has led to rapid expansion of the aseptic market with 2020 revenues projected to be $6.4 billion in the U.S. alone (Lindell 2017). The use of these materials coupled with packaging machines with ever faster lines speeds have come with advancements in packaging sterilization. Early systems used steam in 1950s. However, hydrogen peroxide gained favor as sterilant for paperboard and HDPE bottles since in 1970s,. In the early 2000's, peroxyacetic acid was also utilized to sterilize PET bottles. Recently, Tetra Pak announced a new carton filling machine that utilizes E-Beam sterilization technology to treat the packaging material with production capacities exceeding 40,000 packages per hour (TetraPak 2019).

Foods may also be commercially sterilized by other novel technologies discussed in other chapters of this book.

25.2 Aseptic Processing

25.2.1 Principles and Technology

Most commercial aseptic processing systems are continuous, closed systems in which, the product is heated, held at an elevated temperature to accumulate enough lethality to render it commercially sterile and then cooled before final packaging. A simple aseptic product sterilizer is sketched in Fig. 25.1. Major components of a simple aseptic product sterilizer include a flow control device, heating heat exchanger, hold tube, temperature indicating device, temperature recording device, temperature controller, cooling heat exchanger, back-pressure device, and a flow diversion system. These parts are described in more detail below.

25.2.2 Components of the Aseptic System

25.2.2.1 Feed Tank

The feed tank or bulk tank is also called the product supply tank. This tank usually has a mixer to keep the product suspended while feeding the system. The outlet of this bulk tank is connected to a pump. The level sensor on the tank is used to control the level in the tank so that the pump does not run dry and cause unnecessary damage to the pump. Temperature in the feed tank can be controlled by having a jacketed

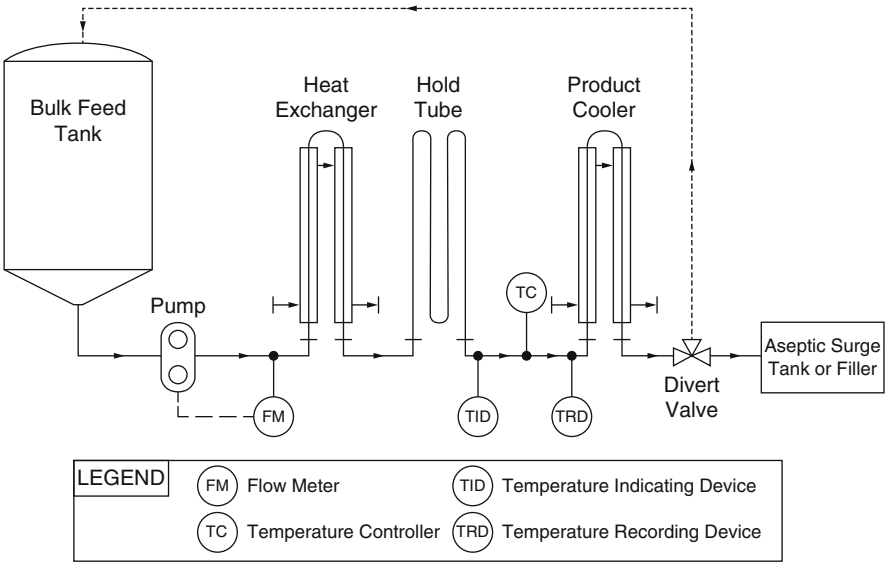

Fig. 25.1 Simple diagram of an aseptic product sterilizer

tank or a steam coil. Coolants (e.g. water or ethylene glycol) can be circulated to keep the product under refrigeration or steam can be used to keep the product at an elevated temperature. To avoid any incipient spoilage, for low acid food production, the feed tank should be kept above 130 °F or lower than 45 °F for extended runs and rinsed in between filling of different batches into the tank. For any other temperature of the feed tank, a time limit should be defined based on type of product and kind of microorganisms can grow in the product.

25.2.2.2 Positive Displacement Pumps

Food grade pumps for the aseptic system are used to push the product throughout the system. Because of the process design and lethality requirements in the hold tubes, positive displacement provides a means to provide a constant flow rate and hence a defined fluid velocity and residence time in the hold tube. Each stroke or revolution of the pump pushes a fixed amount of fluid regardless of other factors; thus, it positively displaces a fixed amount of fluid in the system. Different types of pumps are discussed below.

(i) *Piston (reciprocating) pump*: This is a positive displacement pump driven by a reciprocating action with a camshaft, drive belt, pistons and cylinders. Each piston draws a defined quantity of product into the cylinder and pushes it forward through the exit of the pump. Homogenizing valves are a type of piston pump and are most often used to break up the larger particles in the product such as fat globules in milk. The first stage homogenization is usually at 3.45×10^6 Pa and the second stage is up to 13.79×10^6 Pa. In some cases, homogenizing valves are mounted after the pre-cooler and referred to as remote homogenizing valves. Since, the product is sterile after the hold tube and in the pre-cooler, remote homogenizing vales must be equipped with sterile barriers to protect against any post-process contamination.

(ii) *Rotor or lobe pump*: These are rotary pumps that displace fluid positively throughout the system at a constant velocity. These pumps contain two rotors with two to three lobes each. The rotation of the pump creates a vacuum at the inlet of the rotors. As the rotors turn, they draw fluid into the cavity formed between the lobes and the pump housing. The clearance between the lobes and the pump housing is very low to allow for positive displacement of the fluid without leakage between the lobe and cavity wall. Lobe pumps are suitable for processing products containing larger particles as it maintains the particle integrity.

(iii) *Progressive cavity pump*: Progressive cavity pump provides a fixed flow rate. The rotor of the pump is made of steel and coated with a smooth, but hard surface. The body, or the stator, is made of molded elastomer located in the metal tube. The screw design of the rotor sits tightly with the inner housing of the pump. The rotation of the rotor forms pockets in which the product is carried forward. Due to its gentle handling, progressive cavity pumps maintain particle integrity of the food particles.

(iv) *Peristaltic (or tube hose) pump*: Peristaltic pumps are also called as tube hose pump as the main supply of product is through a flexible tube or hose. There is no direct contact of product and the pump housing. The design of the pump is such that the rotor with the rollers squeeze the tube that creates a vacuum that draws the product inside the tube. The pocket of product moves forward and when it reaches the outlet end of the pump, the tube return to the normal shape and discharge the product forward. These pumps are typically used for chemical dosing, clean-in-place (CIP) detergent and sanitizer dosing, and also for peroxide dosing for filler sterilization.

(v) *Centrifugal pump*: Centrifugal pumps are suitable for low-pressure application with high flow rates. These pumps are not positive displacement pumps and are generally not used as a metering pump in the aseptic system. If used as a metering pump, a centrifugal pump must be used in combination with a flow meter in order to control the flow rate of the product in the processing system and specifically the hold tube. However, due to its large volume displacement, centrifugal pumps are very effective CIP pumps. The impeller is attached to a shaft and the product is drawn into the center of the impeller. The impeller accelerates the fluid via centrifugal force along impeller vanes and to the outlet. Centrifugal pumps are also used as a stuffing pump for the main positive displacement pumps, as the positive displacement pumps can never be run dry or it will cause severe damage to the internal components of the pump. However, centrifugal pumps are not self-priming, and the suction line and pump casing must self-priming or be filled with fluid before operation.

25.2.2.3 Heating Heat Exchanger

There are two main categories of heat exchangers used in aseptic systems:

(i) *Indirect heat exchanger*: In indirect heat exchangers, the heating medium, typically hot water, never comes in direct contact with the product. On the contrary, the heating medium heats up a metal (stainless steel 316 L) interface between the heating medium and product. The metal in turn transfers heat to the product through the internal product contact surface. There are several designs of such heat exchangers, double tube, triple tube, coil-in-shell, swept surface and corrugated tube designs of better heat transfer rates.

(ii) *Direct heat exchanger*: The product in direct heat exchanger come in direct contact with the heating media, usually steam. The steam used for product heating must be of culinary quality. The steam line is fitted with a check valve to protect against product entry into the steam line in case of higher pressure on the product side. There are two basic designs of direct heat exchangers: steam injection and steam infusion systems. In direct steam injection system, the steam is introduced through a nozzle into the product pathway. In the steam infusion system, product is introduced as a spray of small droplets into a chamber filled with steam. In both cases the product heats up very rapidly quickly as opposed to the indirect heat transfer heat exchangers.

25.2.2.4 Hold Tube

The hold tube is where the product achieves the predefined lethality for a product. US FDA requires that the hold tube must be sloped upwards with ¼ inch per foot. Additional heat cannot be applied to the hold tube, but it can be insulated to protect the heat from ambient cooling. Flow rate and diameter and length of the hold tube dictate the minimum temperature at the end of the hold tube that is needed to achieve the target lethality.

For product sterilization, the thermal sterilization process is critical for controlling *Clostridium botulinum* (*C. botulinum*) and other spoilage organisms of non-health significance in low-acid food products (pH > 4.6 and water activity >0.85). Since pH will control growth of microorganisms in high-acid and acidified foods, the thermal process for these products is designed to control vegetative pathogens and heat resistant enzymes that degrade quality over the shelf life of the product.

25.2.2.5 Temperature Indicating Device

A temperature indicating device (TID) needs to be installed at the exit of the hold tube or wherever the temperature is a critical factor in the process. The TID should have its own independent sensor different from the recorder/controller sensor. An accurate temperature controller must be installed and be capable of ensuring that the desired product sterilization temperature is maintained. Though not explicitly mentioned in FDA regulations, the temperature recording device (TRD) should be independent of the temperature controller.

25.2.2.6 Cooling Heat Exchanger

For systems where direct heat exchangers are used, during cooling, the steam condenses and dilutes the product and this excess water must be taken out. Usually a vacuum flash chamber is used to cool the product and the evaporated water is removed from the product. The general approach taken in these systems to ensure that the water added during steam injection is fully removed, the product is cooled to the same temperature as when the steam was introduced into the product. For indirect heat exchanger systems, the cooling heat exchangers are similar to the heating heat exchangers and the only difference is that the media is switched to either glycol or cold water. Since the product is sterile while going through the coolers, there is a requirement of product pressure needs to be higher than that of the cooling media to prevent non-sterile cooling media from recontamination of the sterile product (U.S. Food and Drug Administration 2011). This differential pressure should be greater than 6894.76 Pa.

25.2.2.7 Divert Valve

A divert valve is used to direct product exiting the product sterilizer away from the aseptic tank and filler in the event that the upstream product is not sterile due to for example a temperature-drop in the hold tube. This device protects the aseptic tank and filler from contamination.

25.2.2.8 Sterile Barriers

The sterilization process of the aseptic systems provides commercial sterility of the equipment. However, at certain times during operation, movement at the sterile/non-sterile boundaries of aseptic zones is necessary (e.g., valve stems). The maintenance of sterility requires protection against any contamination of moving parts from the environment and hence needs an active barrier. There are different types of barriers such as active steam and sterile condensate barriers. Barriers must also be sterilized prior to production and sterility of the barrier must be monitored and controlled throughout the production. If the temperature of the steam barrier falls below the minimum specified, there should be a defined time limit below which the barrier loses its sterility. Steam barriers are used in locations such as aseptic tank product valve, agitator shaft on aseptic tank, valve cluster and homogenizer piston seals. The barrier must have a hygienic design and be suitable for cleaning.

25.2.2.9 Aseptic Surge Tank

Aseptic surge tanks are used to temporarily hold the product while filler is not running and filling the product in package. The sterility of the aseptic tank is maintained by keeping a positive sterile air or nitrogen pressure on top of the product. The over pressure can also be used to feed the product to the filler.

25.2.2.10 Air Filtration

Sterilizing air by filtration is used to maintain commercial sterility in critical areas of the aseptic system. Critical areas are those in which air contacts the sterile product or sterile packaging environment, which are therefore a potential risk for microbial post-process contamination. Failure to keep microbial contamination from entering these areas may lead to product insterility. The critical locations where sterile air is needed in the aseptic process include:

- Overpressure of aseptic product tanks
- Overpressure of the sterile zone of the aseptic packaging equipment
- Heating or drying of the packaging materials

- Head-space injection into packages
- Blowing preforms and transport of bottles

Cartridge filters (membrane and depth) and HEPA filters are the two main filter types commonly used for air sterilization in critical areas of aseptic processing systems. Cartridge filters are most commonly used for low-volume compressed air applications typically found on aseptic tanks and aseptic packaging machines. Though cartridge filters may be used on high volume blower air supplies, economic considerations typically limit their use in this application. Sterilizing grade cartridge filters will produce sterile effluent in the filtered air. HEPA air filters are typically used for high-volume low-pressure applications needed to maintain the integrity of the aseptic zone of an aseptic filler. HEPA air filters have an extremely high capture efficiency of 99.97% at submicron-sized (0.3 μm) particles in air, which provide air free of viable microorganisms in aseptic filling lines.

25.2.2.11 Aseptic Filler

The aseptic filler is the heart of an aseptic operation. In brief, packaging materials are sterilized by sterilizing method and then product is filled, and the packaging material is hermetically sealed before exiting the filler. The sterility of filler is maintained by positive sterilize air overpressure. The details of the sterilization of the aseptic fillers and packaging materials is provided later in this chapter.

25.3 Recent Advances in Aseptic Processing

Advances in novel processing systems, sensors, and modeling software are discussed in this section. Novel aseptic systems that have been developed for processing large particle foods in the absence or with only a minimal volume in the liquid phase is discussed. Technological advances in sensors give industry the tools needed to properly validate these complex processes are described below. These recent advances in aseptic processing are largely aimed either at controlling or measuring residence time of discrete particles, which has hampered advancement in this area in the past.

25.3.1 Multiphase Product Processing

Aseptic processing has been gaining popularity due to its superior quality as compared to the traditional thermal processing technologies (Nelson 2010). It has been widely adopted for the processing of homogeneous products such as low acid purees and beverages. However, the processing of multiphase products has not

enjoyed the commercial success as the homogeneous counterpart. The main obstacle was the validation of such a process for multiphase product. Research in the 90s through the government-industry-academia consortium (CAPPS) provided the necessary methodologies for validation of aseptic multiphase food production. The commercialization that was expected as an outcome of this extensive study did not happen, with the exception of the Campbell's Soup Company (Butschli 2005). Till date, there are only handful of processors that are either actively processing and producing such products or are still evaluating the technology. What remained as a hurdle is the lack of tools for conducting the validation of the process for a multiphase product. There are several key factors to consider while designing a process for multiphase product (Lechowich and Swartzel 1996; Kumar et al. 2007). The two most challenging areas are: residence time distribution (RTD) and microbiological validation.

With the recent advancements in sensing technology, residence time distribution can be determined using a non-intrusive magnetic signal (Simunovic et al. 2004; Simunovic et al. 2007; Swartzel and Simunovic 1999; Swartzel and Simunovic 2000). This technology relies on the signal produced by a particle embedded with magnet while traveling through the pipes. The signal strength can be transmitted through the stainless-steel pipes. However, the magnet can change the density of the particle being studied and would not provide the real residence time of the particles in the aseptic system. To overcome this challenge, a simulated particle can be used to study the RTD. The simulated particle can be manufactured with a plastic material such as polyetherimide and polypropylene. The density of the manufactured particle needs to be in the range of food particles and its thermal conductivity and specific heat should such that it heats slower than the real food particles. This manufactured particle is also referred to as the worst-case particle (Jasrotia et al. 2008). The general recommendation for performing the RTD is to capture a minimum 299 particles (Lechowich and Swartzel 1996), as it provides the 95% confidence level to capture the 1% of fastest moving particles. The experimental design of the RTD studies should also include the other factors that could impact the RTD. Some of the factors that should be considered are listed below;

- Processing temperature
- Density range of particles
- Flow rate of the system
- Viscosity of the product
- Particle size

Sensors for the RTD studies should be installed at multiple locations including end of pre-heater, heater, hold tube, pre-cooler and final cooler. The overall experimental design in combination with sensor locations will provide the robust data for the RTD.

Once the RTD of the system is defined, microbiological validation of the process is the next step. Multiphase simulation can be modeled using heat transfer module of COMSOL, Inc. (Burlington, MA, USA) based on the input needed to simulate the heat transfer in particles. A direct measurement of the process temperature at the

center (worst-case location) of the particle is not possible with the current sensors. Mathematical models are used to predict the lethality achieved by the worst-case particle. However, the model needs to be validated before it can be used to establish the thermal process. The input parameters to the model include the particle size, thermophysical properties, viscosity, fluid-to-particle heat transfer coefficient, and heat exchanger and hold tube dimensions.

The thermal process conditions established by the mathematical model needs experimental validation. Properly characterized inactivation kinetic parameters (D and z values) for spores of the surrogate organism *Clostridium sporogenes* PA 3679 are used for the microbiological validation. Experimental design for the microbiological validation should also consider factors as listed above in RTD design. The spores should be planted at the center of the manufactured particles. A successful trial should not produce a positive result for the 10^5 CFU/mL concentration of PA 3769 spores if the model prediction was 5 log reduction.

25.3.2 Advances in Heating Technology

Heating for the multiphase product is achieved by indirect heating heat exchangers. In traditional heat exchangers, double tube, coil-in-shell or scraped surface heat exchanger, the heating media most often is hot water. However, as the particle size increase the quality deterioration is higher for the carrier fluid due to conductive heating inside the particles. With the advances in industrial microwave (Coronel et al. 2008) and ohmic heating (Ito et al. 2014), the multiphase products can be heated rather quickly as compared to the traditional heat exchangers. Microwave heating provides a means to heat the larger particles (> 0.5 inches) volumetrically and do not rely on the conductive heating (Coronel et al. 2008).

25.3.3 Advances in Cooling Technology

Advancements in cooling heat exchangers for multiphase products are rather limited. The heating technology with microwave and ohmic heating does not have a counterpart in cooling. This limits the quality improvement that could be achieved for multiphase products. There is an opportunity for future research in cooling technology area (Stoforos 2017; Stoforos 2014).

25.3.4 Aseptic Dosing of Sensitive Ingredients

Sensitive compounds such as probiotics and flavors that cannot tolerate higher temperatures can be added after the thermal process step. However, this addition

needs to be done in the aseptic zone of the system usually after the cooling heat exchangers and prior to the aseptic tank storage and filling. The dosing system consists of in-line sterile filters (<0.3 microns) that can eliminate the microorganisms from the solution. Most important criteria in such systems is to maintain the aseptic integrity of the system, any contamination from the environment can cause food spoilage issues and potentially impact public health.

25.3.5 Risk-Based Process Design

It is a common practice in manufacturing to establish processes by including 'worst-case' margins of safety based on historical process data. However, this approach almost always results in a high sterilization value, which leads to an over-cooked product of lesser organoleptic and nutritional quality for the consumer. Thus, risk-based process design has been applied to setting the sterilization time for a low-acid product, to yield potentially improved sterilization value determination that could readily provide improvements in the organoleptic quality which benefit the consumer, as well as operational efficiencies and utility savings (Membré and Van Zuijlen 2011).

25.4 Sterilization of Aseptic Packaging Materials

In aseptic packaging systems, packaging material sterilization is very essential, because the sterile products, which are packed and sealed in sterile packages, are not subjected any longer to thermal treatment. Therefore, it is important that both food product and package must be sterile and then hermetically sealed with a sterilized closure under a sterile environment, called the aseptic zone. The aseptic zone starts at the stage where the pre-sterilized or the non-sterilized package is introduced into filling machine in order to sterilize the package. For pre-sterilized packages such as irradiated pouch and bag for bag-in-box style, only the outer surface needs to be sterilized while for non-sterilized packages, the inner and outer surfaces must be sterilized. The aseptic zone ends at the place at which the package is hermetically sealed before exiting the sealing station.

Packaging sterilization system including design and operation is the main element of aseptic machine. It is important to maintain the sterility of aseptic environment and to protect the packaging material from microbial recontamination during aseptic filling and sealing operations (Cerny 1992). Package designs and the types of packaging materials are also essential to achieve the effective sterilization for aseptic packaging. In addition, it is very essential that packaging materials must be able to withstand the sterilization conditions.

According to the National Food Processors Association (NFPA), the aseptic system for low-acid foods production must meet the criteria: (1) establishing data

to ensure that the equipment provides and maintains the product sterility; (2) the control and monitoring devices should located in proper place; (3) the level and residue of any chemical sterilants used in sterilization should be controlled properly; (4) the on-line monitoring can ensure the proper seals; and (5) on-site testing can help to validate the capability of the sterilization system (Ito and Stevenson 1984).

Since the packages are sterilized separately from food, this benefits to widen the option to use several different packaging materials and formats. Metal cans were used for the first aseptic system by James Dole Corporation (Robertson 2012). Paperboard carton and thermoplastics such as high-density polyethylene (HPDE), low-density polyethylene (LPDE), linear low-density polyethylene (LLDPE), polyethylene terephthalate (PET), high impact polystyrene (HIPS), polypropylenes (PP) and laminated aluminum foil packaging film are now being used for aseptic packaging. Even though carton and plastic are more difficult to thermally sterilize than metal can due to their low conductivity, these package types have become common packaging container for aseptic foods in current market. Glass containers are not used in practice for commercially produced aseptic low acid foods, though several sterilization methods have been developed for glass container (Hersom 1985).

A 12D process for *C. botulinum* has historically been recognized as a public health performance standard for low acid canned foods (Stumbo 1973). The decimal reduction time (D-value) is the time required to kill 90% of the microbial population (one log reduction). In setting the public health performance standard for aseptic packaging materials, the FDA concluded that the risk of failure of the container sterilization process to eliminate *C. botulinum* should the same as the risk for the aseptically processed food to be packaged into the container (Read Jr 1978). The evaluation was conducted for milk in response to a petition filed by Brik Pak, Inc. (FDA 1979). Spore load (general population) was assumed to be 1000 spores/mL and calculations were based upon a 1000 mL container. Thus, the spore load per container was taken as 10^6 spores/container. The ratio of *C. botulinum* spores to all spores in a general population was taken as 1:1 million, which resulted in the approximation that there would be one *C. botulinum* spore per container. The application of a 12D process would thus reduce the risk of the probability of a non-sterile unit (PNSU) due to *C. botulinum* to 10^{-12}. Similar estimates were made for the container. Spore load was taken as approximately 1 per container. Applying the same 1:1 million *C. botulinum* spore to spores ratio, the number of *C. botulinum* spores per container was calculated to be 10^{-6}. Therefore, if a 6D process was applied to the packaging material, the resultant 10^{-12} probability of a non-sterile unit (PNSU) due to *C. botulinum* contributed by the aseptic package would be the same as that of the product.

Given the resistance of *C. botulinum* to hydrogen peroxide, a minimum of four decimal reduction (4D) of an appropriate surrogate organism is required to demonstrate commercial sterility (Davidson and Weiss 2003). Surrogates commonly used to validate aseptic packaging equipment based on the sterilization method employed. In commercial sterilization validation of packaging material with a surrogate organism, a decimal reduction by heat treatment is approximately 3–6 D depending on the

type of heat sterilization. A reduction of 5–6 D is achieved by chemical and gamma irradiation treatments while UV-light and Infrared irradiation can attain a reduction of 2–3 D (Reuter 1993).

Generally, to select the sterilization method in commercial use, the cost consideration and regulation requirements are key elements (Fox 2012). Design of the package and the type of packaging materials can also limit the type of sterilants and the sterilization process. Moreover, the sterilization agents must be consistently effective and controllable. Sterilization of packaging material can be achieved by heat, radiation, and chemical independently or in combination of these methods.

25.4.1 Heat

Heat sterilization is to use the thermal treatment to inactivate microorganisms and estimate inactivation parameters (Dolan and Mishra 2013; Dolan et al. 2013). The contact time and temperature depend on the resistance of the target microorganisms. The sterilization by heat can be used as either moist heat (saturated steam) or dry heat. The heat method is suitable only for heat resistant packaging materials, but is often employed to sterilize machine components such as pipes and valves in aseptic zone.

25.4.1.1 Saturated Steam

Saturated steam is the moist heat method, which is created by heating the water to the boiling point under the pressure (without the present of air or any gases). It is the most reliable sterilization process. This steam treatment requires a pressurized chamber due to the spore inactivation temperature requirement. In early 1920s, saturated steam was used as sterilization method for metal cans and lids in the United States (Reuter 1993). It can also be used to sterilize aseptic packaging materials for low-acid foods.

This sterilization method is currently used to sterilize HIPS cups, which is a form of polystyrene (PS) and lidding film in the Oystar Hassia aseptic form/fill/seal machine for aseptic products such as coffee creamer (David et al. 2012). After the sheet of packaging material (web) is fed into the aseptic filler, the chamber de-aeration valves are opened, and the steam pressure is built up inside the closed chamber. The web is subjected to the saturated steam at 150–165 °C before the plastic sheet is molded to form the container shape at thermoforming station. After forming, the sterile packaging containers are moved into the filling station to be filled with the food. In the meanwhile, lid material is also similarly sterilized by steam prior to feeding into the sealing station in aseptic zone to seal the containers. The sterilization time depends on the temperature and log cycle reduction requirements.

Moist heat is more effective sterilization method compared to dry heat at the same temperature. Nevertheless, it is not a suitable sterilant for paper-based packaging

material since it can cause the delamination, blistering, and impair the heat-sealing layer of the laminated plastic film on the paperboard (Toledo 1988; Ansari and Datta 2003). Saturated steam might also not be suitable for any thermoplastics, which cannot withstand the high-pressure steam temperature. Another drawback of this method is that the condensation from steam process that might remain inside the container, resulting in the dilution of the product (Robertson 2012).

25.4.1.2 Hot Water

Hot acidified (citric acid) water is used as a sterilizing agent of aseptic packaging for high acid food (pH < 4.6) (David et al. 2012). This sterilization method was used in CrossCheck aseptic packaging machine, which was manufactured by Mead Packaging and Rampart Packaging companies. It was successful to produce single-serve aseptic applesauce in the preformed plastic cups with laminated foil lid by Seneca Foods (Fox 2012; Sam Saguy and Graf 1990). The packaging containers are submerged through a bath of hot citric acid water at 82 °C and then dried with the sterile nitrogen during an inverted position before moving to the filling station. Kodera (1983) reported that the combination of hot water and UV can achieve a 6-log reduction of *Bacillus subtilis*.

25.4.1.3 Superheated Steam

When water is heated above its boiling temperature point, it is turned into dry steam, which is also called superheated steam. In 1950, superheated steam was used as the sterilization method for the metal cans and lids in the Martin-Dole aseptic canning process. This continuous sterilization method initially starts by applying the superheated steam at atmospheric temperature approximately 220–226 °C for 45 seconds in order to sterilize external and internal can surfaces. The process takes about 35–45 seconds (Davies 1975; Larousse and Brown 1996).

25.4.1.4 Hot Air

Hot air or dry heat is also used as a sterilant for the aseptic packaging. It is preferred over superheated steam for sterilization of laminated paperboard carton. The initial temperature of 315 °C is applied in order to reach the surface temperature of packaging material at 145 °C for 3 minutes (Reuter 1993; Toledo 1988). Since the thermal transfer rate is slow in dry hot air, it requires more time to achieve equivalent microbial inactivation as compared to moist heat. For example, at the same level of sporicidal effectiveness, moist heat sterilization requires 121 °C for 20 minutes whereas dry heat sterilization requires 170 °C for 60 minutes (Buchner 1993; Massey 2005; Robertson 2006). Due to the use of the high temperature, the most polymeric packaging materials do not have the capability to withstand, causing the

change of their properties. Therefore, the dry hot air is only suitable for heat resistant packaging materials and acidic product (pH < 4.6) such as juice.

25.4.1.5 Heat of Extrusion Process

During package formation, heat co-extrusion process of granulated plastic approximately 180–230 °C can be used as sterilization method to sterilize the surface of packaging material (Robertson 2012). Hassia Oystar Erca form-fill-seal filler uses this method to sterilize the roll-fed polypropylenes before forming the cups and lidding material. Due to the non-uniformity of heat distribution of extrusion, the packaging containers from this treatment should be used for acidic food (pH < 4.6). For low acid food (pH > 4.6), additional treatment with hydrogen peroxide or peracetic acid should be used to sterilize the food contact surface.

25.4.2 Radiation

25.4.2.1 Irradiation

Ionizing radiation is a non-thermal process, which is commonly used to sterilize foods, medicals, pharmaceuticals and packages by exposing the decided absorbed dose to the products for a limited time (Murano 1995). FDA approved three ionizing radiation sources to use for pasteurization and sterilization: gamma (γ) ray (Cobalt-60, and Cesium-137), electron beam (E-Beam) and X-ray (Komolprasert 2007). In aseptic packaging, gamma rays and e-beams are commonly used in commercial scale.

Irradiation treatment is not complicated and does not leave any residue. Hence, it is an alternative sterilization method for the packaging materials that cannot withstand with heat and chemical sterilization processes and/or has intricate shape, which is difficult to sterilize such as fitment of aseptic bag-in-box pouch and sprouted stand-up pouch for juices. It can treat surface sterilization of packaging materials including web, prefabricated packages (bottles and pouches), caps and bulk packaging container. For prefabricated packages, the empty clean containers are formed and sealed in the clean environment before treating with ionizing irradiation. The irradiation dose of 25–30 kilograys (kGy) is the most effective and commercial use to ensure the sterility.

Cost consideration and the irradiation effect on the polymeric packaging materials causing the changes of material properties and discoloration are the primary concerns for this method. For the use of irradiation sterilization with biomaterials, both gamma and E-beam does not affect barrier, physical, and biodegradation properties of cellophane and polylactic acid (PLA) films (Benyathiar et al. 2016; Benyathiar et al. 2015). As corporations grapple to improve sustainability by for example decreasing energy and water use, Tetra Pak commercialized E-Beam as a

technique to sterilize aseptic packaging materials (TetraPak 2019). E-Beam affords substantially greater throughput than existing cartoning machines utilizing hydrogen peroxide, reduced operation costs, and consumes less water and energy. Packaging materials and all adjuvants used in irradiation for foods must meet all specifications and limitations of the applicable FDA regulations and must be authorized by FDA in order to be marketed in the U.S. for food contact (Komolprasert 2007; Paquette 2004).

25.4.2.2 Emerging Radiation Technologies

25.4.2.2.1 UV-C Light

Ultraviolet (UV) light is a region of the electromagnetic spectrum between visible light and X-rays in the wavelength of 10–400 nanometers (nm). At the wavelength of 200–315 nm, the UV rays have the microbial destruction effect. However, the most microbicidal effect by destroying the nucleic acids (DNA) of microorganism cells is between 248 and 280 nm, also known as UV-C range. The UV-C light at a wavelength of 254 nm which is obtained by a low- pressure mercury vapor lamp has a germicidal effect to inactivate bacteria, yeast, molds, viruses and protozoa (Robertson 2012; Meulemans 1987). Vegetative microorganisms are sensitive to UV radiation whereas spores of molds (e.g., *Aspergillus niger)* and bacteria (e.g., *Bacillus* species) tend to be resistant to the action of UV light (Setlow 2001; Begum et al. 2009). It has been studied that UV-C radiation (254 nm) can inactivate the vegetative forms of *Bacillus subtilis*, *Enterococcus faecalis*, *Candida albicans* and *E. coli*. Nonetheless, UV radiation of a 222 nm generated by KrCl-excimer lamp can inactivate *Bacillus subtilis* spores much better than a 254 nm (Claus 2006; Claus et al. 2003; Munakata et al. 1986; Warriner et al. 2000). The total UV dose and the initial microbial load also affect the microbial log reduction.

In aseptic packaging, the high intensity UV radiation is used for surface sterilization of packaging materials (Bachmann 1975; Maunder 1977). There is, however, uncertainty in the use of UV alone due to its effectiveness depending upon the distance between the light resource and material surface properties. This treatment is also sensitive to shadow effects from dust particles on packaging surface and packaging geometry due to its poor penetration (Cerny 1992; Joyce 1993). The use in conjunction of UV with low concentration hydrogen peroxide solution provides the improvement in sporicidal activity (Hersom 1985).

25.4.2.2.2 Pulsed Light

Pulsed light technology is an alternative method for aseptic package sterilization in order to minimize the use of chemical sterilants. This technique has been discovered and studied to eliminate microorganisms by Japanese scientists since 1970. In 1990, the use of this technology to sterilize aseptic packaging material was developed

(Dunn et al. 1991). Pulsed light is a short duration of the strong electrical energy generated in a capacitor with intensity approximately 20,000 times greater than the sunlight on the earth's surface. The emitted white light pulse has a broad spectrum from the UV to the infrared (200–1100 nm). The duration of pulses ranges from 1 μs to 0.1 s. and the flashes of light are usually between 1 and 20 pulses per second for food processing application (Dunn 1996) and an energy density in the range of 0.01–50 J/cm^2 (Condon et al. 2014). In comparison to heat and chemical treatments, pulsed light sterilization is a faster process and leaves no residues on food surface material. More details for this technology can be found in another chapter of this book.

Similar to other light treatments, the insufficient light exposure and shadowing from packaging geometry can limit the effectiveness. Even though pulsed light cannot penetrate the opaque materials, it can be transmitted through several packaging materials including LLDPE, LDPE, HDPE, PP and nylon. Shadowing effect, which is caused by material surface roughness, can also limit the effectiveness of this light treatment. Surface roughness of packaging material, which is greater than the micrometer range such as polyethylene-coated aluminum foil paperboard laminate, can also prevent the light treatment comparing to materials which have less surface roughness such as LDPE and HPDE (Ringus and Moraru 2013; Chen et al. 2015).

25.4.2.2.3 Infrared Heating

Infrared (IR) ray in the wavelength (λ) 0.8–15 $\times$ 10^{-6} can be used to sterilize the packaging surface such as laminated aluminum lidding films (Ansari and Datta 2003). When the material surface contact with the IR resource, the radiation energy is converted into heat resulting in raising temperature on material surface. Like UV, this treatment is suitable for the smooth surface material without shadow effect from any dust particles and packaging geometries.

25.4.2.2.4 Cold Plasma

Cold plasma or nonthermal plasma (NTP) is another non-thermal method that has gained attention as an alternative sterilization method for microbial decontamination of food packaging materials. It is electrically energized matter which can be generated by a variety of species such as gas molecule in fundamental or excited states, both positive and negative ions, free radicals, electrons and photons at room temperature of 30–60 °C (Misra et al. 2011; Ratner et al. 1990). Cold plasma can be generated by several devices such as dielectric barrier discharge (DBD), resistive barrier discharge (RBD), corona discharge, glow discharge, radio frequency discharge (RFD) and atmospheric pressure plasma jet (APPJ). DBD and APPJ are the most widely studied forms of plasma system (Ehlbeck et al. 2010). It was found that DBD plasma was effective in decreasing the bacterial food pathogens on different packaging materials (Puligundla et al. 2016).

Cold plasma sterilization can be used to sterilize packaging materials including plastic cups/bottles and lidding films without leaving any residues. Literature studies showed that cascaded dielectric barrier discharge (CDBD) with air can inactivate several microorganisms including *Aspergillus niger*, *Bacillus atrophaeus*, *Bacillus pumilus*, *C. botulinum* type A, *Clostridium sporogenes*, *Deinococcus radiodurans*, *Escherichia coli (E.coli)*, *Staphylococcus aureus*, *Salmonella mons* on PET foils (Muranyi et al. 2007). Low-pressure microwave plasma sterilization with below 5 second can reduce 10^5 CFU of *Bacillus atrophaeus* and 10^4 CFU of *Aspergillus niger* on PET bottles (Deilmann et al. 2008). More details for this technology can be found in another chapter of this book.

25.4.3 Chemical

25.4.3.1 Hydrogen Peroxide

In 1981, the publication of a final rule 21 CFR 178.1005 permitted the use of hydrogen peroxide (H_2O_2) sterilization of packaging material specifically polyethylene food contact surfaces (FDA 1981). FDA also set a maximum residual hydrogen peroxide level of 0.5 ppm in the food at the time of packaging (Davis and Dignan 1983; FDA 2013). This level was established on the basis that within 24 h of packaging the food product, the levels will fall below 0.5 ppm. Since these amendments to FDA regulations, hydrogen peroxide has been the primary disinfectant used to sterilize packaging materials.

Three commercial methods using H_2O_2 sterilization of packaging materials are dipping, spraying and rinsing process. According to FDA regulation, after the treatment, H_2O_2 solution much be removed from the surface of packaging material which can be done by hot air, steam or infrared radiation.

(i) *Dipping (bathing):* The bath method is suitable for the web fed packaging materials. Roll stock of the packaging material is continuously fed through a bath of 30–35% H_2O_2 solution then mechanically squeezed by the squeeze roll and/or blown with sterile hot air in order to remove the remaining H_2O_2 solution on the materials before forming container shape, filling the food product and sealing the packaging containers.

(ii) *Spraying:* Vaporized 35% H_2O_2 with combination of hot air is commonly used instead of the liquid droplets. H_2O_2 vapor is sprayed from the nozzles onto the prefabricated packaging materials and then removed from the container by hot air before filling the food product. The treatment time, drying time and temperature depend on the container volume and machine speed.

(iii) *Rinsing:* This process is used to sterilize the several types of packaging container such as glass and blow molded plastic. It is also good method to sterilize prefabricated container with an intricate shape.

The effectiveness of H_2O_2 sterilization related to microbial inactivation depends on the concentration of H_2O_2 solution, temperature of H_2O_2 solution, exposure time and microbial load on packaging surface (Cerny 1992). H_2O_2 can be combined with other sterilization agents such as UV and heat to improve the effectiveness of microbial degradation (described in more detail in Sect. 25.6 of this chapter).

H_2O_2 can be used with several types of packaging materials. However, there is a concern when it is used with PET containers due to the absorption into polymeric material. Lately, Sidel aseptic filler for PET bottle has come up with the design to use H_2O_2 sterilization on PET preforms and then use the heat from blow molding process to evaporate the H_2O_2 from the preforms before blowing PET bottle. This technique is called "dry preform decontamination". Another consideration in the use of H_2O_2, it is a fire hazard and can cause skin damage and irritation of eye and respiration. It is very important to have an airtight system, training for chemical handling and supply logistics, and H_2O_2 detectors to provide a safe work environment.

25.4.3.2 Peracetic Acid

Peracetic acid or peroxyacetic acid (PAA) is another approved chemical by FDA to use as a sterilizing agent for aseptic packaging in both liquid and vapor forms (FDA 2018). PAA is highly effective to microorganisms at low concentrations. Thus, concentrations of PAA solution and contact time are the key parameters. In 2000's, the use of PAA gained favor as a means to sterilize PET bottles for aseptic products. Blakistone et al. (1999) determined that *Bacillus cereus*, another pathogenic spore former, was more resistant to PAA than *C. botulinum* and thus, should be consider as a pertinent pathogen for aseptic packaging systems using PAA as machine or packaging sterilant.

PAA can be used at low temperature (40 °C), which is an advantage over H_2O_2 resulting in 5 times shorter sterilization time. However, PAA can cause corrosion and damage to the equipment if high concentration is used. PAA can also cause skin damage and irrigation of eye and respiratory issues (Joslyn 2001). Airtight system is very important for the use of PAA and employees should have PAA leak detectors to check the safe working environment. A vinegar like off flavor can develop in some food products from the presence of small amounts of PAA. Because H_2O_2 is a primary component of PAA, residual limits imposed for H_2O_2 are followed for PAA as well. However, since current petitions to FDA require a water rinse after PAA treatment, H_2O_2 residuals are rarely a concern.

The efficacy of sterilizing agents is validated using surrogate organisms. These biological indicators are given in Table 25.1.

Table 25.1 Biological indicator to assess the reliability of sterilization for aseptic packaging systems (IFTPS 2011)

Sterilization agent	Target microorganism
Superheated steam	*Geobacillus stearothermophilus*[a], *Bacillus polymyxa, Clostridium sporogenes*
Dry heat	*Geobacillus stearothermophilus*[a], *Bacillus polymyxa*
Wet heat	*Geobacillus stearothermophilus*[a], *Clostridium sporogenes*
H_2O_2 + Heat	*Bacillus atrophaeus*[b], *Bacillus licheniformis, Geobacillus stearothermophilus*[a]
H_2O_2 + UV	*Bacillus atrophaeus*[b]
Heat of extrusion	*Geobacillus stearothermophilus*[a]
Gamma or E-beam irradiation	*Bacillus pumilus*
PAA	*Bacillus atrophaeus*[b]

[a]Previously named *Bacillus stearothermophilus*
[b]Previously named *Bacillus subtilis, Bacillus subtilis var. niger or Bacillus globigii*

25.5 Aseptic Packaging Systems

There are several types of commercial aseptic packaging equipment that uses specific packaging materials including fill/seal, form/fill/seal and thermoform/fill/seal machines. In this chapter only paperboard carton and plastic materials are discussed due to the current commercial use for aseptic products. Four categories of aseptic packaging systems can be classified based on material types and the method of forming the packaging container.

25.5.1 Paperboard Carton Systems

Carton containers for shelf-stable foods are commonly made of layers of different materials including (outer) polyethylene, paperboard, aluminum and (inner) polyethylene in order to improve barrier properties to carton paper. Recently, renewable materials such as sugarcane have found their way into cartons to replace oil-based polymer layer. Carton-style packages are produced by two different methods.

25.5.1.1 Fill-Seal-Prefabricated Carton

Preformed brick cartons, or sleeves, are manufactured from packaging factory by being die cut, creased, completed sealed longitudinally and distributed in the flat form. The fill-seal aseptic filler is used to process aseptic food products in this carton type. When packages are fed into filler, the sleeves or lay-flat-form of the cartons are

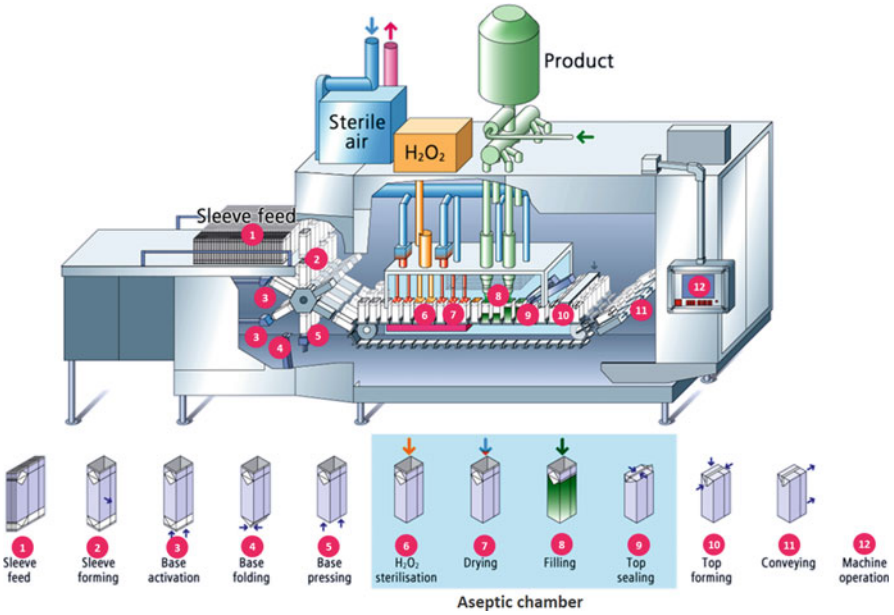

Fig. 25.2 SIG Combibloc aseptic fill-seal machine for prefabricated carton. (Courtesy of SIG Combibloc Group Ltd.)

shaped and sealed at bottom just prior to filling step (Fig. 25.2). Both outside and inside carton surfaces are sterilized by the combination of 35% solution of vapor H_2O_2 and hot air.

25.5.1.2 Form-Fill-Seal Carton

In these systems, the paperboard carton enters the aseptic form-fill-seal machine in the form of roll stock (web). The web paperboard carton is fed into aseptic machine and is sterilized by a H_2O_2 bath (30–35% concentration) and then formed to the box by longitudinal seal after hot-air is used to remove H_2O_2 from the material surface before filling steps as shown in Fig. 25.3.

25.5.2 Bottle Systems

HDPE, PP and PET are common thermoplastic materials used in aseptic beverage. Plastic bottles can be aseptically processed in two different ways:

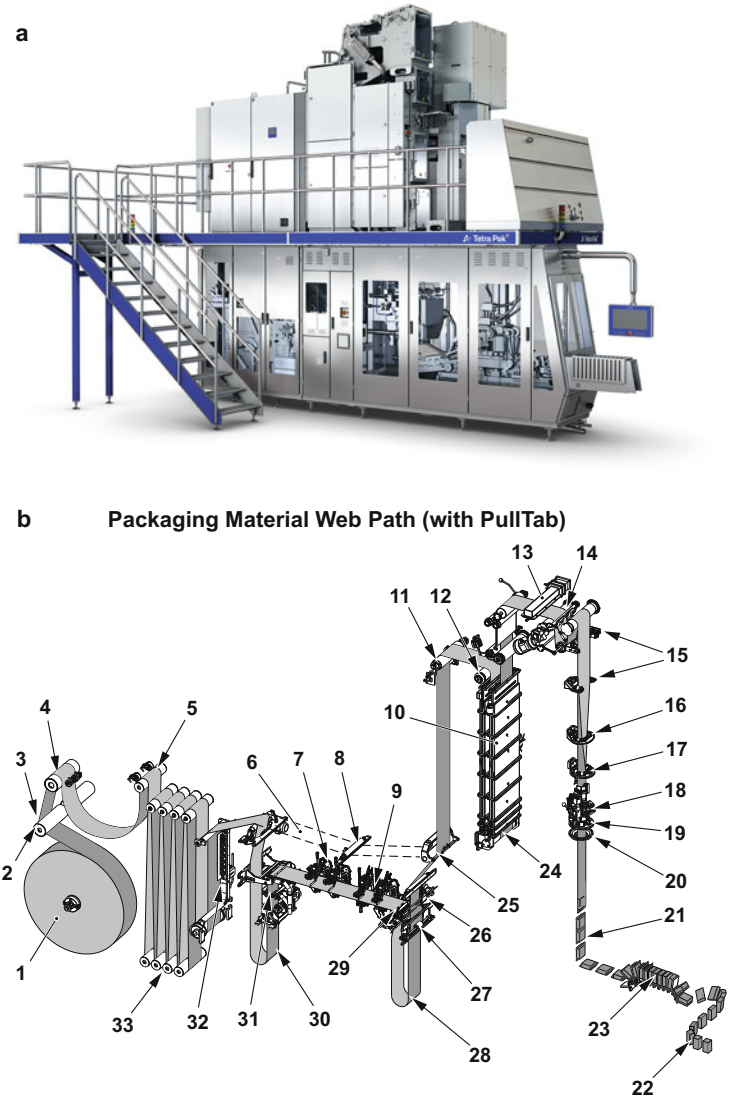

Fig. 25.3 (**a**) Tetra Pak A3 Speed Aseptic form-fill-seal carton filler and (**b**) packaging material pathway through the aseptic filler. (Courtesy of Tetra Pak)

25.5.2.1 Fill-Seal Preformed Bottles

HDPE and PET can be pre-formed as a ready to use containers. Fill-seal aseptic filler is used to process. Since these containers are made by packaging factory and shipped to the food processor, there is a chance of microbial contamination on food and packaging contact surface. Thus, these non-sterile bottles must be sterilized both inside and outside surfaces (outside surface in aseptic zone) when they are fed into the filler. PAA and vapor H_2O_2 are used as sterilization agent depending on the filler manufacturers. In this aseptic system, packaging geometry, amount and uniformity of H_2O_2 vapor delivered from each nozzle and the flow distribution through the bottle interior are very important factors to be considered. Bosch, JBT (Stork), Serac, Shibuya manufacture aseptic packaging machines utilize preformed bottles sterilization method.

25.5.2.2 Blow Mold-Fill-Seal Bottles

Recently, Sidel commercialized an aseptic packaging machine with blow molding process of PET preforms. A dry decontamination sterilization technique is applied to sterilize PET preforms before transferring to the blowing station with lower amount of H_2O_2 vapor. This results in much lower consumption of H_2O_2 per bottle as compared with preformed bottle systems. Heat from the oven of blow molding process provides the opportunity to remove H_2O_2 residue from material before forming the container. This system is a continuous process for which is more complicated to maintain the aseptic zone than comparable other aseptic filling machines.

25.5.3 Cup Systems

In aseptic food, PP and HIPS are typical plastic packaging materials to be used in the cup form. They can be processed in either preformed or thermoform-fill-seal systems.

25.5.3.1 Fill-Seal Preformed Cup

Similar to the aseptic fill-seal preformed bottle, the plastic cups, which are already formed as a container, are sprayed with approximately 35% H_2O_2 solution for both outer and inner surfaces and then applied with sterile hot air to remove the H_2O_2 solution prior to filling and sealing processes. The lidding material is also used the same sterilization method. Several commercial aseptic filling equipment for the preformed cup including Ampack Ammann (German), Benco (Italian) and Metal

Fig. 25.4 Ampack FCL aseptic fill-seal machine for preformed cup. (Courtesy of Robert Bosch Packaging Technology GmbH)

Box (the United Kingdom) are used to produce pudding, dairy products and fruit conserve with fruit pieces and layered yogurt (Szemplenski 2013). A schematic diagram of aseptic fill-seal machine for preformed cup is shown in Fig. 25.4.

25.5.3.2 Thermoform-Fill-Seal Cup

In this system, roll stock of HIPS is fed into the aseptic filler to thermoform the container shape and then filled with sterile product before sealing with sterile lidding film. Bosch and OYSTAR Hassia aseptic fillers use saturated steam technique to sterilize packaging materials (Fig. 25.5). Shelf-stable coffee creamer and cold brew coffee in cup style container are processed from this system.

25.5.4 Pouch Systems

Pouch systems historically were offered in bulk sizes for bag-in-box, drums and totes.

25.5.4.1 Fill-Seal Preformed Pouch: Single Serve Packaging

More recently, single-serve pouch sizes have gained favor. Preformed pouch is typically made of multilayer or aluminum laminated films. They are completely

Fig. 25.5 Bosch TFA 4830 aseptic thermoform-fill-seal cup machine. (Courtesy of Robert Bosch Packaging Technology GmbH)

sealed (all sides) under clean environment to minimize the microbial contamination and then pre-sterilized by irradiation process before shipping to food processor. This flexible pouch is generally packed as a roll stock or individual pouch on the rails. Scholle has developed the aseptic stand-up pouch with a spout, plug, and cap combination and filler for single served juice and beverage as illustrated in Fig. 25.6. Gamma irradiation is used to sterilize the pouches prior to shipping to producers. The sterilization of the pouches and spout in the machine is accomplished by the use vapor H_2O_2.

Ecolean aseptic filler also produces single served stand-up pouch. Flexible pouches are packed as a roll stock and pre-sterilized with E-beam irradiation. When fed into the aseptic filling machine, this roll stock is again treated with H_2O_2 vapor on outer surface before cutting off the top part of the pouch for filling process. Pouches are heat-sealed again before leaving the aseptic zone.

25.5.4.2 Fill-Seal Preformed Pouch: Bulk Packaging

For aseptic bag-in-box systems (Fig. 25.7), the preformed pouch, which is available in different gallon sizes with fitment attached on the pouch to provide the convenience, is normally pre-sterilized by irradiation. There are different fitment styles depending on the application. In these systems, Scholle, Rapak, Dupont, Liquibox, ELPO to name a few, the spout is sterilized by steam or H_2O_2 before the product is filled into the pouch and resealed.

The filling operation depends on the fitment styles. If the fitment is attached with a cap, the cap is removed after sterilizing and recapped after filling. For the spout with double membrane, the filling nozzle punches through the outer membrane to fill the product inside the bag and then inner membrane is heat sealed by the machine.

Fig. 25.6 Scholle aseptic fill-seal machine and preformed pouch. (Courtesy of Scholle IPN)

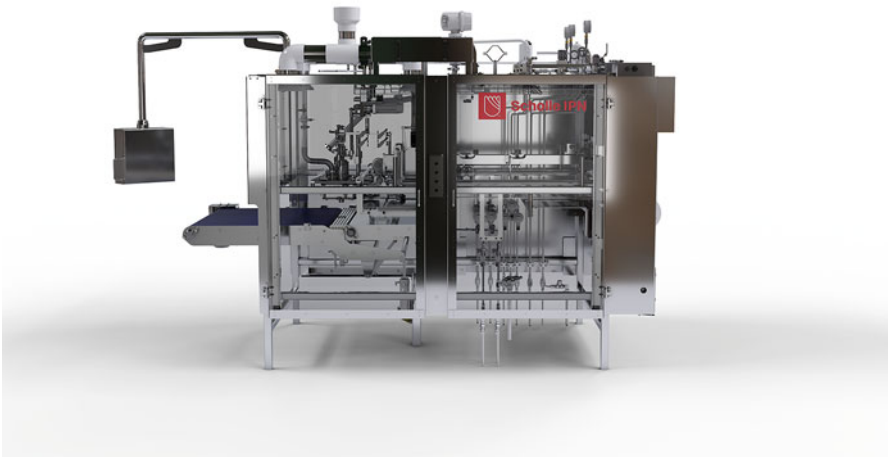

Fig. 25.7 Aseptic bag-in-box filling machine. (Courtesy of Scholle IPN)

Fig. 25.8 Fresco aseptic form-fill-seal pouch. (Courtesy of Fres-co Inc.)

25.5.4.3 Form-Fill-Seal Pouch

Several fillers, Fres-co, Cryovac and Hassia, use web-fed poly-laminate roll stock to form, fill and seal aseptic pouches. Similar to web-fed carton machines, roll-stock typically moves through a heated H_2O_2 bath to sterilize inner and outer surfaces of the pouch and then is dried with sterile air before it is formed filled and sealed in an aseptic zone (Fig. 25.8).

25.6 Conclusions and Future Trends in Aseptic Processing

Quality of food products processed by aseptic processing tends to produce higher quality, shelf stable products than traditional canning processing. Because aseptic products can be packaging in a wide variety of package types and sizes it offers great flexibility to processors and wide appeal to consumers. As the demand for clean label food increases, aseptic processing is poised to continue its rapid growth in different

categories of food products. Though industry has been slow to apply aseptic processing to multiphase foods, consumer demand for minimally processed, nutritious foods of high quality will lead to many more multiphase and particle foods being processed aseptically in the future.

Big data analytics continues to transform several industries and its application in aseptic processing could be of tremendous advantage. One of the main applications will be in the preventive maintenance area, which is critical to aseptic manufacturing facilities. Another application would be in quality enhancements by providing continuous input to the PLC (Programmable Logic Control) based on product type.

Advancements in sensor technology will enhance the overall impact of aseptic processing. Current methodology of fouling detection in aseptic systems relies on the increasing demand on the steam supply to heat the product. Future innovations in sensor technology will enable processors for early detection of fouling. This can also be useful in determining how long the production can run before it needs to be shut down for cleaning. Fouling sensor will also help to facilitate better clean in place (CIP). Early detection of fouling can extend the run-time of the system.

Innovation in dynamic (temperature-dependent) sensor technology and online application of quality sensors will improve the quality of aseptically processed food products. PLC systems integration with the sensor technology will provide an opportunity for metadata analysis across entire product portfolio of a processor and will automate the process of quality enhancements based on the lethality requirements.

Sustainability in aseptic processing will be critical for future production of food products. Improving the CIP in combination with the sensor technology has the potential to reduce water and chemical consumption in aseptic processing facilities. Novel innovations such as plasma activated water (Kamgang-Youbi et al. 2009) and micro bubble incorporated water (Dayarathne et al. 2017) can reduce the overall water consumption for the production facility. Innovation in biodegradable packaging material would also contribute towards the sustainability goals (Benyathiar et al. 2016).

Future research should not only focus on the fundamentals of a problem, but also provide practical solutions and tools for successful commercial application. The commercial success is only possible when suitable technology is developed, and training tools are provided to the food processors.

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Chapter 26
Modified Atmosphere Packaging

Kenneth W. McMillin

26.1 Introduction

Traditionally, food packaging, as with most product packaging, has several functions, including containment of the product, protection of the product against contamination and deteriorative effects, communication of information about the product, and if a package for consumers, serving as a marketing tool while providing ease of use and convenience (Yam et al. 2005). It is estimated that 40% of the food produced in the U.S. is never consumed due to damage or spoilage (Gunders et al. 2017). Packaging has helped to reduce food waste by reducing losses and damage during distribution, enabling efficiencies in distribution and storage, improving convenience to consumers, and promoting the use of retail-ready packaging (Verghese et al. 2015). There are many materials available for packaging of food. Wood, fiber, and pottery are used for specialty products while most food products are enclosed in glass, metal, or plastic materials, with the properties and low costs of plastic making them ideal for food packaging (Jenkins and Harrington 1991). Primary packaging should be considered a four-component system comprising the food, the internal environment, the package materials, and the external environment (Buonocore and Iannace 2013). With shelf stable food, packaging may be the major barrier to prevent contamination while with perishable food, other conditions such as temperature control, exposure to light, or food processing technologies are used in conjunction with packaging to maintain desired shelf life and safety of the products. More information about conventional food packaging can be found in another chapter of this book.

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Changes in atmospheres for perishable foods were investigated in the 1930s, scientific studies expanded on horticultural crops in the 1940s and 1950s, but commercial use became widespread in the 1960s and 1970s (Phillips 1996). Therefore, modified atmosphere is the intentional alteration of the gaseous atmosphere during the initial packaging of the product while controlled environments change and then maintain the desired atmosphere, temperature, humidity, and other factors (Zhang et al. 2015). Controlled atmosphere systems are most often used for bulk products and rarely for individually packaged food items unless the package materials have selectivity for oxygen, carbon dioxide, and/or ethylene gases or water vapor. Controlled atmospheres and environments are also employed for horticultural products to control respiration, ripening, and spoilage through oxygen, carbon dioxide, and ethylene regulation systems (Farber et al. 2003). The restriction of gas exchange by barrier packaging is the basis for modification of atmospheres by packaging. In some cases, it is desirable to have microporous membrane packaging to achieve the desired gas partial pressures within the package or to use microperforations in the packaging material for controlling the exchange of gases between the package and exterior (Beaudry 2000; Lange 2002). Modified atmosphere packaging (MAP) is defined as the initial altering of the gaseous environment surrounding the food. This initial gaseous atmosphere will change as a perishable product respires or has other metabolic changes due to inherent and extrinsic influences on the packaged food. Multiple sources provide background information on MAP and food (Blakistone 1999b; Brody 1989a; Church 1994; Inns 1987; Ooraikul and Stiles 1991; Robertson 1993; Smith et al. 1990; Wolfe 1980). This chapter will focus on the fundamental concepts of MAP systems for food and the safety aspects of the many different foods that might be in MAP.

26.2 Modified Atmosphere Packaging (MAP)

The two forms of modified packaging are vacuum and insertion of various gases to delay spoilage and provide safety of the packaged food (Church and Parsons 1995). Vacuum packaging (VP) is considered as a form of MAP since the initial gaseous composition in the package surrounding the food is removed. Air is usually the gas around the food and the extent of its removal depends upon the level and duration of gas flushing and/or vacuum application before the package is sealed. During storage and/or display of packaged food, the atmosphere can change due to chemical and microbiological changes (Dobrucka et al. 2015) as well as enzymatic reactions (Nagarajaroa 2016) of the packed product. The residual gas left in the package, the permeability or transmission rate of vapors through the packaging material, and the temperature, light, and handling environment surrounding the package may affect either the food metabolism, the package barrier stability, or both. Even though respiring foods in VP may continue to consume the residual oxygen and produce carbon dioxide to create a gaseous

modified packaging (Brody 1989b), the effects of this gas are usually minimal compared with intentional insertion of gas mixtures into the package.

In gas exchange or gas flush packaging, air is removed from the package either by vacuum to remove the initial gas and then backflushing with the desired gas mixture or by continuous flushing with the desired gas mixture until the desired amount of air removal and gas insertion is achieved. With either method, the atmosphere within the modified package environment can change depending upon the same factors as for vacuum packaging as well as with any interactions of the food with the specific gas type(s), the amount of the gas in the package, and the partial pressure exerted by the gas. While VP creates an anaerobic environment if sufficient air is removed, the environment of gas modified atmosphere packaging may be aerobic if oxygen is a part of the gas mixture or anaerobic if oxygen removal is sufficient enough to create an anoxic environment. Regardless of the packaging system, control of hygiene and temperature are essential to delay deterioration of the food (Eilert 2005). For the remainder of this chapter, modified atmosphere packaging of either vacuum or gas types will be abbreviated as MAP, vacuum packaging will be abbreviated as VP, and gas modified atmosphere packaging will be abbreviated as GMAP.

26.2.1 Materials and Equipment for MAP

MAP applications rely on barrier properties of the materials to stabilize and maintain the atmosphere that is modified. Although most of the containers for food with barrier properties potentially may be used to create vacuum conditions or provide for GMAP, most VP and GMAP packages are made from plastic materials or plastic materials combined with other types of material due to low cost and functional advantages over other materials (Marsh and Bugusu 2007) despite the variable permeability of different plastics to light, gases, and vapors (Opara and Mditshwa 2013). Most plastics for foods are thermoplastics, polymers that soften upon heating and return to their original condition at room temperature, which is ideal for molding and shaping of food packaging (Marsh and Bugusu 2007). Most thermoplastics are recyclable (Marsh and Bugusu 2007), with common plastic films being low density polyethylene (LDPE), high density polyethylene (HDPE), polyethylene (PE), polypropylene (PP), and laminated films (Opara and Mditshwa 2013). Additional plastics used for food are polytetrafluoroethylene, polyamide (nylon) (Han et al. 2005) polyvinylchloride, polyvinylidenechloride, polystyrene, polyesters, ethyl vinyl acetate (Marsh and Bugusu 2007), styrene butadiene, acrylonitrile butadiene styrene, polymethyl pentene, fluoropolymers, cellulose-based materials, and polyvinyl acetate (Kirwan and Strawbridge 2003). Packages for VP may be thermoformed from roll-stock or premade into pouches while packages for gas modified atmosphere packaging may be thermoformed from roll-stock film to form base webs sealed to lidding film or be pre-formed base trays with lidding film sealed to the tray flanges (McMillin 2008). Antifog compounds that reduce surface tension of moisture may be added to the lidding or transparent films in GMAP to prevent film fogging due to

moisture condensation on the inner surface of the film. Common antifog agents are glycerol esters, polyglycerol esters, sorbitan esters and their ethoxylates, alcohol ethoxylates, and nonyl phenol ethoxylates (Osswald et al. 2006). More details on the manufacture of plastic materials, their characteristics, and their use for foods can be found in the compilations of Greengrass (1999), Marsh and Bugusu (2007), and Bauer et al. (2019). Biobased or biopolymer materials derived from renewable sources are biodegradable, renewable, and edible, but have been limited in use for commercial food packaging by relatively poor mechanical and water vapor barrier properties (Rihm and Ng 2007). These materials can be derived from polysaccharides, proteins, lipids, microbial sources, or biologically-derived monomers (Mangaraj et al. 2018). The properties of biobased polymer matrices vary from those of petroleum-based films (Guillard et al. 2018), but nanoparticles provide bionanocomposites with barrier properties without inhibiting their biodegradability (Youssef and El-Sayed 2018). Nanomaterials are abundant and will comprise many composite packages in the future (Brody et al. 2008).

The two main types of machines for MAP are chamber and pillow wrap. The basic operations are to place the food in a barrier plastic film or pouch, evacuate or gas flush to remove air, and seal the package with heat or adhesive. For VP, the product is either placed in a preformed pouch or encased in film that provides both moisture and gas permeation barriers to maintain a constant environment around the food product. Chamber machines use thermoforming or pre-made trays while pillow wrap equipment is horizontal or vertical form-fill-seal (Hastings 1999). With pouch operations, food in the pouch is placed into an evacuation chamber where a vacuum removes air from the unsealed side (Fig. 26.1). After the specified time and/or level of evacuation, the pouch is heat sealed. GMAP machinery was developed from vacuum packaging in thermoforming or chamber forms (Hastings 1999). Thermoform and vacuum seal equipment use heat on the plastic film to form a cavity into which the food is placed. A second sheet of flat plastic is heat-sealed to the top of the thermoformed package after removal of the atmospheric air by evacuation or continuous gas flushing (Brody 1989b) (Fig. 26.2). GMAP packages can also be made by inserting the food into a preformed barrier tray and sealing a lidding film to the flange after evacuation or be a pouch containing the food for evacuation or gas

Fig. 26.1 Chamber pouch vacuum packager. (Photo courtesy of Supervac Maschinenbau GmbH and Robert Reiser and Company)

Fig. 26.2 A horizontal form-fill-seal packager. (Photo courtesy of Repack and Robert Reiser and Company)

Fig. 26.3 Double tray small semiautomatic tray sealer packager. (Photo courtesy of Ross Industries and Robert Reiser and Company)

flushing before the desired gases are inserted and the package sealed to provide a positive pressure within the package (McMillin 2008) (Fig. 26.3). Pillow-pack packages are made by forming the plastic film into a tube, sealing the two edges together, placing the food inside the enclosed plastic tube, and sealing the other end of the tube. Evacuation, gas flushing, and/or gas insertion before sealing depend upon the food type and desired food characteristics in the MAP. Horizontal form-fill-

Fig. 26.4 Vertical form-fill-seal packager. (Photo courtesy of ProMach Matrix Packaging Machinery)

seal, inverted horizontal form-fill-seal, and vertical form-fill-seal are machine types for pillow-pack food packaging (Hastings 1999). A small vertical form-fill-seal packager is shown in Fig. 26.4.

Laminations of polyester and polyethylene, nylon and polyethylene, polyvinyl dichloride, oriented polypropylene, and other combinations are common materials for MAP (Hastings 1999). A three-layer coextrusion of plastic for the exterior that provides resistance to abrasion, a middle layer for barrier, and an inner layer for package sealing may also be used with all of the packaging systems. With heat sealing, the thermoplastic layers of the two inner film sides are melted together, but for packages that are desired to be easy to reseal or open by consumers, adhesives are used to bind the inner film sides. Detailed descriptions explain the operating principles of each type of MAP packaging equipment and their operational requirements (Hastings 1999; Jenkins and Harrington 1991; Kotsianis et al. 2002; Ooraikul 1991a; Powrie and Skura 1991).

26.2.2 Gases for MAP

Air normally contains 79% nitrogen (N_2), 20.9% oxygen (O_2), 0.03% carbon dioxide (CO_2), and trace amounts of other compounds such as the noble gases

helium, argon, and xenon, but in varying proportions when calculated by weight (Compressed Gas Association 1981). The food type, the microorganisms that might be present, and the amount of time the food is desired to be acceptable for consumption determine the amount of air that is removed for VP or GMAP. For raw refrigerated meat, complete removal of air from VP or anoxic GMAP is necessary as residual air levels as low as 0.5% O_2 can cause the formation of brown metmyoglobin pigment during storage and display due to tissue respiration and decreased reducing conditions (McMillin 2008).

In the U.S., any substance that is intentionally added or comes into contact with food is a food additive and is subject to review and approval by the U.S. Food and Drug Administration (FDA). Compounds may be deemed to be safe as Generally Recognized as Safe (GRAS) substances within the U.S. Code of Federal Regulations Title 21 Parts 182 and 184 (CFR 2019). Substances may also be used if FDA finds no objection to their safety through scientific evidence or if there is proof of common use in food before 1958 with a substantial consumption history by a significant number of consumers. In these cases, FDA publishes a letter of their findings and assigns the substance a Generally Recognized Number (GRN). Since gases for GMAP come into contact with food, some are approved for specific uses with food while other gases have no restrictions on their use. Gases listed in 21 CFR as a secondary direct food additive permitted in food for human consumption are chlorine dioxide (173.300) and ozone (173.368). Gases that are direct food substances affirmed as GRAS are carbon dioxide (184.1240), helium (184.1355), nitrogen (184.1540), nitrous oxide (184.1545), ozone (184.1563), and propane (184.1655). Three gases (hydrogen, argon, carbon monoxide) used with food were recognized as ingredients after 1997 and are not listed in 21 CFR. FDA had no questions as to the validity of the expert decisions on the safety of hydrogen for drinking water, flavored beverages, and soda drinks at levels up to 2.14% by volume (GRN 520) and argon gas for use in wines, fruit juices and vegetables to replace the normal air atmosphere in sealed containers (GNR 57). Carbon monoxide has been approved with multiple GRN numbers, including for use in the packaging of fresh cuts of muscle meat and ground meat as a component of a gas mixture in a GMAP system (GRN 83 in 2002), as a component of a GMAP system for case-ready fresh beef and pork (GRN 143 in 2004), for use in GMAP for red meat products (GRN 167 in 2005), as a dissolved gas at a concentration of 21.4 milliliters per liter of brine/marinade solution injected at no more than 27.8% into beef muscle parts that are vacuum-packed and prepared for case-ready marketing (GRN 194 in 2006), and at a concentration of 0.4% as a component of a GMAP system for fresh ground and muscle red meat (GRN 251 in 2008).

Packaging gases in the European Union (EU) are gases other than air introduced into a container before, during or after the placing of a foodstuff in that container (European Parliament and Council 2019). Labeling must state "Packaged in a protective atmosphere" (Djenane and Roncalés 2018). Food additives permitted for use with foods in the European Union must not, based on available scientific evidence, pose a safety concern to the health of the consumer at the proposed level of use; not mislead the consumer; and show a reasonable technological need not

achievable by other practicable means. Approved GMAP gases for quantum satis ("which is enough") amounts with foods are carbon dioxide (E290), argon (E938), helium (E939), nitrogen (E941), nitrous oxide (E942), oxygen (E948), and hydrogen (E949) (European Parliament and Council 2019).

The main gases for GMAP are N_2, O_2, and CO_2, but other gases that have been proposed for use with various foods include argon, carbon monoxide, chlorine, ethene, ethylene, ozone, propylene oxide, sulfur dioxide, nitric oxide, and nitrous oxide (Heinrich et al. 2016; Jemni et al. 2016). N_2 is an inert tasteless gas often used in GMAP because it is unreactive with most food components and is not readily absorbed at the food surface (Farber 1991) so it can function as a filler gas (Church and Parsons 1995). Helium, argon, xenon, and neon are also inert and have been studied for use as filler gases (Sebranek and Houser 2006). CO_2 has an inhibitory effect on many common spoilage microorganisms, but it can have differing effects with different microorganism species. Gram negative bacteria are more susceptible to inhibition by CO_2 than Gram positive microorganisms, most of which are facultative or strict anaerobes (Gill and Tan 1980). Gram-positive bacteria that produce lactic acid may also grow in GMAP containing CO_2 (Hintlian and Hotchkiss 1986). Generally, 20–40% CO_2 is used in GMAP (Clark and Lentz 1969). While levels of CO_2 less than 20% do not satisfactorily inhibit microorganism growth, levels above 40% may result in package collapse, because CO_2 is highly soluble in fat and water. Solubility is increased with decreased temperatures (Sivertsvik et al. 2002), which are needed for refrigerated foods. The partial pressure of the atmosphere will also influence concentration of CO_2 in the food (Ho et al. 1987). The inhibitory effect of CO_2 on microorganisms depends upon the specific food, gas partial pressure, gas concentration, headspace to product ratio, temperature, food acidity and water activity, type of microorganism, and stage of microorganism growth (Farber 1991). Upper limits for CO_2 range from 1% to 30% as higher levels will cause injury for specific horticultural crops (Watkins 2000).

Oxygen (O_2) at moderate levels will stimulate the growth of many aerobic microorganisms while a high level will impede the proliferation of aerobic bacteria. Anaerobic microorganisms vary in their sensitivity to O_2 levels (Farber 1991). Exclusion of oxygen is generally desirable as it is required for the two major spoilage factors of oxidative reactions and aerobic bacteria metabolism (Church and Parsons 1995). High levels of O_2 generally are accompanied by CO_2 to provide microbial inhibition (Van der Steen et al. 2002). The oxidation of both lipids and proteins in meat in high oxygen packaging (Lund et al. 2011) has caused a shift away from use of high O_2 GMAP for meat in the U.S., but it is still used for fresh meat in the European Union and rest of the world. The most common gas mixtures are 60–80% O_2 and 20–30% CO_2 (Djenane et al. 2003), with a minimum of 5% O_2 partial pressure needed for oxymyoglobin pigment formation (Ledward 1970), 13% O_2 for predominate oxymyoglobin pigments (Siegel 2001), and 55% O_2 to maintain red meat color (Jakobsen and Bertelsen 2000). Low levels of O_2 are recommended in GMAP for foods that are susceptible to anaerobic pathogen growth and/or toxin production to prevent these occurrences. Levels of O_2 are controlled in GMAP for fresh fruits and vegetables to slow, but not stop, respiration processes (Zagory 1995).

Carbon monoxide (CO) is a highly toxic gas and concentrations of 12.5–74.2% are explosive (Blakistone 1999a). However, as noted above, it is approved in the U.S. for specific uses in meat packaging at low levels (0.4%) (Eilert 2005). CO irreversibly binds to myoglobin and hemoglobin pigments to maintain a red meat color (Lanier et al. 1978) and at low levels CO can slow respiration to prevent browning in lettuce (Blakistone 1999a). It is effective against many microorganisms at concentrations as low as 1% (Zagory 1995). Cornforth and Hunt (2008) reviewed the use and concerns of meat GMAP containing CO. A major concern is the masking of spoilage or pathogenicity by the extension of the red meat color shelf life, but low levels of CO are not inhibitory to the growth of spoilage organisms (Sørheim et al. 1999) nor mask offensive odors and/or prevent flavor deterioration (Eilert 2005). However, the controversy about the safety of CO in GMAP has led to disallowance of use in GMAP in the European Union. Use of carbon monoxide at 5–10% should be combined with oxygen levels reduced to 2–4% to maximize fungistatic effects (Kader 1983). At abuse temperatures of 10 °C, CO-GMAP was inhibitory to growth of *Y. enterocolitica*, *L. monocytogenes*, and *E. coli* O157:H7, but was not as inhibitory against Salmonella strains, indicating that temperature control is important during storage, regardless of packaging method (Nissen et al. 2000). The toxicity risk from the packaging process or by consuming meat treated with CO is deemed negligible (Djenane and Roncalés 2018).

Ozone (O_3) is strongly microbicidal against bacteria, fungi, parasites and viruses so it is suitable for washing and sanitizing solid food with intact and smooth surfaces that would minimally compete for O_3 activity (Kim et al. 2003). However, most food has readily available organic constituents that react with O_3 and so may reduce the concentration of O_3 to levels below those required to inactivate microorganisms (Kim et al. 1999, 2003). Ozone efficacy is less in complex systems like food than when organisms are in pure water or simple buffer solutions (Khadre et al. 2001). O_3 minimally reacts with water, but water often contains readily oxidizable organic and inorganic substances that react rapidly with O_3 so O_3 often is relatively unstable in aqueous solutions and decomposes slowly to O_2. The effectiveness of ozone is generally greater against vegetative microorganisms than spores of bacteria and fungi. Low concentrations of O_3 cause nose, throat, and eye irritation and so U.S. regulations have imposed maximal human exposure limits (Khadre et al. 2001).

Helium has FDA GRAS (184.1355) and EU (E939) status and is sometimes used for the detection of package leaks. Argon may inhibit some microorganisms, but Kader and Watkins (2000) did not find evidence that argon, helium, or other noble gases were suitable to replace N_2 in GMAP for fresh produce. Argon-CO_2 (70%:30%) and 40% argon:30% CO_2:30% N_2 mixtures reduced *L. monocytogenes* to less than 0.5 $\log_{10}$ CFU per gram on ham while growth was greater than 0.5 $\log_{10}$ CFU per gram with 20% CO_2:80 N_2 (Heinrich et al. 2016). Sulfur dioxide controls mold and bacteria on soft and dried fruits like blueberries (Rodriguez and Zoffoli 2016) and table grapes (Lichter et al. 2008) when in unbound and non-ionized molecular form and is thus most effective at pH 4 or lower while being selective in its toxic action (Blakistone 1999a). However, SO_2 may cause product bleaching and allergenic sulfite residues (Suppakul et al. 2003).

Gas mixtures in GMAP vary with the food product and with the processing and storage conditions before or after packaging. Cooked or shelf-stable foods utilize different gas mixtures than raw and/or perishable foods (Farber 1991). Highly respiring foods like some fruits or vegetables or high fat foods that are subject to rancidity require specific atmospheres to balance spoilage, pathogenicity, nutrient retention, and appearance (Subramaniam 1999). Some products require only a single gas, such as 100% CO_2 for hard cheese and 100% N_2 for dairy cakes, fresh pasta, and dried or roasted foods (Blakistone 1999a). Some recommended gas mixtures are 20–35% CO_2 and 75% N_2 for poultry, 60% CO_2 and 40% N_2 for oily fish, 30% CO_2 and 70% N_2 for soft cheese, 60% CO_2 and 40% N_2 for nondairy cakes, and 60–70% CO_2 and 30–40% N_2 for bread (Blakistone 1999a). Other products have different gas mixtures used in their packaging (Farber 1991). Some products require O_2 for safety (20–30% for fish), to assist in respiration control (3–5% for some fruits and vegetables), and for appearance (60–85% for mammalian meat to maintain red oxymyoglobin pigments) (Blakistone 1999a). However, as oxygen may promote lipid oxidation that can initiation pigment oxidation, CO is now used for stabilizing the red color in many raw refrigerated meat and fish products (Djenane and Roncalés 2018).

26.2.3 General Safety Concerns for MAP

MAP generally results in a longer shelf-life compared with packaging in air, but there may be increased risk from microorganism growth or toxin production (Church and Parsons 1995) or from the gaseous atmosphere itself. The gases used in GMAP are GRAS (generally recognized as safe) or allowed for use by FDA and so are of minimal concern because of the safety record. As indicated in the previous section, gases approved for GMAP in the EU carry an "E" designation. The risk from CO that would be inhaled during package manufacture or from consumption of meat treated with CO is considered to be negligible (Cornforth and Hunt 2008; Djenane and Roncalés 2018). However, there is a lack of legal permission for use of CO with meat in the EU (Djenane and Roncalés 2018).

The ability of MAP to inhibit spoilage microorganisms is documented, but pathogenic microorganisms may be less affected, so there is a concern that food in MAP may become hazardous before a consumer detects spoilage of the food. Common food pathogens are *S. aureus*, Salmonella, Enterococci such as *Y. enterocolitica* and *E. coli*, Clostridia such as *C. perfringens* and *C. botulinum* (Hintlian and Hotchkiss 1986), *L. monocytogenes*, *B. cereus*, Shigella, and viruses (Church and Parsons 1995). *L. monocytogenes*, nonproteolytic *C. botulinum*, enterotoxigenic *E. coli*, *A. hydrophila*, and *Y. enterocolitica* are examples of pathogenic microorganisms that grow at refrigeration temperatures (Dodds 1995). Therefore, MAP can extend the shelf-life of products before they are considered to be spoiled so psychrotrophic aerobic pathogenic microorganisms such as *A. hydrophila*,

L. monocytogenes, enterotoxigenic *E. coli*, and *Y. enterocolitica* may have an extended time in which to grow (Cutter 2002).

Microorganism growth and/or toxin production are influenced by the food water activity, temperature, oxidation-reduction potential, pH, nutrient content as well as extrinsic factors such as the gaseous atmosphere and partial pressure (Cutter 2002). Absence of oxygen surrounding foods in VP may permit growth and toxin production of *Cl. botulinum* while suppression of aerobic spoilage bacteria may allow growth of pathogenic aerobic bacteria such as *A. hydrophila*, and *Y. enterocolitica* (Cutter 2002). A headspace of 20–60% CO_2 will inhibit spoilage organisms (*Pseudomonas* spp., *Acinetobacter* spp., and *Moraxella* spp. and slow the growth of *B. thermosphacta* and lactic acid bacteria while outgrowth of *L. monocytogenes, B. cereus*, and *C. botulinum* may occur (Cutter 2002). *S. aureus* and Salmonella were inhibited at 10 °C and had no increase in growth at 20 °C during 10 days in 60% CO_2:15% O_2: 15% N_2 (Silliker and Wolfe 1980). This indicates the need to consider all of the factors that might influence microorganism growth and/or toxin production in packaging and shelf life studies. Other studies have shown inhibition of Salmonella by 100% CO_2, but not by VP (Silliker and Wolfe 1980) and decreased respiration and increased generation time of Yersinia and inhibition of *E. coli* growth in CO_2 (Eklund and Jarmund 1983). Yersinia and Salmonella were reported to be more controlled by temperature than by a CO_2 atmosphere (Farber 1991). Levels of 30% CO_2 and greater slowed the growth of *L. monocytogenes* in atmospheres of 20% O_2 (Hendricks and Hotchkiss 1997). Of concern with some foods in anoxic MAP packaging is *Cl. botulinum* because of its ability to produce neurotoxin. Studies have indicated that elevated levels of CO_2 were not inhibitory to clostridia (Hintlian and Hotchkiss 1986) and CO_2 enhanced the germination of three strains of *Cl. botulinum* spores (Foegeding and Busta 1983) and *Cl. perfringens spores* (Enfors and Molin 1978). A high CO_2 concentration decreased growth of non-proteolytic *C. botulinum* type B, but greatly increased the expression and production of toxin (Lövenklev et al. 2004). CO_2 appeared to have effective antimicrobial effects on *Bacillus cereus*, while *C. jejuni* survival was not affected by any gas atmospheres (Farber 1991). Mixtures of 20% CO_2:80% N_2 reduced numbers of *E. coli* on ham (Heinrich et al. 2016). The relative effects of CO_2 on gram positive and gram negative bacteria were summarized by Farber (1991).

As previously indicated, O_3 can be an effective sanitizer when there are low levels of organic materials to react with the gas. It is generally more effective against vegetative bacteria cells than bacteria or fungal spores and provides more microorganism destruction when surfaces are hydrated (Khadre et al. 2001). Argon at 40% and 70% levels inhibited *L. monocytogenes* and *E. coli* on ham (Heinrich et al. 2016). Oxygen at moderate levels will generally stimulate the growth of aerobic bacteria and although it can inhibit the growth of strict anaerobic bacteria, anaerobic microorganisms have a wide variation in their response to O_2 (Farber 1991). O_2 may be included in GMAP for foods susceptible to *Cl. botulinum* contamination, with package headspace levels of 1% O_2 reported to be the critical level for *Cl. botulinum* germination and growth (Whiting and Naftulin 1992). However, it is necessary to determine both the redox potential and the partial pressure of oxygen to adequately

determine the growth of *C. botulinum* in a medium or a food (Lund et al. 1984). Multiple barriers or hurdles to control microbial stability for foods in GMAP susceptible to pathogenic or spoilage microorganism contamination are provided by temperature and/or other factors (Farber 1991). Information on the microbial aspects, gas mixtures, machine types, and packaging materials are available for red meats and offal, fish and seafood products, poultry products, dairy products, and fruits and vegetables in VP and GMAP (Farber 1995).

26.3 Foods with MAP

Different food products are associated with different pathogenic microorganisms and quality parameters and thus have differing requirements for shelf life, appearance, and palatability that must be balanced with safety considerations in determining the appropriate MAP system. Foods that are packaged in VP have primarily been those that are highly perishable or not easily deformed when displayed in the packaging. Respiring foods will continue the metabolic processes in VP, consuming the small amount of O_2 residual in the tissues to increase the vacuum level and produce CO_2 and moisture, thus creating a GMAP in some aspects (Brody 1989b). Major food groups that are packaged in GMAP are muscle foods, produce, bakery products, and precooked foods (Brody 1989b) while beverages, dried foods, and dairy foods also have some GMAP applications (Dodds 1995).

26.3.1 Muscle Foods

MAP for muscle foods requires a barrier to both moisture and gas passage through packaging materials to create a stable environment (McMillin 2008). Plastic materials create attractiveness, hygienic conditions, and convenience (Renerre and Labadie 1993) and the relatively inexpensive price and functionality (Marsh and Bugusu 2007) make them the dominant packaging materials for meat, poultry, and fish products. The types and properties of plastic materials used for raw primal and retail mammalian cuts and processed meat items vary with the specific species and meat cut (McMillin 2008). Primal cuts of mammalian species are often in VP while VP use is limited to specific raw retail cuts. High O_2 packaging is common in the EU and some other countries, while GMAP containing CO or the more traditional overwrap (moisture impermeable, vapor permeable) packaging are used for raw mammalian meat cuts in the U.S. Processed cured and/or heat-treated items are frequently in VP while some cured cuts and sausages may be in GMAP containing mixtures of CO_2 and N_2 (McMillin 2008).

Poultry carcasses and parts are seldom in VP or GMAP, often being frozen or hard chilled in semi-permeable packaging. Growth of *Y. enterocolitica* and

A. hydrophila in chicken breast meat was slowed with 100% CO_2 and 80% CO_2:20% N_2 (Ozbas et al. 1996). Enterobacteriaceae on precooked chicken meat was inhibited with 30% CO_2 in gas mixtures (Patsias et al. 2006). Additional impacts of gases on beef, pork, and poultry are in Arvanitoyannis and Stratakos (2012) and McMillin (2008). In ground beef at 10 °C, growth of *E. coli* O157:H7 was almost totally inhibited in 60% CO_2:40% N_2:0.4% CO and 70% O_2:30% CO_2, Salmonella counts were higher in 60% CO_2:40% N_2:0.4% CO than in 70% O_2:30% CO_2, and growth of *Y. enterocolitica* and *L. monocytogenes* were not increased in 60% CO_2:40% N_2:0.4% CO, indicating that temperature is important for control of pathogenic microorganisms in contaminated ground beef (Nissen et al. 2000). Meat and poultry in VP and GMAP are safe when proper refrigeration temperatures are maintained (Narasimha Rao and Sachindra 2002).

C. botulinum type E is associated with marine food products as it has the ability to produce toxin at refrigeration temperatures as low as 3 °C in low oxygen atmospheres (DeWitt and Oliveira 2016). The shelf life and characteristics of marine and seafood products vary with the species and packaging atmospheres (DeWitt and Oliveira 2016). Packaging with high levels of CO_2 can delay growth of *L. monocytogenes*, but this is not sufficient even with refrigerated temperatures to control the pathogen in some fishery products (Sivertsvik et al. 2002). Vacuum and gas mixtures of 50% CO_2:50% N_2, 80% O_2:20% CO_2, and 2.5% O_2:7.5% N_2:90% CO_2 delayed, but did not stop, the growth of *L. monocytogenes* on rainbow trout fillets (Yilmaz et al. 2009). Flounder fillets in VP and 100% CO_2 developed toxicity from *Cl. botulinum* after 20 and 25 days, respectively, while film with an O_2 transmission rate of 3000 $cm^3\ m^{-2}\ 24\ h^{-1}$ did not prevent spoilage at 4 °C, but did prevent toxin formation. At 10 °C, spoilage occurred before toxin production at day 8 (Arritt et al. 2007). There was no effect of headspace O_2 (balance CO_2) or vapor transmission rate of the film on the development of toxigenesis from *C. botulinum* type E on fresh rainbow trout fillets so additional barriers such as temperature are essential to ensure the safety of these fish (Dufrene et al. 2000a, b). The growth of *Cl. botulinum* does not depend on the total exclusion of O_2 and the inclusion of O_2 as a packaging gas does not ensure the prevention of growth or toxin production (Sivertsvik et al. 2002).

The low levels of CO used with meat and fish do not have much effect on microorganisms (Djenane and Roncalés 2018).The lag phases were extended and the growth rates of *E. coli*, Achromobacter, and *P. fluorescences* were extended while *P. aeruginosa* was unaffected by 25–30% CO (Gee and Brown 1981). Edible films, polylactic acid films, and nanocomposites are not in common use for meat, but reclosable packaging and high pressure-processing are increasing, particularly for ready-to-eat items (McMillin 2017). Moisture absorbing pads and oxygen scavenging sachets are the most frequent active packaging technologies used with muscle foods (McMillin 2017).

26.3.2 Fruits and Vegetables

Fresh-cut fruits and vegetables are minimally processed and in a raw state ready for eating or cooking (Oliveira et al. 2015). There is an increased demand for these products, prompting the industry to develop additional methods like GMAP to actively or passively control or modify the atmosphere surrounding the products (Farber et al. 2003). Fresh fruits and vegetables are metabolically active for long periods after harvesting, either through endogenous respiration or due to physical injury, microbial populations, dehydration, and temperature (Kader et al. 1989). The gas composition influences anaerobic and aerobic respiration as do the differences in climacteric nature (rates of respiration and ethylene production with ripening) among different types of fruits and vegetables (Powrie and Skura 1991). During storage, CO_2 is produced as O_2 is consumed during respiration so microbial activity is usually reduced by decreasing O_2 and increasing CO_2 above atmospheric levels (Jemni et al. 2016). It is as difficult to describe the many permutations of GMAP for fruits and vegetables as it is to make the decision on the appropriate modified atmosphere technique for a specific fruit or vegetable. Factors for consideration of suitable atmospheres include cultivar and maturity, harvest and postharvest handling, pre-treatment processing, washing, tissue operations, temperatures, prevention of contamination, and food additive use (Powrie and Skura 1991). Detailed descriptions of packages and packaging procedures for specific fruits and vegetables are in Garrett (1999), Powrie and Skura (1991), Prince (1989), and Zagory (1995).

Shrink-wrapping of individual horticultural commodities does not produce sufficient atmosphere modification from respiration due to high film permeability. This then provides less advantages for climacteric commodities that depend upon respiration to develop the desired atmosphere than for commodities where moisture control is more important (Ben-Yehoshua 1989; Prince 1989). Each fruit and vegetable type has different gas mixtures for balancing spoilage and safety and maintaining quality (Oliveira et al. 2015; Zhang et al. 2015). Low O_2 conditions may promote growth of pathogenic microorganisms that are initially present on produce products (Boz et al. 2018). GMAP does not reduce growth of *Listeria* on fruits and vegetables (Berrang et al. 1989) and mixed salads made from raw vegetables have been implicated in *L. monocytogenes* food poisoning (Ho et al. 1986). The possibility of *C. botulinum* spores in vegetables would increase the health risk in anaerobic MAP environments (Lilly et al. 1996). Cross-contamination with *E. coli* O157:H7 may occur during processing, handling, and marketing of vegetables (Phillips 1996). Salmonella spp. and Enterobacteriaceae growth is reduced with increased levels of CO_2, but storage temperature must also be controlled (Sawaya et al. 1995). VP caused reduction in pH, increased CO_2, decreased O_2, and increased microbial growth in lettuce, cabbage, broccoli, carrots, and green beans, with botulinal toxin detected in spoiled broccoli and lettuce samples, but not in cabbage, carrot, or green bean samples. It was suggested that the probability of botulinal toxin production prior to spoilage in these vegetable types was less than 1 in 100,000 (Larson et al. 1997). Care must be taken in reducing spoilage microorganisms that

might provide an opportunity for pathogenic organisms to grow. Inhibition of spoilage organisms with UV light allowed botulinal toxin formation in air packaged melons before overt spoilage (Larson and Johnson 1999). Cut produce has typical headspace gases of 2–10% O_2 and 10–20% CO_2 (Marston 1995), which may facilitate spoilage before toxin production by *Cl. botulinum* (Farber et al. 2003).

26.3.3 Bakery Products

Bakery products include bread, unsweetened rolls and buns, doughnuts, varieties of pies (meat, dessert, pizza), crusts, crackers, and cookies, with the classification based on product type, method of leavening, or moisture content/water activity (Smith and Simpson 1995). Most bacteria growth is limited to those bakery products with a high moisture content since bacteria require a relatively high water activity for growth (Smith and Simpson 1995). Much attention has been given to the staling, moisture loss or gain, and spoilage of bakery goods (Seiler 1989) rather than to the safety aspects of MAP. Some products such as non-pasteurized pasta are packaged in 70–80% CO_2 and 20–30% N_2, so there is concern that temperature abuse and the high CO_2 levels might stimulate pathogenic bacteria or toxigenesis. Storage of pasta at 3 °C for 2–4 weeks stimulated *C. botulinum* type B toxigenesis upon subsequent storage at 8 °C (Notermans et al. 1990). Growth of *C. botulinum* requires conditions of very low oxygen tension, which seldom occurs in commercial bakery products in MAP. Few bakery products have the water activity, pH, and nutrients to promote growth of these organisms (Seiler 1999). Bacterial food poisoning outbreaks due to bakery products are rare and usually traced to cream-filled cakes with *S. aureus*, *B. cereus*, or Salmonella (Ooraikul 1991b). Microbiological characteristics differ for dough or batter products, cake or pastry items, layer cakes, and pies or products with filling (Ooraikul 1991b).

26.3.4 Dairy Products

A tight fitting package from CO_2 flushing or VP limits the amount of internal O_2 and headspace area for cheeses (Fierheller 1991). Hard and semi-hard cheeses, like cheddar, are commonly packed in 100% CO_2 or CO_2 and N_2 (Hotchkiss et al. 2006) with form-fill-seal or tray and lidding equipment (Subramaniam 1999). Gas mixtures with 10% or 20% CO_2 with and without 10% O_2 and balance N_2 increased the lag time, but did not inhibit the growth of *L. monocytogenes* in mold-ripened cheese at refrigerated temperatures (Whitley et al. 2000). CO_2 resulted in slight inhibition of *L. monocytogenes* growth on cottage cheese at 4 and 7 °C (Chen and Hotchkiss 1993), while Fedio et al. (1994) reported that CO_2 inhibited growth of *L. monocytogenes* in cottage cheese. *L. monocytogenes* and *B. licheniformis* were not affected by dissolved CO_2 in yogurt while *E. coli* decreased to nondetectable

levels (Karagul-Yuceer et al. 2001). Injection of CO_2 at low levels into pasteurized milk retarded spoilage at refrigeration temperatures and did not increase the risk of botulism at refrigeration or abuse temperatures (Glass et al. 1999), but had no effect on *B. cereus* outgrowth (Hotchkiss et al. 2006).

26.3.5 Pre-cooked Food

The major concerns with precooked foods are microbial development and oxygen-related difficulties such as rancidity, discoloration, and loss of flavor (Coulon and Louis 1989). Most of the concerns for safety of precooked foods in either VP or GMAP are the same as for the other food products, but the potential for foodborne illness might be greater from pathogen growth or toxin production if contaminated pre-cooked foods are eaten with no further preparation or reheated to sublethal temperatures. The two main types of packaging for precooked foods are semirigid packages produced on thermoforming equipment and flexible packs made on form-fill-seal machines or bulk packagers. Mixtures of N_2 and CO_2 comprise the basic gas components for precooked foods in GMAP (Coulon and Louis 1989). Toxin production by *C. botulism* A and B on hamburger, sausage, and turkey sandwiches under refrigeration was inhibited by N_2 atmospheres while toxin was produced on hamburger and sausage sandwiches at room temperature (Kautter et al. 1981). Sausage, hamburger, and turkey sandwiches in N_2 environment did not have toxin produced by *S. aureus* at 8 and 12 °C, but sausage and hamburger sandwiches had detectable toxin after 2 and 4 days, respectively, at 26 °C (Bennett and Amos 1982). These results confirm that abusive storage or display temperatures influences enterotoxin production by pathogenic microorganisms differently in different precooked products. Packaging for stuffed pastry, pizza, egg rolls, and pasta are described in Coulon and Louis (1989).

26.3.6 Beverages, Intermediate-Moisture, and Dried Foods

The safety of these foods in MAP is of less concern than shelf-life and palatability considerations as most have undergone one or more preservation processes prior to packaging. VP is not suitable for products that are crushed or broken from the evacuation process (Fierheller 1991), but the choice of flexible or rigid packaging depends upon the specific food and desired characteristics. If CO_2 is used, generally the stability of the products is increased with higher levels of CO_2 (Fierheller 1991) and mixtures of varying levels of CO_2 and N_2 are common to create anoxic environments within GMAP. Alcoholic beverages, fruit juices, and soft drinks often have gases injected to provide pressurization within containers or have the packages or bottles flushed with gas immediately before sealing. GMAP is used for some intermediate moisture foods, dehydrated foods like coffee and tea, and snacks (Subramaniam 1999).

26.4 Technologies for Use with Modified Atmosphere Packaging

Improvements in packaging to lower costs, make more food available, and minimize waste depend upon antimicrobial agents, the packaging, and the packaging production process (Nicoletti and Del Serrone 2017). Smart packaging provides functional attributes through mechanical, chemical, and electrical driven functions that add benefits to the food product and subsequently to users (Opara and Mditshwa 2013). Intelligent packaging monitors the condition of the food or surrounding environment to communicate the status to users of the package (Kerry et al. 2006), while active packaging has components deliberately incorporated to release or absorb substances into or from the food, environment surrounding the food, or the packaging materials (Labuza and Breene 1989). When determining the appropriate packaging and any active, smart, and/or intelligent packaging concepts for a specific food, the total influences on food losses and waste must also be considered. These include removal of excess packaging, reduced package material weight or volume, concentration of liquid products, refillable packaging, renewable packaging, recycling or reuse, convenience for users, portions in suitable sizes, minimization of container damage, and communication with users (Wikström et al. 2018).

There are many technological improvements that can be used in combination with MAP (Wilson et al. 2019). Table 26.1 lists some of the major smart and intelligent technologies that would improve the functionality of MAP, giving the types of compounds or mechanisms and examples of the active components of the technology. Gas absorbing/scavenging and releasing/emitting capabilities would help to maintain the desired package environment as it otherwise might be changed by food or microorganism metabolism, package permeability, or external factors such as light, temperature, or handling. The implementation of active control of gaseous content of GMAP would provide the advantages of controlled atmosphere storage to individual rather than bulk packages or containers. The absorption, scavenging, emission, or release of flavors and odors, antimicrobial agents, and antioxidant materials would extend the shelf life, maintain quality, and/or improve safety of foods in GMAP. The application and availability of sachets, packets, film, or other methods for delivery or retrieval of the desired components will determine their use with GMAP.

Indicators or sensors of package or product tampering and integrity are important to maintain product safety and consumer confidence in MAP. Product indicators or sensors for time and temperature, gas atmosphere, and microbial sensors would indicate relative quality and shelf-life status of specific products for use by processors, retailers, and consumers. Traceability, antitheft, and product authenticity capabilities linked to MAP would assist in more immediate responses to product recalls, improve inventory management, and mitigate labelling disputes. The advantages and disadvantages of each specific approach will determine the suitability for individual foods and types of MAP. All of the listed technologies may have application with food in MAP, but most require additional improvements in functionality, economic viability, or marketplace advantages for commercial implementation.

Table 26.1 Active and intelligent packaging technologies for use with modified atmosphere packaging

Technology	Type of compound	Examples of active components
	Carbon dioxide	Activated carbon, zeolites, carbonates, hydroxides, glycinate
	Ethylene	Potassium permanganate, minerals, nanoparticles, zeolites, activated charcoal, silicon dioxide
Absorbing/ scavenging	Flavors	Baking soda, activated charcoal
	Moisture and fog	Silica gel, clay, zeolite, alumina silicate, humectant salts, sorbitol, calcium oxide, polysaccharides, propylene glycol, cellulose, fibers
	Oxygen	Iron, ascorbic acid, photosensitive dyes, unsaturated hydrocarbon dienes, palladium, polymers, enzymes
	Antimicrobial	Essential oils, spices and extracts, carvacrol and thymol, enzymes, bacteriocins, antibiotics, polylysine, chitosan, citric acid, sorbic acid/sorbates, lactic acid/lactates, regenerated cellulose, allyl isothiocyanate, metal oxides
Releasing/ emitting	Antioxidant	Butylated hydroxy toluene, α-tocopherol, quercetin, ascorbic acid, citric acid, green tea extract, spice extracts, beet root residue powder, anthocyanins, pulp and pulp extract, propyl gallate, organophosphate, thioester compounds
	Carbon dioxide	Ferrous carbonate, sodium bicarbonate and ascorbic acid, metal halides
	Ethanol	Encapsulated ethanol
	Flavor	Compound(s) specific to desired flavor(s)
Tamper evidence/ package integrity	Inks, glues, plastics, sensors	Seals, printed labeling, indicator labeling, decorative foils, closure shrink sleeve, gas indicators,
	Time-temperature	Mechanical deformation, color change, enzymes, acid base, polymerization, photochemical, molecular diffusion
Product indicators	Gas sensors	Redox dyes, pH dyes, enzymes, metabolites, crystals, fluorescence, metal oxides, organic conducting polymers
	Microbial sensors	pH dyes, volatile and nonvolatile metabolites, electrochemical, enzyme redox
	Labels, tags, chips	Dyes, holographic images
Traceability/ antitheft	Radiofrequency identification tags (RFID)	Electromagnetic or electronic receivers and/or transmitters
Product authenticity	Images, logos, barcodes, tagged materials	Labels, chips, physical markers, food-grade chemical compounds
	RFID	Electronic receivers and/or transmitters

Adapted from Ahmed et al. (2018), Ghaani et al. (2016), Han et al. (2018), Müller and Schmid (2019), Restuccia et al. (2010), Yildirim et al. (2018)

26.5 Conclusion and Future Trends

Vacuum (VP) and gaseous modified atmosphere packaging (GMAP) provide increased shelf life for many food products by maintaining desired nutrient, palatability, and appearance characteristics. The safety of packaged food may be negatively affected in any type of packaging that alters the environment surrounding the food. The presence and growth of pathogenic microorganisms, toxin production, compounds from the packaging materials, gases that are inserted or produced by respiration, and noxious compounds produced by the food inside the altered environment may cause safety concerns. Many different packaging materials, machinery, and gases are available for use in vacuum or gaseous modified atmosphere packaging of foods. The selection of packaging method is highly dependent upon the desired product traits during transport and storage, retail display, and consumption. Technologies like smart, intelligent, and active packaging improve or are synergistic with the applications of VP and GMAP for food. VP or GMAP does not remove the need to control the many situations in which food can become contaminated, stored under improper conditions, be inadequately preserved or heated, or otherwise become unsafe to consume.

Future trends will include refinements and implementation of many of the listed active and smart technologies, with specificity to individual food items and packaging systems needed for successful use. Increasing the complexity of MAP packaging will introduce additional variables into risk assessments. Since the EU necessitates determination of migration products, each of the new technologies, the use of nanoparticles, or inclusion of functional additives in sachets or packaging materials must be carefully scrutinized. Proper attention to product safety requires addressing consumer perceptions and purchase behaviors while overcoming technical constraints and balancing costs with relative value of the technology. Packaging innovations such as temperature control by self-cooling, self-heating susceptor heating, and use of fibers or perforated plastics as insulating materials will provide added consumer convenience with additional cost considerations.

Developments in intelligent and active packaging that further extend the shelf life and product quality information of food in MAP by reducing food wastage, particularly to increase household use of food that would otherwise be discarded, will be important. The integration of advanced technologies must be balanced with the demands for sustainability in packaging using recycled and/or renewable resources, lighter and thinner materials, and minimizing waste (Han et al. 2018).

Commercialization of any or all of the active and intelligent technologies will allow the food and retail sectors to improve control of the food production chain while meeting the needs of consumers (Restuccia et al. 2010). However, consumer confidence and public trust of the packaging advances will be needed, necessitating effective communication on each technology and overcoming the hurdles of technology transfer, manufacturing scale-up, and addressing regulatory requirements and environmental concerns (Werner et al. 2017). Careful decisions must be made on the integration of packaging innovations that complement or improve the use of MAP for food to avoid negative perceptions and promote acceptance by processors, retailers, and consumers.

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Chapter 27
Advancements in Post-packaging Technologies

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27.1 Introduction

Post-packaging can be defined as methods or technologies that help the product/package unit maintain the shelf life and safety of the product throughout distribution and transport to its final destination and use. These treatments can occur internal to the package or external. For example, active packaging can sense a change in the product and create a change inside the package to improve food safety and quality. Often these technologies involve a physical or chemical reaction that occur in response to an interaction with the product, control product interaction or signal that en event has occurred within the package during distribution, storage or retail display. These technologies, if correctly designed, assist with food safety by serving as an additional hurdle in control of microbial contamination, signal presence of pathogens, insect invasion, spoilage microbiota or mitigate the cause of product spoilage. Not all of the technologies described can perform such direct improvements to food safety but rather monitor, signal and indicate if an event has occurred that could significantly impact the safety of the food or reduce shelf life.

Post-packaging technologies can be divided into four major categories: (1) active packaging, (2) intelligent packaging, (3) cold chain innovations, (4) insect invasion control. In all these categories, researchers in the food industry, academia, and the government are developing new materials and technologies leading to novel developments. However, development of packaging systems and materials has challenges for commercial implementation including but not limited to regulatory concerns. In particular, the packaging materials need to be approved or reviewed by a designated regulatory agency. For example, in the United States, the Food and

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Drug Administration (FDA) may regard food-contact materials or component of a material as a "food additive" under the Federal Food, Drug, and Cosmetic Act (Ettinger 2002). Regulatory consideration as a food additive can be expensive and time consuming so another process called Food Contact Notification (FCN), which can provide a non-objection letter for use in the market faster than waiting for FDA approval. A summary of United States Regulations for Food Contact Materials (Packaging) is provided in Table 27.1. In both cases, rigorous testing to provide proof of safety for human consumption is required. For this reason, researchers should consider these regulations when creating materials that are used for innovative packaging technologies. For more details on the regulations of Packaging and Food Contact Substances see the Food and Drug Administration website. (https://www.fda.gov/food/food-ingredients-packaging/packaging-food-contact-substances-fcs)

Table 27.1 Summary of food packaging regulations in the United States

Year and regulation	Description
1938 Food Drug and Cosmetic Act (FDC)	First major United States Law enacted related to Food Produced for Human Consumption
1958 Amendment to 1938 FDC Act	Defined Additives – which included packaging which falls under indirect additives as.. a*ny substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component. . .of any food. . .if such substance is not generally recognized. . .to be safe under the conditions of its intended use.*.
Food Additive Petition	Lengthy and slow to market
Exemption to Food Additive Petition	Lengthy and slow to market but more desirable route for food packaging materials (food contact substances) Exemption paths: Prior sanction (documented safe use prior to 1958) Generally recognized as safe (GRAS) No Migration Exemption – utilizes extraction studies using food simulants to provide evidence of safety margin, defines function barrier Basic Resin Exemption – general clearance list Non-detectable classification – specific limits and conditions Houseware Exemption – mainly for foodservice applications Threshold Regulation (not exactly an exemption, but is treated like one), specific migration limits based on dietary consumption rates
1997 Food and Drug Modernization Act	Established Food Contact Notification (FCN) System – a way to FDA processing of documents – faster way for companies to get new materials to market Parts of an FCN: Composition and Method of Manufacture Intended Use Conditions Amount of Additive in Food and Entering the Diet Safety of Additive Environmental Assessment

Adapted from: https://www.fda.gov/food/food-ingredients-packaging

Active packaging is defined as "a type of package that changes the condition of the package to extend shelf-life or improve safety or sensory properties of the product" (Vermeiren et al. 1999). In regards to food safety, active food packaging provides functions such as oxygen/carbon dioxide emitting and scavenging, ethylene control, moisture control, and antimicrobial activity (Quintavalla and Vicini 2002). According to European Union (European Commission 2009), an intelligent package contains a component that allows it to monitor the condition of the food packaging during shelf life to provide consumers, manufactures, and retailers reliable and correct information on the conditions of the food, the environment and/or the packaging integrity (Shukla et al. 2015). Moreover, an intelligent package integrates smart features such as sensing, communicating, recording, and detecting, to provide information related to the quality of food. For this reason, intelligent packaging improves food safety, quality, and extends the shelf life of foods (Lorite et al. 2016). In the future, the communication function of intelligent packaging will be greatly extended as they will inform to consumers more details about food (e.g. the level of freshness of a perishable product that can be read-out from the package) (Licciardello et al. 2013).

Cold chain refers to shipment of perishable food products under refrigeration or frozen conditions. Most of these foods have minimal processing such as blanching or pasteurization, and it is critical that the temperature not vary excessively. Temperature abuse conditions can occur and each time a perishable produce experiences such an event, the shelf life or even the safety of the food can be at risk.

All foods are susceptible to insect infestation post-packaging. Some foods will have larvae or eggs naturally present based on field harvest while other foods may attract insects due to their attractive odor. Warehouse and distribution attempt to control invading insects but often long-term storage or distribution via ocean vessel can present situations that make insect control challenging.

The following sections will describe these post-packaging strategies in detail. Some of the studies describe refinement to commercially available technologies (TTI, RFID, Cold Chair and Insect control) but some are still research concepts with potential for commercial implementation. Finally, challenges and future of post-packaging technologies will be discussed.

27.2 Active Packaging

Active packaging is often referred to as a technology that senses a change within the package and makes a change in response to the event. There are several existing active packaging technologies such as oxygen sachets or moisture absorbers. These technologies have been in existence long enough to have few innovations within the past few years, therefore, active packaging strategies that have been the focus of new developments will be covered. Table 27.2 summarizes the types of active packaging that will be discussed in the following sections. Antimicrobial packaging has been of

Table 27.2 Summary of active packaging technologies

Active packaging technology	Types
Antimicrobial	
Essential oils	Clove, basil, garlic, rosemary, ginger, lemongrass, lemon, thyme, eucalyptus, menthol, walnut, oregano, cinnamon, sweet fennel
Organic acid	Lactic acid, citric acid, acetic acid, benzoic acid
Polysaccharide	Chitosan
Biologic agents	Hydrolase – lysozyme Oxidreductases – glucose oxidase Bacteriocin – nisin Bacteriophage
Nano materials	Incorporated with silver, titanium dioxide, zinc oxide, magnesium oxide, copper oxide Carbon nanotubes Nanoclay
Vapor emitting sachets	Ethanol Essential oil – garlic, rosemary, ally isothiocyanate Chlorine dioxide
Pads	Purge control

Table 27.3 Summary of methods used to test efficacy of antimicrobial packaging

Test method	General description[a]	Measurement
Well diffusion	Agar seeded with selected bacteria, create wells in solidified agar, add antimicrobial solution to wells – good screening method to establish minimum inhibitory concentration of antimicrobial agent	Zones of inhibition
Spot on lawn	Agar seeded with selected bacteria, add antimicrobial solution to surface of agar – good screening method to establish minimum inhibitory concentration of antimicrobial agent	Zones of inhibition
Film on lawn	Agar seeded with selected bacteria, add antimicrobial film to surface of agar – good method to determine if antimicrobial agent will diffuse from packaging film A variation – soft agar overlay – antimicrobial film placed on seeded solidified agar with cooled agar over top of film – good for film that won't lay flat on agar surfaces	Zones of inhibition or Zones of clearing under the film
Shake flask or tube	Liquid media inoculated with culture of bacteria, place solution or film into culture and measure optical density over time with plating at each time interval	Log reduction
Food challenge	Selected food inoculated with culture of interest at a known concentration	Log reduction

[a]All methods should include a control with no antimicrobial with each test

interest for several years and a wide variety of test methods are used to determine their effectiveness. Table 27.3 summarizes methods commonly used to develop films and coatings applied to film for effectiveness.

27.2.1 Antimicrobial Films and Coatings

27.2.1.1 Essential Oils

Essential oils are volatile oils obtained from plants. The broad spectrum antimicrobial properties and the generally recognized as safe (GRAS) status of these oils make them a promising agent for active packaging applications. The essential oils can be applied to the product, worked into package coatings formulations, or encapsulated within the packaging material and released over time (Santos et al. 2017). Essential oils such as clove, basil, rosemary, ginger, lemongrass, lemon, thyme, eucalyptus, menthol, walnut, and more have been known to have antimicrobial and anti-oxidant activity (Santos et al. 2017). While essential oils are broad spectrum, different essential oils and various combinations of essential oils effect different microorganisms differently (Santos et al. 2016). Oregano, cinnamon, and sweet fennel essential oils were incorporated into cellulose acetate films. Significant microbial inhibition was observed; Gram negative *Eschercia coli* was more resistant than gram positive *Staphylococcus aureus and Penicillium* spp. (Santos et al. 2016). Pure oregano essential oils were shown to be the most efficacious against *S. aureus*, while the films with a 1:1 mixture of oregano and cinnamon essential oils were the most effective against *Penicillium* spp. (Santos et al. 2016). Encapsulating essential oils protects them and allows a controlled release into the food package environment over time. Essential oils have been encapsulated within milk proteins (Oussalah et al. 2004), alginate (Oussalah et al. 2007), low density polyethylene (Suppakul et al. 2008), whey protein isolate (Botrel et al. 2015), cellulosic resin (Emiroğlu et al. 2007), gelatin (Gómez-Estaca et al. 2010), chitosan (Gómez-Estaca et al. 2010), soy protein (Emiroğlu et al. 2007), cellulose acetate (Santos et al. 2016; Melo et al. 2012), and cassava starch (Souza et al. 2013). While studies have shown limited success, the rollout of these technologies is limited due to volatility of these compounds, low water solubility, and oxidation susceptibility (Santos et al. 2017). Essential oils can leave a slight to strong aroma on the food product. When choosing an essential oil for use as an antimicrobial in concentrations above the detection threshold, care should be taken that the oil chosen compliments the flavor of the food product.

27.2.1.2 Organic Acids

Organic acids have a long history of use as preservatives and are generally recognized as safe (GRAS). Studies of encapsulated organic acids have shown significant action against a broad spectrum of microorganisms (Cruz-Romero et al. 2013). However, these treatments are not effective against lactic acid bacteria and other acidophiles (Ouattara et al. 2000). Packaging materials used to create organic acid based antimicrobial films include chitosan, polyethylene, methylcellulose, whey protein isolate, soy protein isolate, alginate, corn zein, and starch (Quintavalla and

Vicini 2002). Fresh salmon was packed with 100% CO_2, a solution of citric acid (3%), acetic acid (1%), and cinnamaldehyde (200 μg/mL). Both acetic and citric acid alone reduced total plate counts, lactic acid bacteria, sulphur reducing bacteria, and *Enterobacteriaceae* in inoculation samples and samples containing natural flora of the salmon. Synergy between citric acid (3%), acetic acid (1%), cinnamaldehyde (200 μg/mL) and 100% CO_2 atmosphere, when used in combination, completely inhibited bacterial growth during a 14-day storage period at 4 °C (Schirmer et al. 2009). Many recent studies in the literature focus on synergistic combinations of acids and other microbial hurdles (Ouattara et al. 2000; Schirmer et al. 2009; Cagri et al. 2001; Ouattara et al. 2000).

27.2.1.3 Chitosan

Chitosan is a deacetylated derivative of the polysaccharide chitin. Chitin is found in the exoskeleton of arthropods and the cell walls of fungi and is the second most abundant polysaccharide after cellulose. Chitosan is GRAS, non-toxic, non-antigenic, biodegradable, extrudable (heat resistant), and has film forming properties. Chitosan has been studied as a broad spectrum antimicrobial agent; however, the mechanism of action is not fully understood (Reesha et al. 2015; Kim et al. 2011; Friedman and Juneja 2010). In laboratory tests chitosan imbedded in a polyethylene matrix and extruded onto films, migrated from the films and showed a significant reduction of *E. coli*. Researchers used these films to store chilled tilapia. The control sample (not treated with chitosan containing packaging) spoiled and was rejected at day 7, while the fish stored in the chitosan package had a shelf life of 15 days. Aerobic plate counts were significantly lower than the control on day seven, on day 15 the differences in aerobic plate counts were mitigated (Friedman and Juneja 2010). The efficacy of chitosan films is highly variable from study to study.

Chitosan films were formed by incorporating garlic essential oils, potassium sorbate, or nisin. The control chitosan film showed no inhibitory effect but when garlic oil was added inhibition was shown using a film on lawn method. Garlic essential oil, potassium sorbate and nisin when incorporated individually with chitosan all showed zones of inhibition against *S. aureus,* but antimicrobials were incorporated zones of inhibition were produced, but the antimicrobial effect was not attributed to chitosan (Pranoto et al. 2005). The molecular weight of chitosan seems plays a role in the antimicrobial action. Researchers created biopolymer films containing chitosan of varying molecular weights and tested the inhibitory action on various microorganisms. The results from agar diffusion assays showed that *Listeria monocytogenes, Escherichia coli* O157:H7, and *Salmonella* Typhimurium responded differently to the antimicrobial films. *L. monocytogenes* was the most responsive to treatment, however different treatments were more effective for different bacteria. The authors concluded that the molecular weight must be chosen selectively to control the specific target. The study also showed that different concentrations of bacteria responded differently to the treatments (Kim et al.

2011). The local environment exerts a major influence on the antimicrobial activity. Since chitosan is cationic, binding to the non-target negative food surfaces can occur. This sequesters the chitosan away from the target bacteria and can result in decreased efficacy in food trial studies. Since chitin is sourced from shellfish exoskeletons, the incorporation into food packaging would violate halal and kosher religious dietary restrictions. This potentially limits the applications of this technology.

27.2.1.4 Biologic Agents

Many enzymes and biologic products are natural antimicrobials and can be employed in active packaging systems. Four main classes of biologics are of particular interest, hydrolases, oxidoreductases, bacteriocins, and bacteriophages. Many biologics have GRAS status and are employed as processing aids and additives.

Hydrolases such as lysozyme, an abundant egg white protein, degrades the peptidoglycan layer of Gram positive bacteria and fungi. However, Gram negative bacteria are susceptible to lysozyme treatment with added Ethylenediaminetetraacetic acid (EDTA). Oxidoreductases, such as glucose oxidase are found in molds such as *Aspergillus niger* and *Pennicillium* spp. (Hanušová et al. 2013). They act *in situ* by scavenging oxygen (3) and generating reactive molecules such as peroxides (Hanušová et al. 2013). This scavenging of oxygen also helps prevent off flavors and color changes in certain foods. These enzymes can be covalently attached to films (Hanušová et al. 2013) or incorporated as coatings and applied to the surface of films (Mehyar et al. 2018; Nestorson et al. 2008). Enzyme stability is a concern. Conditions such as pH, temperature, mechanisms of attachment, water available for internal binding, and spatial accessibility to active site all contribute to the activity.

Nisin is a bacteriocin, which is a GRAS approved naturally occurring lantibiotic. It has been shown to inhibit a wide array of Gram positive organisms including *L. monocytogenes* (Matthews et al. 2010). Nisin is relatively temperature and chemically stable (Oussalah et al. 2007). Acidic solutions are required for solubilizing nisin blends (Matthews et al. 2010; Suppakul et al. 2008). In Gravure coating trials nisin coatings survived drying temperatures of 79 °C and retained significant inhibition of *M. luteus*. In more extreme conditions, nisin can withstand heat temperatures of up to 121.1 °C for 5 minutes before losing efficacy (Denny et al. 1961). Nisin coated films show bacterial inhibition in spot-on-lawn assays, however when introduced to the complex environment present on food surface the inhibitory effect can be reduced (Matthews et al. 2010). To see significant inhibition a nisin dose larger than the approved dose needs to be selected, which resulted in a 2-log reduction of *L. monocytogenes*. (Perna 2016)). The amount of nisin incorporated into the film did not release entirely, therefore, more work needs to be done to determine whether the amount of nisin that could be ingested would truly exceed the maximum approved dose of 10,000 International Units (IU) per gram of food.

Lytic bacteriophages (phages) are viruses that infect bacteria, replicate, lyse, and create new progeny phages from their bacterial host. These new progeny phages can then go on to infect other neighboring cells, continuing the lytic phage cycle. They are narrow spectrum and each type of phage is specific to their host bacteria. Phage treatments are inert to all non-target bacteria. The bactericidal action coupled with the treatment specificity makes them a promising antimicrobial candidate in specific biocontrol settings (Lone et al. 2016). GRAS bacteriophages are currently sold by a number of vendors (ListShield™ 2018). Free phages are often applied directly to food products. New research focuses on encapsulating phages into novel antimicrobial polymers, which can decrease bacterial growth on food contact surfaces (Lone et al. 2016). The rollout of these technologies has been limited due the low stability of phages. Phages are very sensitive to elevated temperatures (>42 °C) and high sheer force (ListShield™ 2018). Processing phage into films must be very gentle and natural polymers are thought to be suitable candidates. Encapsulated phage studies have shown success in decreasing bacterial counts; however, the activity of the phage significantly decreases over time (Gouvêa et al. 2015).

Bacteriophages were ionically bound to the surface of charged modified cellulose membranes used to store *Listeria* inoculated ready-to-eat (RTE) turkey breast. Under all temperature and environmental conditions 1.79- and 1.63-log-unit reduction after 24 and 48 hours, respectively in *Listeria* spp. cocktail was observed. The authors also performed the experiment with *E. coli* and its specific phages, observing similar results (Anany et al. 2011). Acetate cellulose films containing *Salmonella* phage showed significant inhibition using zone of inhibition studies. Upon storage of the material for 7 days, half of the efficacy was lost. All efficacy was lost by 14 days of storage. Puncture resistance, tensile strength, and modulus of elasticity all decreased in the resulting films (Gouvêa et al. 2015). Stability of the phages within the packaging systems and the effect on the polymer physical properties are two major limitations in regard to phage-based active packaging. As researchers screen new materials for phage stability this technology can progress. Vonasek et al. (2014) encapsulated phages within a whey protein film, this film remained active for 30-day storage period allotted for the experiment. The physical properties of whey protein films are not suitable to modern packaging needs, however incorporating phages into film coatings would give strong barrier polymers an antimicrobial layer. There may also be a potential allergen issue with whey based matrices. Rather than incorporating phages within the packaging material they can be stabilized in a hydrocolloid coating to be applied to a food or film surface.

27.2.1.5 Nano-materials

Nanomaterials are structures which are on the nanoscale. Nanostructured antimicrobials are thought to be very efficient in their antimicrobial activity as they have a high surface area-to-volume ratio when compared with higher scale structures (de Azeredo 2013). Nanoparticles can be derived from a wide array of materials such as silver and metal oxides.

Silver nanoparticles have broad spectrum antimicrobial action against Gram-negative and Gram-positive bacteria, fungi, protozoa, and some viruses; however, the mechanism of action is not well understood (de Azeredo 2013). Due to its thermal stability and low volatility it can be processed into a wide variety of materials (de Azeredo 2013). A number of studies have shown promise in packaging of food. Silver nanoparticles were incorporated into a sodium alginate film which showed significant inhibition in zone of inhibition studies. When the coatings were applied to carrots and pears, the shelf life increased significantly compared to the controls without silver (Mohammed Fayaz et al. 2009). Orange juice stored at 4 °C in low-density polyethylene bottles containing silver nanoparticles showed significant microbial inhibition. After a 56-day trial period the silver nanoparticle bottle showed significant reductions in aerobic plate counts and yeast/mold counts across the storage time for the study. The concentration of silver which migrated into the juice was considered low, at less than 10 ppm (Emamifar et al. 2010).

Metal oxide nanoparticles, such as titanium dioxide, zinc oxide, magnesium oxide, and copper oxide show antimicrobial activity and high thermal stability. It is thought that the action is due to the generation of reactive oxygen species (ROS). Titanium dioxide shows photocatalytic antimicrobial activity under UV exposure. (de Azeredo 2013). Zinc oxide nanoparticles have shown significant inhibition of *L. monocytogenes*, *S.* Enteritidis, and *E. coli* O157:H7. Depending on the concentration this treatment can be bacteriostatic or bactericidal (Jin et al. 2009). Zinc oxide seems to have a strong effect against gram-positive compared to gram-negative bacteria (Emamifar et al. 2010). Titanium oxide nanoparticles are slightly less efficacious than zinc oxide, but have similar stability with regards to processing (Marcous et al. 2017). When titanium dioxide is doped with silver, the photocatalytic activity is increased (Li et al. 2011). Chitosan/titanium oxide nanocomposite films were shown to increase the shelf life of tomatoes. The barrier properties of the chitosan films were increased by the addition of titanium dioxide. Researchers showed that the titanium dioxide films increased the photodegradation of ethylene within the package, increasing firmness and decreasing weight loss for tomatoes throughout the test (Kaewklin et al. 2018). Magnesium oxide nanowires were shown to have bacteriostatic activity versus Gram-negative *E. coli* and gram-positive *Bacillus* spp. Antibacterial activity of nanowires increased with increased concentration (Al-Hazmi et al. 2012). Copper metal oxide embedded in a polypropylene matrix showed significant antimicrobial action against *E. coli*. The efficacy is dependent on the release rate of copper ions from the bulk material and contact time (Delgado et al. 2011).

Other unique nanostructures have observed antimicrobial activity. Gelatin films containing carbon nanotubes showed activity against Gram positive and Gram negative bacteria in film-on-lawn assays. Gelatin/nanotube structure showed altered physical properties such as a lower affinity for water, a higher tensile strength, and a lower elongation at break. Safety concerns regarding carbon nanostructures were expressed, however once incorporated into the film the authors observed no migration into the food system (Kavoosi et al. 2014). Chitosan nanostructures have also

been examined for their antimicrobial activity, nano-chitosan seems to be a more effective antimicrobial than the bulk material (Ilk et al. 2016).

Nanoclay particles can be used to increase the efficacy of existing antimicrobial nanoparticle technologies. It is reported that incorporating nanoclays increased dispersion of the nanoparticles. In addition, it seems to affect the bacterial surface interactions (Ilk et al. 2016; Wei et al. 2013). Care needs to be taken in regard to toxicological aspects and migration of nanomaterials. The small size and large surface area of nanoparticles allow more contact with cell membranes, leading to a greater risk of absorption and migration. As a result, nanomaterials often display different properties than their counterparts. Toxicity data from non-nano counterpart materials cannot be attributed to the nanomaterials in question (Brayner 2008). Zinc oxide is non-toxic; however, nano-zinc oxide has genotoxic potential in human cells (Sharma et al. 2009). There is limited data regarding migration into food products. Environmental concern is also worth noting. If these materials become more widespread nanoparticles would enter the food chain, causing unknown environmental consequences (de Azeredo 2013).

27.2.1.6 Antimicrobial Emitting Sachets

Sachets placed inside a package can be used in a packagess to emit antimicrobial agents into the headspace of a product. Many products that alter the atmospheric conditions of the environment have already been adopted by industry (i.e. desiccants and oxygen scavengers). Novel antimicrobial sachets work in two ways: (1) by generating the antimicrobial agent *in situ* and releasing it (2) through binding of antimicrobial and subsequent release over time (Otoni et al. 2016). Volatile antimicrobials have the advantage of penetration; the gas can diffuse onto the target surface even if there is no direct contact between the target and the polymer.

Ethanol releasing sachets have been developed, which show broad spectrum antimicrobial action. The ethanol is pre-absorbed into silica gel inside the sachet, which is released into the headspace of a package over time (Utto 2014). Moisture in the product or in the headspace of the package is the main trigger to release ethanol from sachet (Utto et al. 2018). Zeolites have also been used as carrier matrices (Smith et al. 1987). Headspace concentrations ranging from 4% to 12% have been proven effective to prevent bacteria and mold growth. When choosing an appropriate sachet, the intended dose depends on the weight, water activity, composition, and desired shelf life of the food. In industry products such as Antimold mild® and Negamold® have been successfully rolled out for use in bakery, confectionery, and dry products (Pereira de Abreu et al. 2012).

Essential oils can also be used in binding-release sachet systems. Garlic essential oils have been successfully encapsulated in B-cyclodextrins and formulated into sachet. Exposure to stored sliced tomatoes significantly reduced mesophilic plate counts and yeast/mold counts. High levels were required for a significant load reduction; however, these samples scored well in sensory evaluations (Ayala-Zavala and González-Aguilar 2010). Sachets made out of porous high density

polyethylene (HDPE) resins were developed to carry cinnamon essential oils. Treatments with these sachets showed reduced growth of filamentous fungi (Espitia et al. 2011). Essential oils can also be carried by foam starch matrices. When rosemary and thyme oil sachets were exposed to mozzarella cheese a 2.5 log CFU/g reduction of *Listeria spp*. was observed at refrigeration temperatures. Inhibitory effects were also observed on the cheese for lactic acid bacteria and total aerobic bacteria (Han et al. 2014).

Allyl isothiocyanate is a volatile compound found in cruciferous plants, and is the major antimicrobial constituent in brown and black mustards. Allyl isothiocyanate is a wide spectrum natural antimicrobial with GRAS status. Allyl isothiocyanate sachets are emitting sachets rather than generating sachets. Carriers reported in the literature include diatomaceous earth, calcium alginate beads, and porous HDPE resin (Otoni et al. 2016). An emitting sachet based on a porous HDPE carrier was used to reduce *Aspergillus* sporulation in peanuts. A 4.81 log reduction was observed after 60 days. Allyl isothiocyanate could no longer be detected after 30 days, with a majority undetectable after 15 days (Otoni et al. 2014). Calcium alginate can serve as a carrier of allyl isothiocyanate in antimicrobial sachets. After 5 days of exposure on stored spinach leaves, a 1.6–2.6 log reduction in *E. coli* was observed when stored at 4 °C and a 2.1–5.7 log reduction was observed at 25 °C (Seo et al. 2012).

Chlorine dioxide gas is a broad-spectrum antimicrobial showing significant log reduction of bacteria, protozoa, yeasts, and molds (Popa et al. 2007; Gray 2014). Chlorine dioxide is a strong oxidizer; possessing a similar efficacy to free chlorine. It is also known to oxidize tastes and odors associated with algae blooms in drinking water. Recently it has been shown that chlorine dioxide gas treatment can decrease the respiration rates and ethylene release of tomatoes, further increasing shelf life by prolonging ripening (Guo et al. 2014). While chlorine dioxide is not associated with gross physical damage to cells (Gray 2014), it has been reported to have a bleaching effect on food surfaces at high exposure levels (Ellis et al. 2006). Most chlorine dioxide application strategies in the literature involves bulk gassing of food in sealed spaces through bulky chlorine dioxide generators. Successful applications of chlorine dioxide gas sanitation have been shown on sprouts, blueberries, strawberries, cantaloupes, peppers, tomatoes, spinach, chicken (Ellis et al. 2006; Prodduk et al. 2014; Mahmoud et al. 2008; Mahmoud et al. 2008; Trinetta et al. 2010; Han et al. 2000; Mahmoud and Linton 2008). When bulk gassing food products chlorine dioxide reacts readily and will often leave the center of the stack untreated, furthermore treatment ceases when the chlorine dioxide generator is turned off. Novel chlorine dioxide generating sachets can be applied at the primary package level providing more localized distribution of sanitizer, and therefore better penetration into the bulk stack. Furthermore, these systems release chlorine dioxide over time providing prolonged exposure to the product, thus providing a sustained efficacy (Popa et al. 2007; Delgado et al. 2011).

27.2.1.7 Antimicrobial Absorbent Purge Pads

Absorbent purge pads are widely used in industry for soaking up exudates in food products. This technology has been widely adopted and is ubiquitous in the meat industry. Exudate accumulation is rich in organic matter and macronutrients, furthermore it facilitates a high water activity environment. All these factors create an ideal environment for colonizing microorganisms. Traditional purge pads limit microbial growth through absorption of the exudate into the pads, reducing water activity and sequestering the microorganisms away from the food surface (Otoni et al. 2016). Through the addition of an active component these active purge pads can provide an additional hurdle by directly targeting the bacteria in the absorbed exudate.

Cellulose-silver nanoparticles were absorbed onto cellulose fiber purge pads. Fresh cut mellon was stored with the purge pads for 10 days under refrigeration. Inhibition was observed in psycotrophic organisms and yeasts after treatment. Considerable silver ions were present in the exudate (Fernández et al. 2010). Copper-cellulose pads have also been developed. Strong inhibition of yeasts was observed during *in vitro* experiments. Antifungal activity was observed when pineapple and melon juices were stored in contact with these experimental materials (Llorens et al. 2012).

Shelf life extension has been observed by spraying exudate pads from the peat industry with oregano essential oils. Sensory evaluations were carried out at various time points to determine shelf life. The treated product had a shelf life extension of 2 days compared to the product without oregano essential oil (Oussalah et al. 2007). Bacteriophage solutions can also be absorbed onto the surface of exudate pads. When incubated at 15 °C for 48 hours with solutions of *Salmonella* Typhimurium a log reduction of 4.36 was reached. Viable phages could be detected on the pad after the 48-hour testing period (Smith et al. 1987). These technologies represent novel biocontrol strategies associated with foods that produce significant exudates.

27.3 Intelligent Packaging

Intelligent package technologies can be categorized into three main categories: (1) indicators, (2) data carriers, and (3) sensors (Kerry et al. 2006). Indicators provide information to the consumer regarding food safety (e.g. an indicator that measures the temperature of frozen fish). Data carrier devices are used for storage, distribution, and traceability (e.g. a data carrier device that records temperature of frozen fish during the distribution). Sensors can be used in translating a physical or chemical property into a detectable signal (e.g. the pH of frozen fish as a sensor of food spoilage) (Lorite et al. 2016). Combinations of these categories can also be produced depending upon the food application. Commercialization of all these technologies varies. Indicators for temperature have been used in food and drug applications for

Table 27.4 Summary of Intelligent packaging technologies

Technology	Type
Time temperature integrator	Enzyme based – signals temperature profile
Radio frequency identification	Supply chain monitoring
Freshness/spoilage indicators	Detection of volatiles, pH change, carbon dioxide or humidity change

many years but sensors that accurately indicate food quality are less well developed. A summary of the intelligent packaging technologies discussed in this section is provided in Table 27.4.

27.3.1 Time-Temperature Devices

In food safety, one of the most important factors is controlling temperature to prevent the growth of microorganisms that cause foodborne illnesses. In order to prevent foodborne illness outbreaks, the FDA's Food Code categorized some foods as "time/temperature controlled food safety foods", which are foods that must be time/temperature controlled for their safety (FDA Food Code 2013). Consequently, the food industry must control and ensure that the temperature of "time/temperature-controlled food safety foods" must be maintained during all stages of supply chain (Aung and Chang 2014). Some intelligent packaging technologies can indicate whether foods have not met these temperature profile requirements. The current temperature devices that are commercially available are data carrier devices, smart radio frequency identification (smart-RFID), and time-temperature integrators (TTIs) (Rahman et al. 2018).

As stated earlier, data carriers are used for storage, distribution, and traceability. For example, an intelligent package with a data carrier that records the temperature of food during distribution. Smart-RFIDs are small electronic devices that use electromagnetic fields to automatically identify and track tags attached to food packages and measure the temperature of the package (Lorite et al. 2016). A TTI is a color change label that exhibits visual color changes depending on the time-temperature history thus predicting the food quality status. One of the newest TTI integrates a biosensor which provides digital information about food quality instead of just analog information (i.e. color change) (Rahman et al. 2018). The TTI is based on a glucose biosensor platform. The TTI system is composed of glucose oxidase, glucose, a pH indicator, and a three-electrode potentiostat (glassy carbon, Ag/AgCl, and platinum wire), which produces an electrical signal as well as color change. This TTI can function as a time-temperature integrator system that could be extended to that of a biosensor compatible with any electrical utilization equipment (Rahman et al. 2018).

Other researchers created a biodegradable prototype of a TTI with a prospective application in food tracking that incorporates Bluetooth (Salvatore et al. 2017). This TTI is a fully biodegradable temperature sensor, whose layout and ultrathin format confer a dynamic response of 10 ms and high mechanical stability. The active layer is made of magnesium and a commercially available polymer that acts as encapsulation, given its easy processing, biocompatibility, and small swelling rate. An array of sensors is integrated into a fluidic device made of the same polymer to yield a smart biodegradable system for flow mapping. The proper encapsulation extends stable electrical operation to 1 day and the connection to a Bluetooth module enables wireless functionalities with 200mK resolution. Due to these characteristics, the TTI has a prospective application in food tracking. Monitoring temperature when tracking a food is a critical factor (Salvatore et al. 2017).

Wang et al. (2018) created a prototype of a TTI that can indicate the frozen state and thermal history of foods using nanocomposites of chitosan and gold nanoparticles; optimally synthesized nanocomposite of chitosan and gold nanoparticles may be used as a detector for freezing conditions, indicated by pink to dark grey color change upon freezing for 1 day. This color change can be used to help ensure the quality of foods that may be affected by frozen storage (e.g. seafood, meat). Despite the longstanding recognition of TTIs as effective temperature device for monitoring, their commercialization is still in early stages due to multiple limitations such as legislative rules, accuracy, and quality indication by visible color change (Wang et al. 2018; Salvatore et al. 2017; Kim et al. 2016b; Dario et al. 2008).

Radio Frequency Identification (RFID) is more commonly used compared with TTI; many efforts have been made to improve RFID technology such as integrating different sensors like temperature, humidity, light, sound, and gas sensors with the use of RFIDs (Matindoust et al. 2016). In addition, RFIDs do not require passing through chemical baths so biodegradable and food compatible materials can be used (Lorite et al. 2016). In other words, when creating a RFID system, some of the materials can be food-contact materials, facilitating the process of accepting the RFID by the FDA. For this reason, the process of accepting a new RFID is faster and some researchers are developing new RFID technologies. For example, extra security to RFID system by adding user-defined access code on tag; turning on/off the tag operation; selecting the sent information by the user; interactive packaging; interactive advertisement; and low-cost user interface for application (Matindoust et al. 2016). One of the new RFIDs is one that integrates a critical temperature indicator (CTI), which would provide more information about the distribution chain temperature conditions and ensuring food safety. Lorite et al. 2016 developed a novel and smart RFID assisted CTI for supply chain monitoring. The developed CTI is based on the melting point of the non-toxic and transparent solvent dimethyl sulfoxide and a color change by adding a dye compound in the system. The CTI-smart sensor integrates the microfluidic-CTI to a RFID tag in order to remotely detect the melting of the solvent once the critical temperature is reached.

Researchers are developing new technologies of RFID and TTI as they are excellent tools that can be implemented to control temperature and other critical

factors related with foodborne illnesses. For example, TTI monitors the temperature of foods, a critical factor related with foodborne illnesses; specific temperatures increment the growth of microorganisms that causes foodborne illnesses. When comparing RFID and TTI devices, the TTI have several advantages including small size, low cost, and easy operation (Rahman et al. 2018) whereas one of the advantages of using a RFID is that the process of accepting an RFID by the FDA is faster because it is easier to use food-contact materials for RFID as RFID does not required passing through chemical baths.

27.3.2 Freshness and Spoilage Sensors

Consumers are increasingly looking for food products that are safe to prevent foodborne illnesses. Particularly for fresh produce and ready-to-eat foods (e.g. fresh cut fruits in take away cups). Fresh produce and ready-to-eat foods are in demand due to the growing consumer desire for easy to prepare and healthy foods. However, one of the major concerns is that fresh products and ready-to-eat foods have a higher risk of having microorganisms present that cause foodborne illnesses. Most fresh, ready-to-eat foods have not been thermally processed, which is one of the main controls to kill or reduce microorganisms that cause foodborne illnesses (Lorite et al. 2016). Therefore, food safety in fresh products and ready-to-eat foods must be ensured by controlling the deterioration process (including temperature) and quality according to the type of food product, the package, and the conditions in the supply chain (Licciardello et al. 2013). Freshness and spoilage sensors are an appropriate and easy tool for detecting the freshness or spoilage of fresh products and ready-to-eat foods. Researchers have created different type of sensors. For example, a sensor that evaluates fish spoilage by detecting the total volatile basic nitrogen and a rise in the pH (Aghaei et al. 2018); sensors that detects volatile basic nitrogen (Shukla 2015) and pH in meat (Kuswandi and Nurfawaidi 2017; Kuswandi et al. 2015); sensors that detect carbon dioxide (Bibi et al. 2017; Vargas-Sansalvador et al. 2017; Borchert et al. 2013), and sensors that detect humidity (Mills et al. 2017; Bridgeman et al. 2014; Mraović et al. 2014).

While sensors measure chemical factors (e.g. pH, carbon dioxide, humidity) to detect if a food is fresh or spoiled, humans use their sensory organs (i.e. olfaction, taste, sight, and touch). Overall, sensors are more accurate indicators to detect if a food is fresh or spoiled compared with the sensory organs of humans. Sensors measure chemical factors that are related with the biochemical reactions when food spoils. In contrast, sensory organs do not measure the chemical factors related with biochemical reactions of spoiled food. In fact, sensory organs are a subjective measurement that can vary from person to person and can therefore be difficult to design a signal sensor that would detect what a human can sense.

Currently developed spoilage sensors detect different chemical factors. For example, a color pH sensor detects the basic volatile compounds (i.e. volatile amines) when fish spoils. It is a simple and practical tool to detect if the food is

fresh as the pH color sensor can be read directly using a visual cue for the consumer (Pacquit et al. 2007). One disadvantage of using this single-style sensor is that sometimes a single sensor is difficult to determine the onset of detection related to spoilage threshold, where indicator could trigger spoilage when (Kuswandi et al. 2015). In order to eliminate this error in the sensor, researchers developed a dual sensor based on based on two pH indicators (i.e. methyl red and bromo cresol purple) to monitor beef freshness. These sensors have an accurate response to the beef freshness and have intense color changes when the beef is spoiled. Specifically, the methyl red changes from red to yellow, while the bromo cresol purple changes from yellow to purple. The dual sensor is placed in close proximity to the beef samples inside packaging, in order to detect the increasing of volatile amine generated by spoiled beef. This type of sensor has very distinct color change from red to yellow for methyl red in the surrounding of the dual sensor label, and pale yellow to purple for bromo cresol purple in the center. The pH is measured along with the dual sensor responses. When the dual sensor changes color, the pH changes leading to a more accurate sensor of spoiled beef as the sensor is measuring two chemical factors (Kuswandi and Nurfawaidi 2017).

Sensors can have been designed to measure factors that are related with the shelf life of a food (e.g. carbon dioxide and humidity). Carbon dioxide is produced by microorganisms and product of respiration of foods and vegetables. Consequently, detecting carbon dioxide concentration in some types of foods indicates presence of microorganisms that cause foodborne illnesses. Carbon dioxide sensors are solvent-based sensors that are based on the acidity of carbon dioxide. However, these sensors are not used in food packaging due to the long-term instability, arising from decomposition of the commonly used quaternary ammonium hydroxide derivatives (Vargas-Sansalvador et al. 2011). Researchers have created a carbon dioxide sensor without the quaternary ammonium hydroxide derivates; they created a water-based sensor that is prepared using metal cresol purple sodium salt as the indicator, glycerol as plasticizer and sodium hydrogencarbonate as a buffer in a matrix of hydroxyethyl cellulose. In such a way, the lifetime of the carbon dioxide sensor is increased due to a long-term stability (Vargas-Sansalvador et al. 2017). Wheat gluten has been used like a material in carbon dioxide sensors (Bibi et al. 2017). The dielectric properties of wheat gluten were modified in contact with carbon dioxide at high relative humidity (90%) and a temperature of 25 °C due to a structural change in the sensing material, where amino groups act as receptors to carbon dioxide molecules. Results showed that the dielectric permittivity and loss increased with carbon dioxide concentration indicating that the protein structure (i.e. wheat gluten) was modified. However, further studies are still required for the evaluation of the total potential of using wheat protein as carbon dioxide sensor (Bibi et al. 2017).

Sensors in food packaging can also detect the humidity; for example, a color temperature-activated humidity sensor with a color change based on the irreversible aggregation of methylene blue encapsulated within the polymer (hydroxypropyl cellulose) (Mills et al. 2017). The blue color and heat-treated film (methylene blue and polymer) responds to an ambient environment with a relative humidity

exceeding 70% at 21 °C within seconds, returning to their initial purple color. This color change is irreversible until the film is heat-treated once more. This humidity sensor can be used in food packages of some types of food (e.g. dry foods) that are at risk of growth of microorganisms that causes foodborne illnesses due to the high relative humidity (Mills et al. 2017).

Sensors to detect fresh and spoiled foods could have benefits by signaling when food is experiencing a temperature event that could reduce shelf life or provide conditions for foodborne pathogens to grow by potentially adjusting storage conditions after signaling and thereby prevent food from spoiling (Matindoust et al. 2016). Another benefit is the low cost and fast response compared to other type of detection methods (e.g. identification of microorganisms and emitted vapors) (Matindoust et al. 2016). Also, these sensors are easy-to-use devices as they have immediate results (e.g. color change) and do not require laboratory tests or training of the person that is using them (Bridgeman et al. 2014; Borchert et al. 2013). Finally, these sensors have irreversible reactions leading to a record of the event that caused the sensor to signal (Bibi et al. 2017; Mills et al. 2017).

27.4 Cold Chain Advancements

The distribution of fresh, minimally processed fruits and vegetables often involves long-haul in trailers fitted with refrigerated units referred to as reefers. Overseas distribution can involve shipment with refrigerated units attached to intermodal containers which can be lifted directly from the ship onto a tractor with trailer wheels and framework to link with the intermodal for over the road transit. The refrigerated unit may connect to an electrical source or be powered with diesel fuel, but in either case, the cooled air needs to be distributed throughout the trailer or intermodal interior as evenly as possible. Even cooling presents a challenge depending on how the inside of the trailer or intermodal is configured with regard to flooring that enhances air circulation, air duct work that allows circulation above the product load and the configuration of the load itself.

Trailers or intermodal floors that enhance air circulation from below the load are typically metal T-rails or deep channels that allow circulation below and through the pallet the product sits upon. Pallets may also be designed with stringers spaced apart to allow air flow. Load configurations within the trailer or intermodal have received attention with regard to studies that allow the best load efficiency in concert with air flow. Load configurations referred to as offset, pinwheel and centerline and sidewall all allow for space between pallet stacks to allow cold air to circulate around the products. Corrugated boxes within the pallet load may also have holes or perforations to allow air flow. In cases where air space is provided to improve air circulation, load bars, air bags or dunnage should be placed in areas that might cause loads to shift during shipping.

According the Defraeye et al. (2016), one challenge in shipping fresh produce is whether the product is pre-cooled post-harvest or is loaded prior to pre-cool. If the

product is not pre-cooled, the heat from the produce that is commonly given off as part of post-harvest respiration, must be dealt with inside the trailer or intermodal. Precooling is required in situations where de-infestation of possible invasive insects is performed during pre-cooling, but there are also situations where infestation steps in export citrus is not required and research was needed to optimize ambient temperature loading of citrus for energy efficiency and optimal product quality retention.

27.4.1 Temperature Control Materials

After packaging, foods must endure distribution to the final end user. When in distribution and storage, a packaged food can experience broad temperature fluctuations depending upon the mode of distribution and level of temperature control during shipping and warehousing. In addition, the number of times a product is shipped and stored until it reaches end use also varies. For example, fresh-picked strawberries at peak season may be shipped with less storage to get the product to market within the shelf life range of the product. However, when the season is not a peak and fewer packages of strawberries are available to ship efficiently, they may be stored or shipped in modified atmosphere conditions to extend shelf life until enough units of strawberries can be distributed efficiently and in a cost effective manner. Shelf stable foods are less affected than refrigerated foods and minimally processed to fresh refrigerated item. Frozen foods also have challenges with regard to temperature fluctuations that can affect product quality and shelf life. Currently, the focus is on development of temperature regulating materials to help reduce fluctuations in the cold chain of food distribution.

One approach to address the problem of temperate regulation within shipping and storage of foods is to implement temperature regulating materials (TRM), in some cases more specifically referred to as phase change materials (PCMs). For the purposes of this chapter, the abbreviation TRM will be used. TRMs work by absorbing or releasing heat within a narrow temperature range by changing the phase (physical state) of the material when a temperature event occurs. The most commonly used materials studied for TRM are based on organic materials such as paraffin and fatty acids, which can be incorporated into the primary package that contacts the food or may be part of the shipping container or even the liner of the insulating material used in shipping (Singh et al. 2018). Inorganic materials such as salts or metals may be used but often require a carrying agent since they are usually in liquid form. Therefore, encapsulation of these components has been studied for practical use in food packaging.

In a study performed by Chalco-Sandoval et al. (2014), a combination of polycaprolactone or polystyrene were examined as encapsulation matrices to be incorporated into paraffin using an electrospinning method. The resulting fiber materials were formed into slabs with paraffin and tested for a variety of characteristics including thermal capacity. The study found that the polycaprolactone/paraffin

hybrid slab stored at 4 °C was able to encapsulate a heat storage capacity equivalent to 44% of the weight of incorporated polycaprolactone for 3 months.

One specific example of a TRM for commercial application currently in the U.S. is the Cold Box produced by Pure Temp®, composed of palm, palm kernel, rapeseed, coconut and soybean oils. The product is incorporated into the layer of a re-useable shipping container that includes one-layer corrugated plastic with an internal panel of the TRM that is based on nano-encapsulated material specifically designed for cold regulation. The re-useable container has panels inside to surround the food and keep the food within a safe temperature range during distribution of meals. The containers are used to ship meals to children that are part of a food program to prevent hunger during summer when schools are not in session. The meals are loaded at one location and shipped by car or van (without refrigeration inside the vehicle) via a typical bus route to locations such as parks, churches or community center to allow dissemination to children as part of a summer feeding program. The meals contain fresh, nutritious food that require temperature regulation based on United States Department of Agriculture (USDA) requirements. (PureTemp 2019).

27.5 Insect Invasion Control

Insects are often attracted to food through the seals in a package. For paperboard packages such as boxes and cartons, tape may be applied to all flaps or adhesive patterns can be complex to slow ingress. For flexible packaging some polymers are easier to penetrate than others so a more resistant material can be selected but this option may be too expensive. Another option for laminate structures is to use an insect repellant adhesive that is often synthetic. As manufacturers move toward natural and organic foods, there is a desire to use natural insect control in food packaging. According to Bakkali et al. (2008), many essential oils have natural antimicrobial, antifungal and insecticidal properties. Oils include thyme, cinnamon, oregano, clove (eugenol), rosemary, onion (allyl disulfide), garlic (allyl mercaptan) and star anise (Park et al. 2018; Song et al. 2018; Chang et al. 2017).

One of the disadvantages of using essential oils is that they are volatile; therefore, most research on incorporation of essential oils for insecticides has involved methods to encapsulate, insert in nanoclay or blend with polymers to control release and improve efficacy. Kim et al. (2018) developed and tested halloysite nanotube containing clove oil for effectiveness against Indian mealmoth. The nanotubes with clove oil were encapsulated with polyethylenimine (PEI) and applied to the surface of low-density polyethylene using a gravure process. This material was proven to repel Indian mealmoth while maintaining good mechanical, optical and thermal properties of the film to which it was applied. Rather than nanotubes, Kim et al. (Kim et al. 2016a, b) used bio-based material to encapsulate cinnamon oil. The encapsulated material was applied to a low-density polyethylene similar to Kim et al. 2016a, b study and it was also applied as a solution coating with polypropylene.

Whey protein isolate/maltodextrin combination was found to be the most effective encapsulation formulation to repel moth larvae. The polypropylene solution coating provided slower release of the cinnamon active component compared to the print application (gravure). Song et al. (2018), encapsulated cinnamon oil using polyvinyl acetate then blended with ink, which was applied to polypropylene then laminated to low density polyethylene on a large-scale production. Microencapsulation of the cinnamon oil in the ink maintained the ability to repel Indian mealmoth and the release rate was slower than without microencapsulation.

Films were coated using a patented process with a mix of essential oil to prevent infestation of red flour beetle (Licciardello et al. 2013). Oils included citronella and rosemary to name a few. Since the essential oils used in the study carried strong aromas, the authors tested the effect of the material on the organoleptic properties of the wheat semolina in the package using the coated film and found that there was no aroma carry-over to the wheat semolina. A multilayer film of polypropylene/polyester/low density polypropylene structure with star anise oil used in the adhesive layer between polypropylene and polyester was found to repel Indian mealmoth for more than 3 weeks (Park et al. 2018).

Chang et al. (2017) studied oils from ginger, black pepper, garlic, onion and fennel. Extracts of onion and garlic were also screened for insecticidal effectiveness. The most effective was an extract of onion called allyl mercaptan, which was a core with rice flour as a wall structure of a microsphere. The microsphere was used to create a sachet which was used for packaging brown rice. The package with the sachet was effective for repelling rice weevil and did not have negative effects on the sensory properties of the brown rice (both uncooked and cooked).

27.6 Conclusions and Future Trends

There are a wide variety of innovative post-packaging developments that have taken place over the years. Many focus on natural applications to replace synthetic as well as methods to extend shelf life and better communicate shelf life to the consumer. Unfortunately, many of the active and intelligent packaging applications are not commercially available or limited in application but it is hoped these innovations will help drive the eventual implementation of the most effective solutions. Challenges to implementation for spoilage indicators include accuracy and considerations regarding liability if the indicators trigger spoilage when the food is actually not spoiled. A challenge for all of the antimicrobial essential oils is that they transfer an aroma to the food that may not be desirable depending upon the type of food. One of the main problems with implementation of active and intelligent innovations is that they can be produced on a lab-scale level, but few have been successfully scaled up to commercial production. Cost is the biggest barrier to future implementation of many of the post-packaging technologies discussed but as with many new technologies, economies of scale and new equipment developments eventually allow new materials to find their way to production. Of all the active and intelligent packaging

innovations discussed, spoilage indicators seem to have the greatest interest currently owing to the interest in food waste reduction. It is believed that any strategy that can help reduce food waste has a greater chance for success but work on antimicrobial packaging applications continue as another way to extend shelf life leading to less food waste as well as improved food safety.

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In the original version of this book, the country name in the affiliation of author Kevin M. Keener was printed incorrectly as Canada, which should be USA. This has been updated in this revised version of the book.

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