Advances in Thermal and Non-Thermal Food Preservation

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Edited by Gaurav Tewari and Vijay K. Juneja

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This book is dedicated to food processors, engineers, researchers, entrepreneurs, and students who will take the ongoing research in food preservation to another level in the years to come.

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Preface

Food preservation has been a long-lasting desire of human beings. The significant developments in food preservation started the day fire was discovered by prehistoric humans, which was followed by indigenous methods of food preservation such as pickling, oiling, and salting of different food types, whether raw or processed. Some of the earlier techniques are still in use and are available in several commercial formats. The major developments and needs in food processing and preservation started during wars, when extended shelf life of foods became a necessity. As a matter of fact, several food processing techniques—such as ready-to-eat food in pouches, aseptic processing of milk and liquid foods with particles, and ohmic/electric resistance heating of foods—were developed to achieve extended shelf life of foods for soldiers in wars. A transfer of technology occurred when consumers started demanding a food product with fresh-like characteristics, along with extended shelf life. Over the years, consumers became more and more educated about adding food preservatives and their adverse effects on long-term health. This all added to research and development and finally the commercialization of innovative food preservation techniques. This also gave birth to several non-thermal food preservation techniques, including ultra-high-pressure processing, which has begun to see commercialization since the late 1990s. Irradiation is also getting limited acceptability by consumers for several food products. Thermal food preservation techniques are being revisited and are being modified to provide consumers with a variety of food products with home-cooked meal characteristics.

Researchers not only in the United States but also all over the world have played a major role in the latest developments of food preservation techniques. Europe and Asia have seen more commercial food products with extended shelf life on the shelves of their grocery stores than in the United States. Due to stringent regulatory control in the United States and reluctant behavior of established U.S. food processors in adapting new food preservation techniques, several food preservation techniques are still in their commercial infancy. The ones gaining limited commercial success are ultra-high-pressure processing, retort pouch technology, and, to some extent, irradiation.

Despite the availability of different food processing and preservation techniques discussed in a plethora of books available in stores, a single book with special attention to advancement in thermal and non-thermal food preservation and with special emphasis on commercialization of food preservation techniques was still needed. That is this book.

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1 Basic Food Microbiology

Sadhana Ravishankar and Nicole Maks

Introduction

Microorganisms are present everywhere, and food is no exception. Microorganisms are commonly found in raw commodities and can get introduced into many processed commodities. Some microbes are beneficial in that they cause desirable changes in the food through the process of fermentation. Some cause undesirable changes in the foods that lead to spoilage and such products often become nonpalatable. Some other microorganisms cause human health risk in the form of foodborne diseases or food poisoning, when the food containing these microorganisms is ingested. The science of understanding these three different types of microorganisms is called *food microbiology.*

Food microbiology is an old science. Its history dates back to 1658, when a monk named Kircher, using a microscope, observed minute worms in spoiled meat and milk as well as in decaying bodies. Kircher described these as worms invisible to the naked eye. A few other notable mentions in the early food microbiology era also include Antonie van Leeuwenhoek, Lazaro Spallanzani, Nicolas Appert, and Louis Pasteur, among others. Leeuwenhoek was also one of the earliest persons to observe microorganisms under the microscope. Spallanzani was the first to show "sterility" of a thermally processed product. He showed that when meat broth was boiled for an hour and the container was sealed immediately after boiling, the broth did not spoil, and the appearance of microorganisms was prevented. Appert and Pasteur are the fathers of thermal processing. Appert developed the canning process. Pasteur is called the father of food microbiology since he based his discovery on science, and the process of pasteurization was named after his discovery of thermal processing of wine to inactivate undesirable microorganisms in the 1880s. Pasteur was also the first to discover food spoilage by showing that souring of milk was caused by microorganisms. He also showed that foodborne microorganisms were capable of causing diseases. Food microbiology has been developing since then as a science, and new foodborne disease causing agents have been emerging from time to time. In recent years, the foodborne disease reporting system has improved, and these illnesses are tracked very well by the Centers for Disease Control and Prevention (CDC) and other health agencies. The CDC, in collaboration with the U.S. Department of Agriculture (USDA), the U.S. Food and Drug Administration (FDA), and ten states (California, Colorado, Connecticut, Georgia, Maryland, Minnesota, New Mexico, New York, Oregon, and Tennessee), developed a project called Foodnet (Foodborne Disease Active Surveillance Network). This project will help public health officials better understand the epidemiology of foodborne diseases, estimate the frequency and severity of these illnesses, and identify what foods are involved (CDC 2000a). The CDC has also developed another project, called PulseNet, for identification of foodborne disease causing bacteria through DNA fingerprinting. PulseNet is a national network of public health laboratories that are connected to a central electronic database at the CDC and can do a comparison of fingerprint patterns for identification of foodborne bacteria (CDC 2000b). Both Foodnet and PulseNet are useful in investigations

of foodborne disease outbreaks. These show the technological developments of food microbiology as a science.

It is estimated that foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year (Mead et al. 1999). Known pathogens cause 14 million illnesses, 60,000 hospitalizations, and 1,800 deaths, while unknown agents are involved in 62 million cases, 265,000 hospitalizations, and 3,200 deaths (Mead et al. 1999). The annual economic losses attributed to foodborne diseases associated with medical costs, productivity losses, and business losses due to legal problems may be as large as $5 billion to $6 billion (CAST 1994). Foodborne disease causing agents can be classified into the following: bacteria, viruses, protozoans, toxins, and prions. Food spoilage agents are predominantly bacteria, yeasts, and molds. There are several factors, both in the food product and in the environment, that can affect the growth or survival of foodborne spoilage and pathogenic agents in foods, and food microbiologists develop appropriate measures to control these microorganisms in foods. In this chapter the following topics will be discussed: factors influencing growth and survival of various spoilage and pathogenic agents in foods, various foodborne spoilage and pathogenic agents, methods to detect microorganisms in foods, and methods (processing of food) to control these microorganisms in foods.

Factors Affecting Growth of Spoilage and Pathogenic Microorganisms in Food

Microorganisms in foods can originate from different sources, including plant parts such as fruits and vegetables, animals, birds, seafood, air, soil, sewage, water, humans, food ingredients such as spices and other additives, various food contact surfaces such as equipment, and other miscellaneous sources such as packaging materials, containers, flies, and rodents (Ray 2001). Various factors affect growth and survival of microorganisms in foods. These factors could be intrinsic such as properties of the food or the microorganism itself, or extrinsic such as those of the environment.

Intrinsic Factors

Acidity, pH, and Buffering Capacity

Most bacteria do not tolerate high acidity. Use of fermentation for food preservation is based on this ability of acids to inhibit microbial growth. Weak organic acids such as acetic, lactic, citric, and malic acids have a better antimicrobial action compared to inorganic acids such as hydrochloric acids, and hence such organic acids are more commonly used in foods and are produced during food fermentations. Acids can also interact with other factors or hurdles in inhibiting microbes. The pH is a function of the hydrogen ion concentration in the food. Foods with a pH below 4.6 are called high-acid foods and those with a pH above 4.6 are called low-acid foods. This limit was set because in foods below pH 4.6, *Clostridium botulinum* spores cannot sporulate and produce toxin. The optimum pH for growth of microorganisms is close to neutral (pH 7), and most bacteria do not grow below pH 4.6. Bacteria are more fastidious in their relationship to pH than molds and yeasts, with pathogenic bacteria being the most fastidious (Jay et al. 2005). In general, the optimum pH range for bacteria is 6.0–8.0, for yeasts it is 4.5–6.0, and for filamentous fungi 3.5–4.0 (Adams and Moss 2000). The pH range for growth of molds is 1.5–9.0, for

yeasts 2.0–8.5, for Gram positive bacteria 4.0–8.5, and for Gram negative bacteria 4.5–9.0 (Ray 2001). The buffering capacity of a food refers to its ability to resist any changes in pH; hence, this ability should be considered when acidifying a food with various acids or fermenting a particular food for preservations. Fruits and vegetables usually have a low buffering capacity compared to muscle foods. The high protein content of muscle foods aids in their buffering abilities. Fruits usually undergo more spoilage from molds and yeasts than bacteria, since yeasts and molds can grow at pH below 3.5. Alkalinity with a pH of 11 or above is also detrimental to bacterial growth.

Water Activity

Dry foods are believed to be free from dangerous microorganisms and safe for human consumption, because microorganisms cannot grow and proliferate under dry conditions and they need free water for growth. The water activity of a food is defined as the ratio of the water vapor pressure of the food to the vapor pressure of pure water at the same temperature (Jay et al. 2005). Pure water has a water activity of 1.00. The optimum water activity for growth of most bacteria is above 0.92. The lowest water activity at which most food spoilage bacteria can grow is about 0.90 (Fontana 2000). *Staphylococcus aureus* can grow at water activities as low as 0.86. In general bacteria require higher water activity values for growth than fungi, and Gram negative bacteria have higher requirements for water activity than Gram positive bacteria (Jay et al. 2005). The lowest limit for growth of yeasts and molds is a water activity of 0.60 with no microbial proliferation occurring below 0.60 (Beuchat 1983). The minimum water activities required for active growth of most Gram negative bacteria, most Gram positive bacteria, most yeasts, most filamentous fungi, halophilic (salt tolerant) bacteria, and xerophilic (tolerant to dry environments) fungi are 0.97, 0.90, 0.88, 0.80, 0.75, and 0.61, respectively (Adams and Moss 2000). Water activity is usually used as a preservative factor by the addition of salt (for pickling of vegetables, meat, and fish) and sugar (for fruit preserves, jams, and jellies) (Forsythe 2000). The lowest water activity for any microbial growth is 0.60 and below this value the spoilage of foods cannot be microbiological but may be chemical or due to insect damage (Adams and Moss 2000). The water activity can also interact with other factors such as pH, acid, and nutrients and either inhibit or promote bacterial growth.

Redox Potential

Redox potential (Eh) or the oxidation-reduction potential is the measurement of the ability of a substrate to gain or lose electrons. Redox potential is defined as the ratio of the total oxidizing power (electron accepting) to the total reducing (electron donating) power of a food (FDA 2001b). When electrons are transferred between two compounds, a potential difference is created between them. This difference that can be measured by an instrument and expressed in millivolts (mV) is redox potential (Jay et al. 2005). Highly oxidized products will have positive Eh values and highly reduced products will have negative Eh values. Generally aerobic bacteria can tolerate higher Eh values better than anaerobes that require negative Eh values for growth. Microaerophiles such as lactobacilli and *Campylobacter* can thrive well under slightly reduced conditions. The various ranges of Eh for microbial growth are +500 to +300 mV for aerobes, +300 to −100 mV for facultative anaerobes, and +100 to −250 mV for anaerobes (Ray 2001). Microbial growth in general

in a food reduces the Eh values since oxygen is depleted and reducing compounds such as hydrogen are produced by microorganisms (Adams and Moss 2000). The growth of microorganisms at certain Eh values can also be influenced by other food components such as presence of salts, the poising capacity (resistance to changes in Eh) of food, processing treatment that a food undergoes, and presence of active respiratory enzyme systems (FDA 2001b). Fresh foods derived from plants or animals are usually reduced due to the presence of acids, reducing sugars, and –SH groups of proteins and upon diffusion of oxygen become more oxidized (Ray 2001). Aerobic bacteria utilize the O_2 in the medium as they grow and can lower the Eh values of the environment while anaerobes do not.

Presence of Nutrients

The nutrients present in a food product affect microbial growth because microorganisms need certain nutrients such as carbohydrates, proteins, fats, vitamins, and minerals for growth and metabolism. *Salmonella* Enteritidis has a need for iron to grow well (Clay and Board 1991). Microbes derive their energy mainly from carbohydrates, alcohols, amino acids, and fats. Amino acids and proteins are the major source of nitrogen for microorganisms. Other nonprotein nitrogen sources are urea, ammonia, creatinine, and trimethylamine (Ray 2001). Some of the minerals required for microbial growth include phosphorus, calcium, iron, sulfur, manganese, and potassium in small amounts, which are present in most foods (Ray 2001). Depending on the nutrient content of the food, those microorganisms that can metabolize the most available substrate will likely dominate in that food. Plant-based foods in general are rich in carbohydrates, some proteins, vitamins, and minerals, while animal-based foods are more abundant in proteins, lipids, vitamins, and minerals. Some microorganisms are more fastidious in their nutritional requirements than others. In general, Gram positive bacteria are more fastidious in their nutritional requirements (Jay et al. 2005). *Staphylococcus aureus* and *Listeria monocytogenes* require certain B vitamins for growth (Jay et al. 2005).

Biological Structures of Food

Many raw foods have an outer covering that is a natural barrier to microbial entry and growth. Some such structures are shells of nuts and eggs, fruit and vegetable skins, hides of animals, outer coverings or husks of grains, and the testa of seeds. These outer barriers are usually composed of macromolecules relatively resistant to degradation, provide an inhospitable environment for microbes with characteristics such as a low water activity and low readily available nutrients, and often contain antimicrobial compounds such as short chain fatty acids on animal skin and essential oils on plant surfaces (Adams and Moss 2000). As long as the outer covering is intact, internal contamination will be prevented. However, these outer barriers can become damaged during transportation, handling, processing, and storage, allowing microbial entry and subsequent growth under suitable conditions. For example, once there is a small crack on the egg shell, microorganisms can enter and grow under favorable conditions. Damaged fruits and vegetables spoil more quickly than nondamaged ones. The maturity of plant foods can influence the effectiveness of the protective barriers (FDA 2001b). For example, the skin of very ripe fruits is softer and more prone to damage than nonripe fruits that have a firmer skin and pulp.

Antimicrobials (Natural and Added)

Antimicrobials, whether present naturally or added as preservatives, can affect microbial growth in foods. Many foods have their own inherent antimicrobial properties through certain antimicrobial compounds present in them. For example, certain spices contain essential oils that have antimicrobial properties. Some plant antimicrobial compounds include eugenol, thymol, carvacrol, cinnamic aldehyde in certain spices, allyl isothiocyanate in cruciferous vegetables, allicin in garlic and onion, phaseollin in green beans, oleuropein in olives, flavonoids in some fruits and tea, and caffeic acid in chicory root. Animal-based foods also contain antimicrobials. For example, milk contains lysozyme, conglutinin, lactoferrin, and a lactoperoxidase system; and eggs contain lysozyme, avidin, ovotransferrin, ovoflavoprotein, and conalbumin. Ovotransferrin, avidin, and ovoflavoprotein are proteins that bind to nutrients essential for bacterial growth and thereby prevent growth of microorganisms requiring these nutrients. Often the processing of foods can introduce antimicrobial compounds. Fermentation is one such process, wherein compounds such as hydrogen peroxide, bacteriocins, carbon dioxide, reuterin, diacetyl, or others are produced as metabolites of lactic acid bacteria that can inhibit other microorganisms. Smoking can introduce compounds such as phenols that are inhibitory to microbes. Maillard reaction compounds have antimicrobial properties (Mossel et al. 1995). A number of chemical preservatives are used as additives in foods and are approved for food use by FDA. Salts of organic acids such as lactates and diacetates are used in ready-to-eat meats and have inhibitory activity against certain foodborne pathogens. A combination of pediocin, sodium lactate, and sodium diacetate was found to have inhibitory activity against *L. monocytogenes* in frankfurters (Uhart et al. 2004). Sorbates and benzoates are used as preservatives against yeasts and molds.

Extrinsic Factors

Storage Conditions

The conditions under which a food is stored can greatly play a role in microbial growth. Among those conditions, temperature, time, and relative humidity of storage environment are important and can significantly affect microbial growth and survival. These extrinsic factors can also interact among themselves as well as with other intrinsic factors such as pH and water activity and can influence growth of microorganisms. Understanding such interactions is important in selecting the proper storage conditions for various food products.

Temperature of Storage

Microorganisms can grow over a broad range of temperatures. However, the optimum temperature of growth for most foodborne pathogens is between 30 and 37°C. Microorganisms can also grow at low temperatures but slowly. Based on their growth temperature ranges, microorganisms can be classified as psychrophiles (cold loving; optimum between 12 and 15°C), mesophiles (moderate temperature loving; optimum between 30 and 40°C), and thermophiles (heat loving; optimum between 55 and 65°C). Some of the mesophiles can also grow at low temperatures and are called psychrotrophs (growth at 0 to 5°C; optimum between 10 and 30°C). Many food spoilage microorganisms are psychrotrophs. Examples of psychrotrophs are molds, yeasts, *Pseudomonas, Yersinia, Serratia, Aeromonas, Listeria,*

Clostridium, Bacillus, Leuconostoc, and *Lactobacillus*. Most foodborne pathogens are mesophiles. Few foodborne microorganisms are thermophiles. One example is *Bacillus stearothermophilus*, a Gram positive sporeformer, which can cause spoilage of canned foods. The temperature of storage can greatly affect the lag phase as well as the exponential growth phase of a microorganism.

Time of Storage

The time of storage of a food product is determined by the product shelf life. That is why most retail products have an expiration date or a sell by date beyond which the product may not be safe to consume or in some cases not palatable. The time of storage also depends upon other extrinsic and intrinsic factors that affect microbial growth. When time alone at ambient temperature is considered as a factor for controlling microbial growth, it is better to hold the product for less than or equal to the lag phase of the foodborne pathogen of concern in that product (FDA 2001b). The time and temperature of holding is also important in cooling of cooked products since the growth of pathogens such as *Clostridium perfringens* is known to occur during improper or slow cooling of foods prepared in large quantities and stored in huge containers. Even if foods are prepared/cooked in large quantities, they need to be cooled promptly by storing in smaller containers for ease and speed of cooling. For most food products, time and temperature of storage go together and both should be considered essential for microbiological safety of a food product.

Relative Humidity of Storage

The relative humidity of a storage environment may alter the moisture content of a food product. The higher the relative humidity of the environment, the greater the chances for the water activity of the product to increase due to the exchange of moisture from the environment into the food product, and this may have a negative effect on the product shelf life. If the change in water activity is such that microbial growth is permitted, it could cause spoilage or render the food unsafe due to growth of pathogens. For example, improperly wrapped meats tend to undergo surface spoilage more quickly due to the high relative humidity in the refrigerator (Jay et al. 2005). Hence, storage at appropriate relative humidity is important. If the storage environment is dry or less humid and the product loses moisture, it may not affect the microbiological safety of the product; however, this may have negative effects on the sensory attributes. It is better to store foods that can easily undergo surface spoilage from molds, yeasts, or bacteria under conditions of lower relative humidities. Using gases could be an alternate measure to prevent surface spoilage in packaged foods.

Type of Packaging and Packaging Atmosphere

The type of packaging and packaging atmosphere can greatly influence microbial growth. Often gases are used in packages since they can inhibit microorganisms. For example, CO_2 could be lethal to aerobic microorganisms, and O_2 can inhibit anaerobic growth. Ozone is another gas used for sanitizing the surface of fresh produce as well as equipment. Ozone at 0.15–5.0 ppm concentrations has been shown to inhibit spoilage bacteria as well as yeasts (Jay et al. 2005). Other gases in vapor form are also used for sanitation purposes.

Various types of packaging using gases for preservation include modified atmosphere packaging (MAP), controlled atmosphere storage (CA), direct addition of CO_2 (DAC), and hypobaric storage (Loss and Hotchkiss 2002). The gases usually used in MAP include CO_2, O_2, and N_2 in various proportions depending on the type of product and microorganism of concern. The solubility of these gases depends on various other factors such as the storage temperature and food composition. For example, CO_2 has increased solubility at low temperatures and low salt concentrations (FDA 2001b). With modified atmosphere the interactions of various factors such as temperature, product to headspace gas volume ratio, package barrier properties, type and load of the microflora, and food composition play a major role in microbial inhibition (Loss and Hotchkiss 2002).

Processing and Packaging

The type of processing a food undergoes is one factor that determines the microbiological safety of the product. For example, canned foods are processed to a temperature high enough to inactivate vegetative cells as well as spores of pathogens and most spoilage bacteria and thus can be stored at room temperature for a long period of time. On the other hand, minimally processed foods have a shorter shelf life. Packaging after processing is important since recontamination of processed foods needs to be prevented. A good package should be able to prevent microbial entry. Handling of the products after processing and prior to packaging should be done carefully to avoid recontamination of the product. During processing, time and temperature of processing are important. If the required temperature and time are not reached, the process will be inadequate, allowing growth of surviving microorganisms.

Other Factors

Actions and Implicit Factors of Microorganisms

Raw foods are not sterile and can harbor a variety of microorganisms. The types that dominate can depend on the numbers present, the utilizable substrates present in the food, and the accumulation of metabolic products. Some bacteria can grow in the presence of other bacteria, having a synergistic interaction, while some others cannot, having an antagonistic interaction. Certain compounds such as bacteriocins produced by certain bacteria or acids produced by lactic acid bacteria can inhibit coliforms and promote growth of succeeding flora required for fermentations. In sauerkraut fermentations, leuconostocs grow initially and produce lactic acid, which lowers the pH of the product, and this allows growth of lactobacilli that can bring about changes associated with product flavor. At times some microflora are able to utilize nutrients required for growth of some others and compete better. For example, coliforms and *Pseudomonas* utilize amino acids and streptococci utilize certain vitamins required for *Staphylococcus aureus,* thereby inhibiting the growth of this pathogen (ICMSF 1980). In some instances, the growth of one microorganism can remove the inhibitory component and allow growth of another microorganism. For example, in mold ripened cheeses, mold growth can increase the pH, allowing pathogens such as *L. monocytogenes* to grow, which could compromise the safety of these products. The physiological state of a bacterium can affect its growth and survival. For example, exponential phase cells are easier to inactivate by many processes or treatments than stationary phase cells. Also, cells preadapted to certain stresses can resist some lethal stresses better than

nonadapted cells. Acid-adapted cells of *L. monocytogenes* survived better at low pH values of 4.0 and 3.5 than non-acid-adapted cells (Ravishankar and Harrison 1999), and acid-adapted cells were more tolerant of an activated lactoperoxidase system than nonadapted cells (Ravishankar et al. 2000). Cell to cell signaling and secretion of proteins have been known to occur in microbial populations as a warning of stress to the neighboring cells.

Preparation and Handling by the Consumer

The way a consumer prepares or handles a food product can affect its microbiological stability. Some products may require a particular temperature of storage once the packaging is opened, and consumers should be made aware of this. Many shelf-stable products held at room temperature need refrigeration once opened. Also, proper handling of the foods by consumers is important to avoid cross contamination, especially for foods that are consumed without further treatment. For example, the cutting boards and knives used to chop or slice raw meat should not be subsequently used to chop salad vegetables without further cleaning, since this could cause contamination of salad vegetables with harmful microorganisms that may be present in raw meat. It is always better to consume the food products by or before their expiration date or sell by date.

Product History and Traditional Use

The previous history and traditional use of a product tell about the microbiological safety of a product. For example, a product that has been implicated in a foodborne disease outbreak needs to be handled or processed and stored carefully to avoid such occurrence again. However, if there is any change in the product end use, processes, formulation, physical structure, processing, distribution, or storage, the storage time and temperature need to be revalidated, and history cannot be used as the basis for determining safety (FDA 2001b). Also, it should be considered if the food product or one of its ingredients has been involved historically as a vehicle of foodborne disease outbreaks due to abusive handling or storage at ambient temperature, or if adequate temperature control of the product has been the sole factor for preventing foodborne illness (FDA 2001b).

Microbiology of Food Spoilage

Spoilage refers to changes in a food product that make it sensorially unacceptable for human consumption. The change could be either physical damage (change in viscosity), chemical reactions (oxidation, pH or other changes), microbiological (off-odors, off-flavors, off-colors, slime formation), or changes due to insect and rodent damage. Microbial spoilage is more common than chemical spoilage, and it is estimated that 25% of the global food produced annually is lost postharvest or postslaughter due to microbial spoilage (Anonymous 1985). Food spoilage is a worldwide problem. In the developed nations, spoilage due to psychrotrophs, yeasts, and molds is predominant, while in developing nations insect and rodent problems are more common (Huis in't Veld 1996). The reason could be that in the developed nations refrigeration facilities are commonly available, and that is not the case in developing nations where prepared food is consumed immediately and not stored for longer periods. Spoiled food is not poisonous and, therefore, spoilage is a quality issue, not a safety issue (Forsythe 2000). However, the presence of

large numbers of certain indicator microorganisms that can cause spoilage may also be indicative of the presence of certain pathogens.

Food spoilage has been studied extensively and microorganisms causing food spoilage in different types of foods have been well characterized by food microbiologists. Though both foodborne pathogens and spoilage microorganisms undergo similar kinds of stresses in foods, spoilage microorganisms tend to withstand harsh conditions better, often develop resistance to chemical preservatives and sanitizing agents, and are able to outnumber foodborne pathogens both in quantity and types (Roller 1999). Gram et al. (2002) describe three different types of interactions as survival strategies among food spoilage bacteria. One type of interaction is antagonism caused as a result of competition for nutrients (such as iron that can be mediated by bacterial siderophore production), and there is subsequent suppression of less competitive species. The second type of interaction is called metabiosis, whereby there is a change in the spoilage profile of a microorganism due to the supply of nutrients from another microorganism present in the same food. The third type of interaction is bacteria cell to cell communication in which certain Gram negative bacteria are able to coordinate certain phenotypic traits such as hydrolytic enzymes by communication via N-acyl homoserine lactones.

During food spoilage, microbial growth produces certain metabolites causing biochemical changes in the food product. Dainty (1996) has described a number of compounds including volatile fatty acids, indole, hydrogen sulfide, metabolites of sorbates, 4-vinylguaiacol, D-alanine, gluconic and 2-oxogluconic acids, L- and D-lactic acids, acetic acid, ethanol, biologically active amines, methane, trimethylarsine, and other volatile compounds as metabolites produced during spoilage of food by microorganisms. Microorganisms produce enzymes in foods and the action of these enzymes can cause changes in the spoiled food. *Pseudomonas* can produce proteases and lipases, which can cause off-flavors in milk and other dairy products (Frank 1997). Whitfield (1998) has given an excellent review of the compounds responsible for off-odors and off-flavors produced in foods during microbiological spoilage. Along with the compounds, the specific microorganism responsible for formation of the compounds has also been described for most spoilage examples. This review clearly implies that for detection of specific spoilage, it is important to understand what kind of metabolites are associated with which microflora (Whitfield 1998). Also, the relationship between chemical and microbial spoilage needs to be better understood.

Methods for detection of specific spoilage microorganisms include spoilage potential, spoilage activity, yield factor determination, and chemical spoilage profiles (Dalgaard 1993). Each of these methods is based on correlating the bacterial concentrations with the off-odors or metabolites formed in the spoiled products. In chemical spoilage profiles, comparison of natural spoilage is done with controlled or experimental microbial spoilage. Foods that are high in nutrient content, especially proteins, have a pH close to neutral (low acid food) and a high moisture content, and hence, tend to spoil faster than nonproteinaceous foods or foods with low protein content. Also, some foods that do not undergo processing treatment such as fresh foods (seafood and meats) spoil faster than processed foods since they may have an initial load of spoilage microflora (in low numbers), which can grow and increase during storage, causing off-flavors, off-odors, or slime and spoilage. According to Forsythe (2000), on the basis of spoilage susceptibility, foods can be categorized as nonperishable or stable (for example, flour due to low water activity), semiperishable (for example, apples can spoil slowly due to improper handling and storage), and per-

ishable (for example, raw meat, due to high water activity and high pH). The growth of some spoilage microorganisms can be prevented by refrigeration and certain special types of packaging such as modified atmosphere packaging and vacuum packaging. Refrigeration, however, cannot completely prevent spoilage since the psychrotrophic microorganisms can still grow but rather slowly. The psychrotrophic microflora are comprised of bacteria, yeasts, and molds; however, yeasts and molds in general do not compete very well with bacteria except in situations where bacteria cannot predominate; for example, in foods with high sugar or acidity (Huis in't Veld 1996). The food spoilage microorganisms can be broadly classified as Gram negative bacteria, Gram positive (sporeformers, lactic acid bacteria, and others) bacteria, yeasts, and molds.

Gram Negative Food Spoilage Bacteria

Generally foods with pH close to neutral, high in moisture content, and stored aerobically undergo spoilage due to Gram negative bacteria. Among the Gram negative bacteria, *Pseudomonas* is the most predominant spoilage bacterium. It can cause psychrotrophic spoilage in a variety of foods such as milk, red meat, poultry, and seafood. *Pseudomonas* is widely present in the environment and can contaminate foods from several sources and utilize a variety of substrates (Huis in't Veld 1996). Spoilage by *Pseudomonas* can result in slime formation and pigments on the spoiled food (Dainty 1996; Dainty and Mackey 1992) and off-flavors and off-odors, especially in animal products due to metabolism of nonproteinaceous nitrogenous compounds (Huis in't Veld 1996). Other psychrotrophic Gram negative bacteria include *Aeromonas, Shewanella, Vibrio, Hafnia, Moraxella, Serratia, Flavobacterium, Erwinia,* and *Pantoea*. Vibrios are common spoilage microorganisms in fish and other seafood. *Erwinia carotovora* and *Pseudomonas* are responsible for about 35% of spoilage of vegetables (Forsythe 2000). *Pseudomonas* can cause postprocess contamination of pasteurized milk (Eneroth et al. 2000). Off-odors in pasterurized milk are produced by *P. putida* and *Yersinia intermedia* (Whitfield et al. 2000). At temperatures above 10°C, nonpsychrotrophic bacteria such as those belonging to the *Enterobacteriaceae* family will predominate and cause spoilage. In these cases, there is production of acid, gas, slime, rope, bitter flavors, and off-odors (Huis in't Veld 1996). Due to its red pigmentation, *Serratia marcescens* can cause "bloody bread" in bread (Brackett 1997).

Gram Positive Food Spoilage Bacteria

The Gram positive psychrotrophic spoilage microflora include species of *Brochothrix, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Micrococcus,* and *Vagococcus. Carnobacterium piscicola* is associated with fish spoilage. *Brochothrix thermosphacta* is found in raw meats, and MAP, as well as vacuum packaging, allows the growth of this microorganism (Huis in't Veld 1996). Enterococci are often used as indicator microorganisms. Lactobacilli and lactococci cause fermentations in the foods, which may be desirable for some foods. However, fermentations producing acids and other metabolites may be undesirable in some foods such as vacuum-packaged meat, poultry, and cured meats (Borch et al. 1996; Dainty et al. 1983). Lactic acid bacterial fermentation is also undesirable in fruit juices. For example, *Lactobacillus kunkeei* causes spoilage of grape juice through fermentation (Edwards et al. 1998). *Micrococcus* is a relatively heat-resistant microorganism, is present in raw milk in low numbers, and can survive pasteurization and

cause spoilage of milk. Beer spoilage called "rope" is caused by *Pediococcus* and *Acetobacter,* and lactic acid bacteria produce diacetyl in beer, which gives an undesirable flavor (Forsythe 2000). Lactobacilli are specific spoilage microorganisms identified in beer (Sakamoto et al. 2001), and *Pediococcus* species are important beer-spoiling microorganisms (Jespersen and Jakobsen 1996). *Leuconostoc, Lactobacillus, Streptococcus, Micrococcus,* and *Bacillus* were isolated from spoiled doughs, with the majority of the microflora being leuconostocs (Elliott 1980).

Spoilage by Sporeformers

The major sporeforming spoilage microorganisms belong to the genera *Bacillus* and *Clostridium*. Some strains of these microorganisms can cause psychrotrophic food spoilage. For example, psychrotrophic *Clostridium* causes spoilage of vacuum-packed beef and ham (Dainty 1996) and vacuum-packed cooked beef and pork (Hansen et al. 1995; Lawson et al. 1994). Psychrotrophic *Bacillus weihenstephanensis* can cause "sweet curdling" of refrigerated pasteurized milk (Jay et al. 2005). Psychrotrophic *Bacillus cereus* can cause sweet curdling and "bitty cream" in pasteurized milk stored under refrigeration conditions (Forsythe 2000). Sweet curdling is coagulation without significant acid or off-flavor and is caused by protease produced by the spoilage bacterium (Frank 1997). Bitty cream is formed due to the degradation of fat globule membrane by lecithinase (produced by *Bacillus cereus*), resulting in aggregation of fat in the cream (Frank 1997). Psychrotrophic *Clostridium* causes "blown pack" spoilage of vacuum-packed meats (Broda et al. 1996). *Clostridium tyrobutyricum* and occasionally *C. sporogenes* and *C. butyricum* cause gas formation called "late blowing" or "late gas" in aged cheeses (Frank 1997). *B. subtilis* and *B. licheniformis* can cause "ropiness" in bread, which is characterized by a stringy brown mass within the bread loaf (Brackett 1997). *Alicyclobacillus acidoterrestris* is an acid-tolerant sporeforming bacterium causing spoilage in fruit juices (Walls and Chuyate 2000). *Bacillus* and *Clostridium* also cause spoilage of canned food products. Some of the sporeforming bacteria causing spoilage of canned foods are *Bacillus stearothermophilus, Bacillus coagulans, Bacillus polymyxa, Clostridium thermosaccharolyticum*, and *Desulfotomaculum nigrificans* (formerly called *Clostridium nigrificans*). These mainly cause spoilage of canned foods arising out of underprocessing or damage to the can. *B. stearothermophilus* and *B. coagulans* cause "flat-sour" spoilage. There is no bulging of the cans and acids are produced from carbohydrates present in the food product. *C. thermosaccarolyticum* and *B. polymyxa* cause "swells" in the can. The swell could be a "hard swell" or a "soft swell." In hard swells the can bulges due to gas and explodes. There are two types of soft swells: "flipper" swells and "springer" swells, where one end of the can is bulged out. In flipper swells, if you press the bulged end, the other end pops out. In springer swells, if you press the bulged end, the other end does not pop out. *D. nigrificans* causes spoilage known as "sulfide stinker," in which the spoiled product has the smell of rotten eggs. In canned condensed milk "sweet coagulation" similar to sweet curdling is caused by *B. coagulans, B. stearothermophilus,* and *B. cereus* (Frank 1997).

Food Spoilage by Yeasts and Molds

Yeasts and molds can spoil a wide variety of food products including those with low pH, high sugar or salt content, or low water activity. These microorganisms can act on a

number of substrates including carbohydrates, proteins, lipids, and organic acids (Huis in't Veld 1996). Yeasts and molds cause spoilage of fruits, vegetables, and bakery products, and they produce pectinolytic enzymes that soften the plant tissues, causing rot (Forsythe 2000). The spoilage by yeasts and molds is most visible either in the form of spores or slime, often pigmented usually on the surface. Yeasts that can grow in high concentrations of sugar such as found in jams, jellies, and preserves are called osmophilic yeasts, and they can cause spoilage of these products. *Saccharomyces* and *Torulopsis* are examples of osmophilic yeasts. Yeasts associated with spoilage of dried fruits include *Zygosaccharomyces rouxii, Hanseniaspora, Candida, Debaryomyces,* and *Pichia* species (Brackett 1997). *Zygosaccharomyces bailii* can cause spoilage of salad dressings. Yeasts can spoil sweetened condensed milk and butter, and some yeasts involved in dairy product spoilage include *Kluyveromyces marxianus*, *Debaryomyces hansenii, Candida famata, Candida kefyr, Rhodotorula mucilaginosa, Yarrowia lipolytica,* and *Candida parapsilosis* (Fleet 1990; Frank 1997; Rohm et al. 1992). Molds causing spoilage of cheeses include *Penicillium, Aspergillus, Alternaria, Mucor, Fusarium, Cladosporium, Geotrichum,* and *Hormodendrum* (Frank 1997). *Rhizopus, Penicillium,* and *Aspergillus* can cause spoilage of bread. *Mucor, Rhizopus,* and *Thamnidium* cause spoilage of meat (Forsythe 2000). Thermally processed fruit products can be spoiled by molds such as *Byssochlamys, Neosartorya*, and *Talaromyces* (Brackett 1997). Molds can cause spoilage of grains and grain products as well as adverse changes in appearance (due to colored spores), flavor, and aroma (due to some volatile compounds) of these products and can cause an increase in the free fatty acid value (Brackett 1997).

Foodborne Disease Agents

Salmonella

Salmonella is a motile Gram negative rod-shaped bacterium. It is widespread in animals and environmental sources including water, soil, insects, and animal feces. The genus *Salmonella* is divided into two species, *S. enterica* and *S. bongori* with over two thousand serotypes, and *S. enterica* is subdivided in six subspecies where serotypes isolated from humans, agriculture, and foods usually belong to subspecies *enterica* (D'Aoust 2001). The two most widespread serotypes that cause human salmonellosis are Typhimurium and Enteritidis (CDC 2003).

The consumption of undercooked meats, poultry, dairy products, raw eggs, and egg products contaminated with *Salmonella* is often implicated in cases of salmonellosis. Other foods that have caused outbreaks include alfalfa sprouts, infant formula, orange juice, and ice cream (D'Aoust 2001). After the pathogen is ingested, it penetrates and passes from the lumen into the epithelium of the small intestine, which then becomes inflamed. It is estimated that 1.4 million cases of salmonellosis occur each year in the United States, with only approximately forty thousand being reported each year (CDC 2005). All age groups are affected, with young children, the elderly, and immunocompromised individuals most susceptible to severe infections. Symptoms of salmonellosis include fever, abdominal cramps, and diarrhea 6–48 hours after infection, with acute symptoms lasting 1–2 days. The infective dose can be as few as 15–20 cells and is dependent on age and health of the host (FDA 1998).

Listeria monocytogenes

Listeria monocytogenes is a motile Gram positive rod-shaped bacterium. It is a facultative anaerobe and can grow over a wide temperature range including refrigeration temperatures. *L. monocytogenes* can grow in the pH range of 4.39–9.4 and is salt tolerant. *Listeria* is ubiquitous in nature, occurring in soil, decaying vegetation, and water. Between 1 and 10% of humans may be intestinal carriers of *L. monocytogenes*, and it has been found in other mammalian and bird species (FDA 1998).

Although *L. monocytogenes* has been recognized as a human pathogen, it was not until the 1980s that it was known as a foodborne pathogen (Ryser and Marth 1999). *L. monocytogenes* is an intracellular pathogen that causes listeriosis. The bacterium invades the gastrointestinal epithelium; enters the host's monocytes, macrophages, or polymorphonuclear leukocytes; becomes bloodborne; and grows. Because it is present in phagocytic cells, this permits access to the brain and transplacental migration to the fetus in pregnant women (FDA 1998). Most clinical isolates of *L. monocytogenes* belong to serotypes 1/2a, 1/2b, and 4b. Listeriosis occurs as sporadic disease and epidemic outbreaks. The CDC estimates that up to 2,500 cases and 500 deaths occur from listeriosis each year in the United States (Mead et al. 1999).

Some foods involved in listeriosis outbreaks include coleslaw, soft cheeses, raw milk, and ready-to-eat deli meats and hot dogs. Foods become contaminated with *L. monocytogenes* by using unpasteurized milk in products and by postprocess contamination. There is a "zero tolerance" policy for *L. monocytogenes,* meaning that ready-to-eat foods contaminated with *L. monocytogenes* at a detectable level are considered adulterated.

Immunocompromised individuals, the elderly, pregnant women, and neonates are most susceptible to listeriosis. The infective dose of *L. monocytogenes* is not known and may be dependant on the strain and an individual's susceptibility, but it is thought that ingestion of fewer than one thousand microorganisms may cause disease (FDA 1998). In adults, listeriosis is characterized as invasive and noninvasive. Invasive illness, which has an onset time of a few days to 3 weeks, is characterized by septicemia, meningitis, and encephalitis. In pregnant women, intrauterine or cervical infections can occur, which may result in spontaneous abortion or stillbirth (FDA 1998). Noninvasive listeriosis has an onset time of 18–20 hours, is characterized by gastrointestinal symptoms such as nausea, cramps, vomiting, and diarrhea, and is accompanied by fever, malaise, and headache (Donnelly 2001).

Escherichia coli *O157:H7*

Escherichia coli is a Gram negative, facultatively anaerobic, rod-shaped bacterium. It is found in the lower intestine of warm-blooded animals. *E. coli* O157:H7 is a serotype referred to as enterohemorrhagic *E. coli* O157:H7 (EHEC). It was first recognized as a pathogen in 1982 (Riley et al. 1983). After a large multistate outbreak in 1993, it became recognized as an important threatening pathogen (Rangel et al. 2005). EHEC are acid tolerant and able to survive acidic conditions in foods and passage through the stomach.

There are approximately 25,000–73,000 cases of illness and 60–100 deaths due to *E. coli* O157:H7 each year in the United States (FDA 2001a; Mead et al. 1999). Some foods implicated in outbreaks are alfalfa sprouts, undercooked ground beef, lettuce, and unpasteurized juices. The infective dose may be as little as ten cells. *E. coli* O157:H7 produces

a verotoxin and causes severe damage to the lining of the intestine, hemorrhagic colitis that is characterized by acute abdominal cramps, and bloody diarrhea (FDA 1998). Vomiting is common but fever is rare. Illness onset is usually 3–4 days but ranges from 1–9 days, and illness lasts 2–9 days (Feng 2001). In about 2–15% of *E. coli* O157:H7 cases, hemolytic uremic syndrome (HUS) occurs (Dundas et al. 2001). HUS is characterized by renal failure and is more prevalent in children. Most children recover without permanent damage. In adults, HUS is accompanied by fever and neurological symptoms and is often referred to as thrombotic thrombocytopenic purpura and can have a mortality rate as high as 50% in the elderly (FDA 1998).

Campylobacter jejuni

Campylobacter jejuni is a Gram negative, slender, curved rod that is motile and microaerophilic. It is sensitive to drying, freezing, acidic conditions, and salinity, and therefore sensitive to environmental stresses. *C. jejuni* has been isolated from healthy cattle, chickens, birds, and flies and is sometimes present in streams and ponds (FDA 2001a). The intestines of poultry are easily colonized with *C. jejuni*. In commercial operations, most chickens are colonized within 4 weeks (Humphrey et al. 1993).

C. jejuni was first identified as a human pathogen in 1973, and it is the most diagnosed cause of human gastroenteritis with undercooked poultry and cross contamination from raw poultry being the major risk factors (Altekruse et al. 1999). The illness caused by *C. jejuni* is referred to as campylobacteriosis. There are an estimated 2.1 to 2.4 million cases of campylobacteriosis each year in the United States (Tauxe 1992). Most cases of campylobacteriosis are sporadic. Outbreaks have different epidemiological characteristics from sporadic infections and usually occur during the spring and autumn (Tauxe 1992). Raw milk, untreated water, and raw clams are some foods implicated in *C. jejuni* outbreaks (FDA 1998).

All individuals are susceptible to *C. jejuni*, but children and young adults are more frequently infected (FDA 1998). Those who become ill suffer diarrhea, abdominal pain, and cramping. Diarrhea may be bloody and can be accompanied by nausea and vomiting. Symptoms usually occur 2–5 days after exposure and last up to 1 week. There can be serious sequela in some people. Guillain-Barré syndrome (GBS), a serious sequela of *C. jejuni*, is a demyelating disorder that results in acute neuromuscular paralysis (Allos 1997). It is estimated that one case of GBS occurs for every one thousand cases of campylobacteriosis, and up to 40% of people with GBS have evidence of a recent *Campylobacter* infection (Allos 1997). Also associated with campylobacteriosis is Reiter syndrome, a reactive arthropathy, in which joint pain can last for months or become chronic (Peterson 1994). Both GBS and Reiter syndrome are thought to be autoimmune responses caused by *C. jejuni* infection (Altekruse et al. 1999).

Staphylococcus aureus

Staphylococcus aureus is a Gram positive facultative coccus that grows best under aerobic conditions. Staphylococci can tolerate up to 10–20% salt and 50–60% sucrose, and growth may occur at a water activity as low as 0.86 under aerobic conditions and at 0.90 under anaerobic conditions. The optimum growth temperature is 35°C, but it can grow from 7 to 47°C. *S. aureus* can grow in a pH range of 4.5–9.3 with an optimum between pH 7.0 and

7.5. *S. aureus* is commonly found in the nose and throat and on hands, fingers, hair, and skin of more than 50% of healthy people (Bergdoll 1979). It is also commonly occurs on the skin and hides of animals. Some strains are capable of producing a highly heat-stable protein toxin called enterotoxin that causes illness in humans and is produced when the population of *S. aureus* exceeds 10^5 cells/g (Newsome 2004).

Staphylococcal food poisoning is the name of the illness caused by enterotoxin produced by *S. aureus*. *S. aureus* was first associated with a foodborne illness in 1884 (Bennett 2001b). It is estimated to cause 185,000 cases of staphylococcal food poisoning annually in the United States (Mead et al. 1999). Foods can become contaminated during handling, coughing, or cross contamination with raw products that may contain *S. aureus*. Foods that can support the growth of *S. aureus* include proteinaceous foods such as meats, poultry, fish, milk, dairy products, cream sauces, salads (mayonnaise-based), puddings, and cream-filled bakery products (Newsome 2004). Staphylococcal food poisoning is often associated with institutions such as schools and prisons, where foods are prepared in large quantities.

The onset of staphylococcal food poisoning is quick, usually 2–6 hours. Symptoms are usually acute and depend on individual susceptibility to the toxin, the amount of contaminated food eaten, the amount of toxin ingested, and the general health of the individual. The most common symptoms are nausea, vomiting, retching, abdominal cramping, and prostration, although some symptoms may not occur. In more severe cases, headache, muscle cramps, and changes in blood pressure and pulse can occur. A toxin dose of less than 1.0 μg in contaminated food will produce symptoms, and in highly sensitive people, a dose of 100–200 ng will cause illness (Bennett 2001b).

Bacillus cereus

Bacillus cereus is a Gram positive, aerobic, rod-shaped sporeformer with spores that do not swell the sporangium. This microorganism can grow within a temperature range of 10–50°C with an optimum range of 30–40°C, although some strains are psychrotrophic. *B. cereus* can grow over a pH range of 4.9–9.3. *B. cereus* is widely distributed in nature; it is common in soil and on vegetation and can be isolated from a variety of foods, including dairy, meats, spices, dried products, and cereals.

All people are susceptible to *B. cereus* food poisoning or intoxication. There are an estimated 27,000 cases of foodborne illness each year in the United States due to *B. cereus* (Mead et al. 1999). *B. cereus* has been known as a foodborne pathogen since the 1950s (Bennett 2001a). Because *B. cereus* spores normally occur on many foods and generally survive cooking, germination and growth occur when foods are held at 10–55°C overnight. When the microorganisms grow to large numbers, toxin is produced. The presence of greater than 10^6 microorganisms/g of food is indicative of active growth and proliferation of the *B. cereus* and is consistent with a potential hazard to health (FDA 2001a).

There are two recognized forms of *B. cereus* food poisoning, the diarrheal and vomiting or emetic type. The diarrheal type is caused by a large molecular weight protein, and the emetic type is caused by a low molecular weight protein. The diarrheal type occurs 8–16 hours after ingestion and is characterized by abdominal cramps with profuse watery diarrhea, rectal tenesmus, and occasional nausea lasting 12–24 hours. The emetic type occurs 1–5 hours after ingestion and is characterized by an acute attack of vomiting and can last up to 24 hours (Bennett 2001a). Foods associated with the diarrheal type include ce-

real dishes containing corn and corn starch, mashed potatoes, vegetables, puddings, and sauces; the emetic type is often associated with fried or boiled rice dishes (Johnson 1984).

Clostridium

The genus *Clostridium* is a group of Gram positive, anaerobic rod-shaped sporeformers that are wide spread, commonly found in soil, water, air, and vegetation. There are two species of *Clostridium* that cause foodborne illness. These are *C. perfringens* and *C. botulinum*.

C. perfringens was first identified as a foodborne pathogen in the 1940s (Hobbs 1979). *C. perfringens* is aerotolerant, a rapid grower, and found in the intestines of humans and animals. It is different from other clostridia in that it is nonmotile. Its virulence is a result of production of toxins that are classified into five types (A–E) with type A causing foodborne illness. Each year, the estimated number of cases that occur in the United States is ten thousand (FDA 1998). Failure to rapidly cool cooked foods, especially large volumes, is the main cause of outbreaks with foodservice establishments being the most likely sites for acquiring illness. Meat and poultry products are the most common vehicles of *C. perfringens* (Labbé 2004). Foodborne illness is produced 8–24 hours after ingestion of more than 10^5 cells/g of food (Heredia and Labbé 2001). Vegetative cells that survive stomach acid reach the small intestine, where they multiply, sporulate, and release toxin (Heredia and Labbé 2001). The illness is characterized by severe abdominal cramps and diarrhea that lasts 24 hours, but less severe symptoms can last 1–2 weeks (FDA 1998) and are often confused with staph food poisoning.

C. botulinum was first isolated in 1897 from raw salted ham that caused an outbreak (Lund and Peck 2000). There are seven toxin types (A, B, C, D, E, F, and G) of *C. botulinum,* all of which produce a potent neurotoxin (BoNT). The types of *C. botulinum* differ in their tolerance to salt and water activity, minimum growth temperature, and heat resistance of spores (Pierson and Reddy 2001). Human botulism is caused by types A, B, E, and F. The toxin is heat labile and can be destroyed if heated at 80°C for a minimum of 10 minutes (FDA 1998). The spores are heat resistant and can survive in foods that are incorrectly or minimally processed.

Foodborne botulism results from the consumption of food in which *C. botulinum* has grown and produced toxin. Only a few nanograms of toxin are needed to cause illness. There are 10–30 cases of botulism each year in the United States, mainly due to improperly processed home-canned vegetables, although commercially produced foods are sometimes involved (FDA 1998). The toxin is absorbed and irreversibly binds to peripheral nerve endings. Symptoms may occur 12–36 hours or as long as 8 days after ingestion (Lund and Peck 2000). Initial symptoms probably caused by other products of *C. botulinum* metabolism include nausea and vomiting. Initially, the toxin affects neuromuscular junctions in the head and neck, causing double vision, inability to focus, droopy eyelids, dry mouth, difficulty speaking clearly, and inability to swallow (Lund and Peck 2000). Descending paralysis occurs and leads to death from failure of heart muscles and diaphragm. If not treated, the death rate is high; however, the overall death rate is 10% (FDA 1998; Pierson and Reddy 2001). Foods implicated in recent outbreaks include canned salmon, bottled chopped garlic in oil, and baked potatoes.

Another type of botulism is infant botulism, which affects infants under 14 months. It is thought to be caused by ingestion of *C. botulinum* spores that colonize and produce toxin

in the intestinal tract. Honey has been implicated as a source of spores, as well as nonsterilized foods and nonfood items in the infant's environment.

Yersinia enterocolitica

Yersinia enterocolitica is a Gram negative, facultative anaerobic rod. It is motile at 22–25°C, cold tolerant, and survives in frozen conditions for 12 weeks. *Y. enterocolitica* can grow over a temperature range of 0–44°C with an optimum range of 22–25°C, a pH range of 4.5–8.5 with an optimum of pH 7–8 and 0.5–5% NaCl. Not all isolates are pathogenic. *Y. enterocolitica* is prevalent in soil, water, and some animals, especially pigs. It can also be found in meat, oysters, fish, and raw milk.

Illness from *Y. enterocolitica*, yersiniosis, does not occur frequently, with approximately seventeen thousand illnesses per year in the United States (FDA 1998). Yersiniosis occurs 24–48 hours after ingestion and is characterized by severe abdominal pain often mistaken for appendicitis, fever, diarrhea, vomiting, and headache. Symptoms usually last 1–3 days. Some outbreaks have involved chocolate milk, tofu, and pasteurized milk (FDA 1998).

Vibrio *Species*

Vibrio is a Gram negative rod-shaped bacterium found in estuarine and marine environments. *V. parahemolyticus* is the species most commonly reported as a cause of *Vibrio*-associated foodborne illness (FDA 2001a). Illness occurs within 4–96 hours of ingestion of about one million cells when the bacteria attach to the small intestine and excrete toxin (FDA 1998). Illness is mild to moderate characterized by watery diarrhea and abdominal pain and can be accompanied by nausea, vomiting, fever, and chills lasting approximately 2.5 days. Outbreaks and sporadic cases occur more during the warmer months, and they are usually due to raw, improperly cooked, or recontaminated cooked shellfish and finfish (FDA 1998).

V. vulnificus is the leading cause of death associated with consumption of seafood in the United States (Tamplin 2001). Illness has a mortality rate of 50%, and it is more common in those individuals with an underlying health condition, especially of the liver (Tamplin 2001). Gastroenteritis occurs 16 hours after ingestion, and only 100 cells are needed to cause illness in predisposed persons (FDA 1998). Primary septicemia can occur, causing septic shock in those individuals. No major outbreaks have been attributed to *V. vulnificus*. Sporadic cases occur at greater frequency in warmer months and are usually associated with handling raw oysters.

There are two serogroups of *V. cholerae*, 01 and non-01. The 01 serogroup causes cholera. It may be found in U.S. temperate estuarine and marine coastal waters. Cholera usually occurs from poor sanitation. Because of excellent sanitation methods in the United States it has been nearly eradicated, but sporadic cases occur from shellfish harvested from fecally contaminated coastal waters (FDA 1998). Cholera occurs 6 hours to 5 days after ingestion of approximately one million cells; cholera toxin is produced in the small intestine, leading to mild to acute diarrhea, abdominal cramps, nausea, vomiting, and dehydration (FDA 1998). The non-01 serogroup is less severe. Illness occurs within 48 hours after ingestion of more than one million cells, causing gastroenteritis with diarrhea, abdominal cramps, fever, and occasionally vomiting and nausea; it is unknown how illness occurs, but an enterotoxin is suspected (FDA 1998). *V. cholerae* non-01 is found in U.S. coastal waters

and occurs from the consumption of raw, undercooked, or recontaminated cooked shellfish. There have been no major outbreaks; however, sporadic cases occur along U.S. coasts in warmer months (FDA 1998).

Viruses

Viruses cause a significant portion of reported foodborne illnesses in the United States. Foodborne viruses cause two types of illness that are transmitted by the fecal-oral route, gastroenteritis and hepatitis.

Viral gastroenteritis has slower onset than bacterial gastroenteritis and is characterized by diarrhea, instances of vomiting, and a self-limiting course of illness. Examples of this type of foodborne virus are the Noroviruses. They are transmitted by food, especially shellfish, water, and person-to-person contact. A mild brief illness occurs within 24–48 hours after ingestion and lasts 24–60 hours (FDA 1998). It is estimated the Noroviruses are responsible for about a third of the viral gastroenteritis cases not involving the 6- to 24-month age group (FDA 1998). Rotavirus is another cause of viral gastroenteritis. The infective dose is 10–100 particles, and because it is excreted in large number in diarrhea, infection can be readily acquired through contaminated hands and objects, usually being spread to food by food handlers (FDA 1998).

Hepatitis A is a foodborne virus that causes hepatitis. There are approximately 130,000 infections and 100 deaths each year in the United States due to foodborne hepatitis A (FDA 2001a). The infectious dose is unknown, but it is thought that only 10–100 virus particles are needed. Hepatitis A is usually a mild illness characterized by sudden onset of fever, malaise, nausea, anorexia, and abdominal discomfort, followed several days later by jaundice. The incubation period ranges from 15 to 65 days. The virus is produced in the liver and drains into the intestine via the common bile duct and is shed in the feces for 10–14 days before the onset of illness and rarely more than a week after onset of jaundice (Cliver 2001). Most cases are due to an infected food handler and some food products involved include frozen strawberries and raspberries, lettuce, and shellfish (FDA 2001a).

Protozoa

There are between 107 and 127 animal parasites that may be foodborne, and only those whose infective stage are encysted in or on food, animals, and plants are exclusively foodborne (Jackson 2001). All parasites are invertebrates belonging to the protozoa (single-cell animals) or metazoa, which include the helminths (worms). Generally, less than ten oocysts need to be ingested to cause illness.

Cyclospora cayetanensis is a coccidian protozoan for which humans are the only host. It is associated with sporadic cases and outbreaks involving raspberries, lettuce, and basil (FDA 1998). *Cryptosporidium parvum* is a protozoan that can cause illness by any food that was handled by an infected person and tends to occur more in day care centers that serve food; salad vegetables fertilized with manure and contaminated water are also sources (FDA 1998). *Giardia lamblia* is the most frequent cause of nonbacterial and nonviral diarrhea in North America (FDA 1998). It is more prevalent in children and is often associated with contaminated water, although some outbreaks have been traced to food contamination.

Some of the parasitic worms involved with foodborne illness include *Trichinella spi-*

ralis, a nematode (round worm) associated with meat, especially pork; *Anisakis simplex*, a nematode associated with raw or undercooked seafood; *Taenia saginata*, a beef tapeworm; *Diphyllobothrium* species, a tapeworm associated with raw or undercooked fish; and *Nanophyetus* species, a trematode (flatworm or fluke) associated with raw or undercooked fish.

Toxins

Rapid onset (minutes to hours) of symptoms is an indication of chemical intoxication, and illness can be very serious. Seafood toxins, many of which are potent neurotoxins, cause a significant number of illnesses each year (Hungerford 2001). Four types of shellfish poisoning are paralytic shellfish poisoning (PSP) caused by saxitoxin, diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), and amnesic shellfish poisoning (ASP). The toxin is produced by plankton, which the shellfish filter feed on, and toxin accumulates and is metabolized in the shellfish. There are a number of symptoms including neurological symptoms such as numbness, tingling, burning, incoherent speech, and respiratory paralysis and mild gastrointestinal symptoms (FDA 1998). Fish that feed on plankton can also become toxic, causing ciguatera and scombroid or histamine poisoning. Even though there are control measures in place in the United States, there are still a significant number of seafood poisonings each year (Hungerford 2001).

Intoxication can also be caused by fungi. These toxins are called mycotoxins. *Aspergillus* is one genus of fungi that produce mycotoxins. *Aspergillus* is ubiquitous and grows on a wide variety of substrates under varied conditions and has profuse sporulation (Bhatnagar and García 2001). Mycotoxins affect specific organs such as the liver, kidneys, skin, mucosa, and lymph tissue. Aflatoxin is one example of a mycotoxin causing aflatoxicosis affecting the liver. No cases have been reported in humans in the United States, and cases involving animals are sporadic, although outbreaks have occurred in developing countries (FDA 1998).

Prions

Prions are proteins of normal animal tissue that are misfolded and become infectious. These infectious proteins cause normal proteins to become infectious, leading to illness. Prions are thought to be absorbed during digestion. They are associated with a group of diseases referred to as transmissible spongiform encephalopathies (TSEs). In humans the illness is called variant Creutzfeldt-Jakob (vCJD) and in cows it is called bovine spongiform encephalopathy (BSE). BSE is known as "mad cow" disease. Similar diseases are chronic wasting disease in deer and elk and scrapie in sheep. All these diseases lead to fatal neurodegeneration.

The major concern for humans is the potential contamination of meat products by BSE-contaminated tissues or the inclusion of BSE-contaminated tissues in foods, including dietary supplements; high-risk tissues for BSE contamination include the cattle's skull, brain, trigeminal ganglia (nerves attached to the brain), eyes, tonsils, spinal cord, dorsal root ganglia (nerves attached to the spinal cord), and the distal ileum (part of the small intestine) (FDA 1998).

In North America, there have been eight cases of BSE with three cases in the United States and five in Canada (CDC 2006a). Up to this point, there have been 184,000 con-

firmed cases of BSE in the U.K. (CDC 2006b). There have been 190 cases of vCJD reported worldwide; of these, 160 of those occurred in the U.K., and two cases have been reported in the United States but have epidemiological evidence that the disease was acquired in the U.K. (CDC 2006a).

Detection Methods for Foodborne Microorganisms

Detection of foodborne microorganisms is an important step in producing a safe food. Timely detection helps in recalling and preventing an outbreak. The most commonly used enumeration method is the standard plate count. The food sample is homogenized with a diluent, appropriate dilutions are serially done and plated on appropriate media, and colony-forming units are counted after incubation. Injured cells are enumerated by plating on selective and nonselective media. Another method is direct microscopic count, which involves making a smear on a microscope slide, staining with an appropriate dye, and observing directly under the microscope for the presence of microorganisms. The most probable number method is a statistical probability method where three subsequent dilutions are made in nine or fifteen tubes for the three- or five-tube method, respectively, and the tubes are incubated at 37°C for 24–48 hours. The microbial population in the original sample is determined from standard most probable number (MPN) tables. Dye reduction techniques are based on the reducing capacity of the food sample, and methylene blue or resazurin are the common dyes used.

The rapid detection methods usually involve biochemical reactions or antigen antibody binding assays. A number of rapid test kits such as MicroID, API test kits, and latex agglutination tests are currently available for pathogenic and nonpathogenic microorganisms. A biosensor is the most recent technology for detecting bacteria either based on conductivity or other physical or chemical changes in the sample. By coating a specific antibody on the surface of the sensor, specific bacteria can be detected. Some of the molecular methods of detecting/identifying microorganisms include polymerase chain reaction, ribotyping, microarrays, multilocus enzyme electrophoresis, pulsed field gel electrophoresis, amplified fragment length polymorphism, restriction fragment length polymorphism, and nucleic acid probes. Some immunological methods include ELISA, serotyping, fluorescent antibody, and radioimmunoassay.

Adenosine triphosphate (ATP) assays are commonly used in the food industry to assess the efficiency of the cleaning and sanitation process. This method is based on measurement of ATP using a luminometer. Any soil present on a food contact surface will have ATP, which is detected by luminometer. For surface sampling, swab/swab rinse (using cotton, calcium alginate, or sponge swabs), RODAC (replicate microorganism direct agar contact) plates, agar syringe, sticky film, swab agar slant, and direct surface are used. For air sampling exposure plates, All Glass Impinger and the Andersen sampler are used. Detection methods for microorganisms including bioassays are explained in detail by Jay et al. (2005).

Food Preservation by Various Hurdles

Hurdle Concept

Humans learned to preserve foods well before the time of Spallanzani, Appert, and Pasteur. Fermentation and thermal processing are some of the oldest and traditional methods of preserving foods. However, the many different types of foodborne pathogens were unknown

then. In this day and age, we have the emergence and reemergence of a number of foodborne pathogens. Also, present-day consumers are very much aware of food safety, nutrition, health, and well-being, and they prefer nutritious foods with fresh taste and convenience that are safe. Achieving these using a single treatment is a rather difficult task since the dosage required of one particular treatment can be high and may affect the nutritious or sensory quality of food produced. Thermal processing is an effective technology for preserving foods but has a limitation of nutrient loss, and there are significant changes in flavor, color, and texture of foods. To meet the previously mentioned consumer demands, the concept of hurdle technology for controlling foodborne pathogens evolved. Hurdle technology involves a combination of traditional and novel technologies for controlling microbial growth in a food. When technologies are combined there is a possibility of using each with a lower dosage or less intensity, which would help preserve the fresh taste and nutritional quality of the food while targeting the microorganisms with many control measures more efficiently. The objective of the hurdle technology concept is to inhibit the growth and proliferation of unwanted microbes rather than to inactivate them completely, thus allowing for use of hurdles that are not too extreme (Leistner and Gorris 1995). Hence, hurdle technology is an ideal concept for mild preservation of foods. Often physical treatments are combined with chemical and/or biological treatments in the hurdle concept. About sixty different hurdles have been identified for use in foods to improve their stability and/or quality, and the most important ones are temperature (low and high), low water activity, acidity, low redox potential, competitive microorganisms, and preservatives (Leistner 2000).

Physical Treatments

Foods can be treated by a variety of physical methods. The most common physical treatment is use of temperature. Preservation of foods using low temperature is popular and practiced worldwide, and examples of this are refrigeration and freezing, which are the most common technologies in food preservation. Heat treatment of foods is also common worldwide and heat is one of the most traditional methods of preserving foods. Heat-based or heat-involving technologies include canning or retorting, smoking, steaming, ohmic heating, microwaves, and radio frequency heating. As we can see here there is overlap between heat and electromagnetic technologies. These technologies involve heat as a component combined with electricity or irradiation for food preservation. Irradiation methods involve treatment with ionizing radiation (gamma rays, x-rays), ultraviolet radiation, and microwaves. Controlling microorganisms by using electricity involves technologies such as ohmic heating and pulsed electric field processing. Pulsed food processing technologies include pulsed electric fields, pulsed light fields, and pulsed magnetic fields. High-pressure processing (also called high hydrostatic pressure, isostatic pressure, or hydrodynamic pressure) is another technology gaining popularity in recent years wherein high pressures (100–800 MPa) are used to inactivate microorganisms in foods. Ultrasound is a technology that utilizes high-frequency sound waves or sonication for treatment. Drying of foods is one method of food preservation since microorganisms require water to thrive. A combination of freezing with drying called freeze-drying is dehydration of the food under vacuum from the frozen state. Ray (2001) describes centrifugation, filtration, trimming, and washing as methods for physically removing microorganisms from solid and liquid foods. Most of the technologies mentioned above have been covered in detail in later chapters in this book and hence will not be discussed in this chapter.

Chemical Methods

Chemicals are used in a food processing environment as well as in food to control microorganisms. In a food processing environment wet cleaning is done using chemical detergents followed by sanitizers. Detergents remove soil from equipment and other food contact surfaces and sanitizers sanitize the surfaces. Chlorine and chlorinated compounds, quaternary ammonium compounds, iodophors, peracetic acid, and peroctanoic acid compounds are some of the sanitizers used in the food industry. Some sanitizers have both cleaning and sanitizing capabilities and are called detergent-sanitizers. Fumigation using hydrogen peroxide, formaldehyde, chlorine dioxide, ozone, or other gases is also used for sanitizing food contact surfaces and processing environments. In foods, chemical compounds used are called preservatives. Fermentation occurring in foods is a process occurring as a result of microbial action. Some of the chemical preservative compounds produced by lactic acid bacterial metabolism include acids (lactic and acetic), hydrogen peroxide, diacetyl, and CO_2. One of the major groups of chemical preservatives used in foods includes organic acids and their salts. Examples of organic acids used in foods are acetic, lactic, citric, propionic, malic, fumaric, sorbic, and benzoic acids and their salts. Sodium chloride commonly used as salt in foods has antimicrobial activity. Esters of p-hydroxybenzoic acid called parabens (methyl, ethyl, butyl, and propyl) are used as preservatives in a number of food products including jams, jellies, salad dressings, and beverages (Ray 2001). Certain fatty acid esters such as glyceryl monolaurate (monolaurin) have shown antimicrobial activity in foods (Davidson 1997). Nitrites, especially sodium and potassium salts, are used as preservatives in cured meat products. Phosphates such as trisodium phosphate, tetrasodium pyrophosphate, and sodium tripolyphosphate are used as antimicrobial rinses. The use of sulfur dioxide as an antifungal agent is well known. Sulfites such as potassium sulfite, sodium sulfite, bisulfite, and metabisulfite are used as preservatives in wines, beverages, and fruit products. Sulfur dioxide and sulfites are also used as antibrowning or antioxidant agents in fruits and vegetables. Ethyl and propylene oxides are used as fumigating agents to inactivate microbes and insects in grains, cocoa powder, nuts, dried fruits, spices, and packaging materials (Ray 2001). Antioxidant compounds such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), and t-butyl hydroquinone (TBHQ) have antimicrobial activity. ethylenediaminetetraacetic acid (EDTA) and its salts show antimicrobial activity along with other compounds such as antioxidants, divalent cations, or other membrane acting compounds (Ray 2001). Dimethyl dicarbonate is an antimicrobial liquid compound against yeasts and some bacteria (Davidson 2002). Some of the Maillard reaction compounds are also antimicrobial. Compounds such as phenols, cresols, carbonyls, organic acids, and formaldehyde produced during the smoking process from wood smoke or liquid smoke have preservative action. Packaging using modified atmosphere with gases is used to increase shelf life of certain products. Vacuum packaging is removal of oxygen or air during packaging, which helps in controlling aerobic microorganisms. In modified atmosphere packaging, oxygen is removed, and the package is flushed with N_2 or CO_2 either alone or in combination.

Biological Treatments

The biological treatments are usually antimicrobials from natural sources and can be divided into three categories: antimicrobials from microbial sources, antimicrobials from plants, and antimicrobials derived from animals. The largest group of antimicrobials from

microbial sources is bacteriocins. Bacteriocins are protein molecules released by some bacteria; they inhibit closely related species and strains of the same species. Gram positive bacteria are more sensitive to bacteriocins than Gram negative bacteria since the outer membranes of Gram negative bacteria are impermeable to bacteriocins. However, if the membrane can be damaged by some other treatments such as high pressure, permeability increases and hence combination treatments will be effective against Gram negative bacteria. The use of bacteriocins and the microorganisms that produce them are attractive to the food industry because of the increasing consumer demand for natural products and increasing concern about foodborne disease (Montville and Winkowski 1997). The food applications of bacteriocins are discussed by Chikindas and Montville (2002). Nisin is the most well-known bacteriocin produced by *Lactococcus lactis*. In 1988, nisin was approved as Generally Recognized As Safe (GRAS) by FDA. It is heat stable and is used commercially in cheese spreads and liquid whole eggs. Pediocin is a bacteriocin that has not been approved yet. It is produced by *Pediococcus acidilactici* and *P. pentosaceus* and is stable to heat and pH. A combination of pediocin with sodium lactate and diacetate reduced *L. monocytogenes* population by 2.5 logs in frankfurters stored at 4°C for 3 weeks (Uhart et al. 2004). There are other bacteriocins produced by bacteria, such as Colicin V produced by *E. coli* and Enterococcin or Enterocin produced by enterococci. Reuterin (3-hydroxypropionaldehyde) is a nonproteinaceous antimicrobial compound produced by *Lactobacillus reuteri* during the anaerobic metabolism of glycerol. This compound is stable to heat and pH and has antimicrobial activity against Gram positive and negative bacteria. Bacteriophages can infect specific bacterial species forming plaques. Coliphages and *Salmonella* phages are used as indicator microorganisms in ground beef and poultry meat. Bdellovibrios are microorganisms that can infect and lyse Gram negative bacteria such as *E. coli* and *Salmonella* (Fratamico and Cooke 1996). Some yeasts secrete compounds called killer toxins that can inactive other yeasts, and these compounds have been found in a number of fermented foods such as beer, wine, and soy sauce (Gould 2002).

Plant-derived antimicrobials include essential oils as well as spice and herb extracts. Essential oils are the compounds responsible for odor, aroma, and flavor of spices and herbs and are soluble in ethanol. Many essential oils show antimicrobial activity, though such activity is affected by proteins and fats in foods. Examples of essential oils are eugenol from cloves, thymol from thyme, carvacrol from oregano, and oleuropein from olives. There are some excellent reviews on the antimicrobial activity of spices and herbs (Shelef 1983; Zaika 1988). Saponins are glycosides present in leaves and stems of legumes, cereals, alfalfa, and asparagus. They have good foaming properties and could be utilized in carcass washes. Thiosulfinates are compounds present in Allium species such as onions, garlic, and leeks. Alliin, allicin, and ajoene are some compounds responsible for the antimicrobial activity of onions and garlic. A number of studies have shown the antimicrobial activity of garlic paste, garlic essential oil, garlic oil, and fresh garlic juice against common foodborne pathogens (Friedman et al. 2002; Gupta and Ravishankar 2005; Ross et al. 2001; Uhart et al. 2006; Unal et al. 2001). Glucosinolates are sulfur-containing glycosides present in vegetables of the genus *Brassica* such as cabbage and cauliflower. These compounds upon hydrolysis yield isothiocyanates that have antimicrobial activity. Allyl isothiocyanate in vapor form showed antimicrobial activity (Delaquis and Sholberg 1997; Lin et al. 2000). Flavonoids (flavones, isoflavones, flavanoles, anthocyanins, and catechins) are antimicrobial compounds present in fruits such as citrus, grapes, and apples and in tea. Galangal is the compound responsible for antimicrobial activity of ginger, and sev-

eral researchers have studied the antimicrobial activity of this spice (Friedman et al. 2002; Gupta and Ravishankar 2005; Ibrahim et al. 2004; Ji et al. 1997; Uhart et al. 2006). Studies have shown that carrot has antimicrobial activity against *E. coli* (Abdul-Raouf et al. 1993; Babic et al. 1994). Vanilla is a common flavoring compound in many commercial products. Vanillin is the compound responsible for antimicrobial activity of vanilla. Vanilla has antimicrobial activity against *L. monocytogenes* in some commercial products (Tipparaju et al. 2004).

The antimicrobial compounds derived from animal sources are mainly those present in milk and eggs. In milk, there are some enzyme systems such as lactoperoxidase system and lysozyme and proteins such as lactoferrin and lactoferricin. An activated lactoperoxidase system consists of the enzyme lactoperoxidase, thiocyanate, and hydrogen peroxide. The lactoperoxidase enzyme catalyzes the oxidation of thiocyanate by hydrogen peroxide to hypothiocyanate and other toxic compounds that have antimicrobial activity. Lactoferrin is an iron binding protein present in milk and colostrum that inhibits bacteria by depriving them of iron needed for their growth. Lactoferrin (also called lactoferrin B) is a small peptide molecule produced during acid-pepsin hydrolysis of lactoferrin and has antimicrobial activity against a number of microorganisms. Eggs contain the following antimicrobial compounds: lysozyme, ovotransferrin, ovoflavoprotein, avidin, ovomucoid, and cystatin. Lysozyme is approved for use in a number of food products and is effective against Gram positive bacteria. In Gram negative bacteria, lysozyme could be made more effective with another treatment (physical, such as high pressure, or chemical) that can cause bacterial membrane rupture. Ovotransferrin (also called conalbumin) is similar to lactoferrin in that it binds to iron, which is necessary for bacterial growth and inhibits them. Ovoflavoprotein binds to riboflavin and avidin binds to biotin, making these essential vitamins unavailable for bacterial growth. Ovomucoid and cystatin are protease inhibitors inhibiting serine protease and cysteine protease, respectively, thereby preventing the synthesis of these amino acids needed for bacterial growth.

Hazard Analysis Critical Control Point (HACCP) Program

The HACCP program is a food safety program adopted by the industry to assist in identifying factors that could cause a hazard during processing and preventing or controlling such factors. It is a plan of evaluating the risks associated with different stages in processing and using the results of the evaluation to develop a plan to address these risks. The concept was first discussed by Dr. Howard Bauman in 1971 and became mandated by the USDA and FDA in the 1990s for large establishments followed by medium-sized and small establishments. HACCP is a systematic approach based on seven principles: (1) Conduct hazard analysis—identify the steps in the process with hazards and describe preventive measures; (2) Identify the critical control points (CCPs) in the process (e.g., time and temperature of treatment or storage); (3) Establish critical limits for preventive measures for each CCP; (4) Establish CCP monitoring requirements and establish procedures for using the results of monitoring to modify the process and maintain control; (5) Establish corrective action to be taken when monitoring indicates that there is a deviation from an established critical limit; (6) Establish procedures for verification that the HACCP system is working correctly; and (7) Establish effective record-keeping procedures that document the HACCP system. Thus the HACCP system is useful for assessing the risks associated with food production and documenting the safety of the food production process, thereby

helping in ensuring a safe food supply. HACCP in combination with good sanitation measures and hygienic practices will help the food industry deliver a safe food product to the consumer.

Summary and Conclusions

Food microbiology has been an interesting science to mankind for centuries and continues to deepen its roots in recent years. Microorganisms in foods continue to be a problem both to the safety and quality of food products, and hence food safety continues to be an important issue for the industry. Pathogenic microorganisms are emerging and/or reemerging due to several factors including globalization of the food supply and consumer demand for fresh-tasting, convenient to use, microbiologically safe foods. Spoilage microorganisms affecting the quality and sensory attributes are also a threat to the food supply. There are also other agents causing foodborne disease such as viruses, parasites, toxins from seafood and other sources, and prions causing the brain-wasting disease. There are several factors affecting the survival and growth of microorganisms in foods, and understanding these factors will aid in controlling microorganisms in foods. Detecting microorganisms in foods is an important issue, and there are several methods available. The industry is continuing to develop rapid methods for better detection. There are several technologies (physical, chemical, and biological) used to control or reduce microorganisms in foods. Apart from the traditional processes such as thermal processing, novel technologies such as high-pressure processing, pulsed electric field processing, irradiation, and others are also being developed. A combination of technologies termed the *hurdle concept* is also becoming popular among food technologists. The HACCP approach is also very useful is developing a risk-limited or risk-free food production system. Using appropriate detection and control methods, the industry should be able to produce a safe food supply for the current consumer.

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Part One
Thermal Food Preservation

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2 Thermal Processing of Liquid Foods with or without Particulates

Gaurav Tewari

Introduction

Sterilization of canned foods is essential to ensure safety to the consumer by assuring destruction of microbial growth. Thermal processing of canned foods is the most important means of achieving commercial sterilization of canned foods, therefore heat transfer in canned food systems has been a matter of interest to researchers. During heating of foods, microbial destruction is also accompanied by nutrient degradation, which is of particular concern to the process designer. This problem can be overcome by using high-temperature-short-time (HTST) processes for sterilization of canned foods, as these processes ensure quality retention because of minimization of nutrient degradation and maintenance of degree of sterilization (Stoforos 1988). Agitation of canned liquid foods (with or without particulates [solid-food particles]) results in high heat transfer rates. Thus thermal processes with high heat transfer rates from heating source to the food result in better quality. The traditional method of sterilization (i.e., the retort method) and the aseptic method are commonly used and predominant thermal processing techniques for commercial sterilization of canned foods. Convection is the main mode of heat transfer between heat source and fluid, and also at fluid-particle interface, during these thermal processing techniques.

The traditional method of sterilization of food (i.e., retort method), involves sterilization after filling of food in a can. This results in many problems such as the low rate of heat penetration to the slowest heating point in the container, the long processing time required to deliver the required lethality, destruction of the nutritional and sensory characteristics of the food, low productivity, and high energy costs (Ball and Olson 1957; Ramaswamy et al. 1995; Smith et al. 1990). Hence, there is significant commercial interest in retort pouch technology.

Extensive research has been done in the retort method (Anantheswaran and Rao 1985; Clifcorn et al. 1950; Conley et al. 1951; Deniston et al. 1987; Hassan 1984; Lenz and Lund 1978; Naveh and Kopelman 1980; Peralta Rodriguez and Merson 1983; Quast and Siozawa 1974; Rao and Anantheswaran 1988) to study heat transfer in liquid foods (with or without particulates) because development of a mathematical model to optimize these thermal processes requires the knowledge of heat transfer coefficients (Clark 1978). In the retort method, the main focus has been to determine the effect of various parameters on heat transfer coefficients. The published research related to the determination of heat transfer coefficients in the retort processing technique is reviewed in this chapter, along with commercial developments in the retort pouch technology.

Retort Method: Liquid System

Mathematical Treatment

Under the assumption that temperature of fluid surrounding the can is uniform, an energy balance for liquid inside the can yields (Stoforos 1988):

$$hA_c(T_m - T_f) = m_f C_{pf} \frac{dT_f}{dt} \tag{2.1}$$

where h = overall heat transfer coefficient between the external medium and the internal liquid, if T_m is temperature of heating medium (K), or internal heat transfer coefficient between the can wall and inside liquid, if T_m is temperature of the can wall ($W.m^{-2}.K^{-1}$),

A_c = total can surface area (m^2),
T_f = uniform temperature of liquid inside the can at time t (K),
m_f = mass of liquid inside the can (kg),
C_{pf} = specific heat of liquid inside the can ($J.kg^{-1}.K^{-1}$), and
t = time (s).

Equation (2.1) can be used to determine h during retort processing of liquid-filled cans.

Effect of Mode of Rotation

In the retort method, cans filled with liquid food are heated by a heating medium (water or steam) for commercial sterilization. Rotation of the can affects the interaction between liquid molecules and the can wall; thereby the heating rate is enhanced. Conley et al. (1951) reported the impact of rotation of cans on increase of heat transfer rates and the resulting decrease of sterilization times. Many researchers (Anantheswaran and Rao 1985; Naveh and Kopelman 1980; Quast and Siozawa 1974; Tsurkerman et al. 1971) studied the effect of mode of rotation (axial and end-over-end) on heat transfer coefficients. Quast and Siozawa (1974) and Tsurkerman et al. (1971), working with Newtonian fluids (sucrose solutions) and non-Newtonian fluids (carboxyl-methyl-cellulose), reported that heat transfer rates for cans being axially rotated were two to four times the rates for stationary processing.

Naveh and Kopelman (1980) used specially constructed brass cylindrical cans (filled with glucose syrup) for heat transfer experiments. They examined the effect of end-over-end and axial rotation on overall heat transfer coefficient and reported two to three times greater overall heat transfer coefficient for end-over-end rotation than for axial rotation. The end-over-end rotation involves rotation of the central axis of the can around an axis perpendicular to the central axis of the can; that is, end-over-end agitation is the motion imparted to a can when one end is in contact with the circumference of a revolving drum (Conley et. al 1951).

Quast and Siozawa (1974) reported that there was no significant effect of reversing the direction of rotation on heat transfer rates, whereas Hotani and Mihori (1983) reported that reversing the direction of rotation every 15 to 45 seconds (from clockwise to counterclockwise or vice versa) resulted in higher heat transfer rates and more uniform heating. Hotani and Mihori conducted their tests when the fluid was at temperatures less than 70°C, which may have caused different heat transfer rates because parameters affecting heating characteristics are generally more dominant at early stages of heating when fluid temperature is high. Quast and Siozawa made observations at later stages of heating when fluid temperature reached 96°C.

Anantheswaran and Rao (1985) also studied the effect of end-over-end rotation on heat

transfer rates to Newtonian fluids with two copper cans (length to diameter ratio of 0.73 and 1.37) over the range of 0–38.6 rpm and 0–14.9 cm radius of rotation. The test fluids were distilled water, aqueous sucrose solutions, and glycerine. A laboratory agitating sterilizer was used for heat transfer studies. Steam at atmospheric pressure (757 mm Hg) was used as the heating medium. They reported that end-over-end rotation of the cans improved the heat transfer rates to the test fluids (from 49 to 79%). The above results are attributed to the fact that for a given liquid the more vigorous the mixing, the more the interaction among the fluid molecules, resulting in high fluid velocity and thus higher heat transfer rates.

Effect of Rotational Speed

Clifcorn et al. (1950) performed experiments by rotating a can containing liquid at different speeds to obtain the maximum rate of heat penetration. They found that selection of proper speed may give more turbulence within the can contents and thus high heat transfer rates. They calculated the optimal rotational speed by using the formula $RN^2 = 35{,}196$ (centrifugal force = gravity force), where R is the distance from axis of rotation to the center of the can's contents (inch), and N is the speed of rotation (rpm). They used tomato pulp of varying viscosities, reported that optimum rotational speed is a function of fluid viscosity, and concluded that for more viscous fluids, the rotational speed must be decreased to achieve optimum conditions in order to allow time for the headspace volume to travel around the lower half of the can and through the center. Conley et al. (1951) reported that as the speed of rotation increases beyond the optimum, the centrifugal forces created decrease the mobility of the contents and thus decrease the heat transfer rates.

Naveh and Kopelman (1980) reported that increasing the rotational speed (0 to 120 rpm) resulted in a continuous increase of the overall heat transfer coefficient during heating processes, whereas asymptotic values of heat transfer coefficient were obtained with cans agitating at relatively low speeds of 40–70 rpm during the cooling phase. Their experiments were carried out in transparent plexiglass cylinders filled with glucose solutions of varying viscosities (0.12–18 poise). They attributed their observations to the fact that the viscosity of the liquid is higher during cooling phase compared to that during heating (Clifcorn et al. 1950 made observations during cooling phase). These studies (Clifcorn et al. 1950; Conley et al. 1951; Naveh and Kopelman 1980) were conducted using Newtonian fluids. Anantheswaran and Rao (1985), working with non-Newtonian fluids (aqueous guar gum solutions), found that the overall heat transfer coefficients continuously increased with an increase in rotational speed from 0 to 38.5 rpm. The above observations can be attributed to the fact that the more the agitation, the more the interaction among fluid molecules, the higher the heat transfer rates.

Effect of Distance between Can and Axis of Rotation

Clifcorn et al. (1950) reported that the distance between the center of the can and axis of rotation affects the rate of heating. Conley et. al (1951) studied the effect of speed of rotation on the cooling rate of a can containing 6:1 orange concentrate when cooled from 96°C to 38°C in 21°C water and reported that the optimum speed of rotation was dependent on the distance between the bottom of the can and axis of rotation.

Naveh and Kopelman (1980) reported an increase in overall heat transfer coefficient when rotating cans move from central to off-center axis of rotation. Anantheswaran and Rao (1985)

studied the effect of distance between the center of the can and axis of rotation on the heat transfer rates with 60% sucrose solution during the end-over-end rotation and reported that there is no significant effect of this distance on the heat transfer rates, but their investigation was based on a limited range of distance between the center of the can and axis of rotation (0–14.9 cm). In a commercial end-over-end agitating retort, the heating rates were independent of distance between the center of the can and axis of rotation (Anonymous 1983).

Effect of Headspace

Headspace volume (total volume of can minus volume of the can filled with liquid) is one of the important factors that influences the heat transfer rates during processing of rotating canned liquids. Quast and Siozawa (1974) found that the overall heat transfer coefficient increased significantly with an increase in headspace for viscous fluids, but it decreased slightly for fluids with low viscosity. This can be attributed to the fact that less headspace in more viscous fluids means less agitation, that is, less interaction among molecules of rotating fluids, and thus results in low heat transfer coefficient. Naveh and Kopelman (1980) reported that overall heat transfer coefficient approaches a constant value with increasing headspace volume (2–3% of total internal can volume).

Anantheswaran and Rao (1985) reported that the headspace volume between 3 and 9% did not affect the heat transfer rates. Berry and Kohnhorst (1985) studied the effect of headspace on heat transfer rates by performing tests on commercial cans filled with a homogeneous, milk-based concentrate. A multistage preheat process that increased the product temperature by steps was used in this investigation. After heating to 123.9°C for less than 1 minute, the cans were cooled rapidly by continuously spraying water at the top. Berry and Kohnhorst reported that agitation of the product was enhanced by the presence of headspace. Decreasing headspace resulted in lower heat transfer rates. They also pointed out that the headspace effects were more significant for more viscous products (15 cps).

Effect of Fluid Viscosity

Fluid viscosity is the most important parameter affecting heat transfer coefficient. The effect of viscosity on heat transfer coefficient appears in correlation equation among Nusselt, Reynolds, and Prandtl numbers (Anantheswaran and Rao 1985; Kramers 1946; Ranz and Marshall 1952; Whitaker 1972).

Quast and Siozawa (1974) reported that heat transfer increased for decreasing viscosities. Many other researchers (Anantheswaran and Rao 1985; Peralta Rodriguez and Merson 1983; Rao et al. 1985) also found similar results. This can be explained by the fact that more viscous fluids result in less agitation and thus low heat transfer rates.

Retort Method: Liquid Foods with Particulate

Mathematical Treatment

An energy balance on the can contents yields:

$$U_o A_c (T_{st} - T_f) = m_f C_{pf} \frac{dT_f}{dt} + m_p C_{pp} \frac{dT_p}{dt} \tag{2.2}$$

where U_o = overall heat transfer coefficient ($W.m^{-2}.K^{-1}$),
T_{st} = temperature of the heating medium (say steam) (K),
T_p = temperature of particle (K),
m_p = mass of particles inside the can (kg), and
C_{pp} = specific heat of particles inside the can ($J.kg^{-1}.K^{-1}$).

Boundary condition at particle surface is:

$$m_p C_{pp} \frac{dT_p}{dt} = h_{fp} A_p (T_f - T_p) \tag{2.3}$$

where h_{fp} = fluid-to-particle heat transfer coefficient ($W.m^{-2}.K^{-1}$), and
A_p = surface area of particle (m^2).

Equations (2.2) and (2.3) can be used to determine U_0 and h_{fp} during the retort processing of liquid foods with particulates.

Effect of Mode of Rotation

Many researchers (Deniston et al. 1987; Hassan 1984; Lenz and Lund 1978; Stoforos 1988) determined fluid-to-particle heat transfer coefficient (h_{fp}) for canned liquid foods containing particles processed in agitated retort, but they did not compare the effect of different modes of rotation (end-over-end rotation, axial rotation) on h_{fp}.

Lekwauwa and Hayakawa (1986) determined h_{fp} for spheroidal potato particles in water during end-over-end rotation. They matched the center time-temperature profile of a potato particle predicted using a computer model, developed to simulate thermal responses of a packaged liquid-solid food, with the measured data and determined h_{fp} values between 60 and 2,613 $W.m^{-2}.K^{-1}$.

Chang and Toledo (1990) determined h_{fp} by measuring time-temperature history at the center of 2 cm potato cube heated at 75°C in a stationary retort and found an h_{fp} value of 400 $W.m^{-2}.K^{-1}$. Weng et al. (1991) also determined h_{fp} from water to spherical polyacetal and nylon particle (2 cm diameter) in static retort and found an h_{fp} value of 103 $W.m^{-2}.K^{-1}$.

Effect of Rotational Speed

The heat transfer coefficient increases with an increase in can rotational speed (Deniston et al. 1987; Hassan 1984; Lenz and Lund 1978) because it affects the relative particle-to-fluid velocity, which in turn affects h_{fp}. Lenz and Lund studied heat transfer and lethality in canned liquid (water or 60% sucrose solution) foods containing particles processed in an agitated retort at a steam temperature of 121°C. Fluid-to-particle heat transfer coefficient (h_{fp}) was determined for spherical lead particles, immobilized in the center of a rotating can. Changing the rotational speed from 3.5 to 8 rpm resulted in an average increase of h_{fp} by 150 $W.m^{-2}.K^{-1}$.

Hassan (1984) studied heating of potatoes, Teflon, and aluminum spheres in deionized water and silicone fluids of various kinematic viscosities (1.5×10^{-6}, 50×10^{-6}, and 350×10^{-6} m^2/s at 25°C) in a single can rotating axially. He reported that varying the can rotational speed (9.3–55.5–101 rpm) had no significant effect on h_{fp}. He was unable to explain the reason behind this negligible effect.

Deniston et al. (1987) determined the heat transfer rates to steam-heated, axially rotating cans containing potato spheres in water. They attributed the insensitivity of h_{fp} to can rotational speed (9.3–29.1–101 rpm) to three experimental conditions resulting in small changes in relative particle-fluid velocity: (1) closeness of density of potato particle (1,063 kg/m^3) to that of water, so that particle settling due to gravity was minimal; (2) since the particle was located at the can center, centrifugal force acting on it was small; and (3) stiffness of the thermocouple wire hindered the particle motion.

Stoforos (1988) reported that increasing the rotational speed resulted in higher h_{fp} as long as the increasing rpm affects the relative particle-to-fluid velocity. At high rotational speed (100 rpm), the can contents in his experiments behaved as a solid body due to centrifugal forces, and he found tremendous drop in h_{fp} (from 2,071 to 410 $W.m^{-2}.K^{-1}$) when Teflon particles heated in silicone fluid at about 50°C were rotated at 100 rpm instead of 54.5 rpm.

Effect of Fluid Viscosity

Lenz and Lund (1978) found lower fluid-to-particle heat transfer coefficient (h_{fp}) for particles processed in a 60% aqueous sucrose solution (high viscosity) than for solids processed in water (low viscosity). Hassan (1984) reported that the overall heat transfer coefficient and fluid-to-particle heat transfer coefficient decreased as the fluid viscosity increased. He demonstrated that under equal processing conditions, the overall heat transfer coefficient (U_o) and h_{fp} to Teflon particles were lowered when more viscous fluids (silicone oils of kinematic viscosities 1.5×10^{-6} , 50×10^{-6}, and 350×10^{-6} m^2/s) were used instead of water. The same pattern was observed for aluminum particles. Stoforos (1988) visualized a decrease in U_o and h_{fp} with increasing fluid viscosity. This can be attributed to the fact that more viscous fluids result in less agitation, that is, low particle-to-fluid relative velocity and thus low heat transfer.

Effect of Particle Interaction

The presence of particulate matter during agitated processing alters the flow pattern of pure fluid (Rao and Anantheswaran 1988). The amount of solid in the can influences the relative particle-to-fluid velocity and thus heat transfer rates.

Lenz and Lund (1978) added real food particles (peas, carrot, or radish) of equal diameter to the test system containing lead spheres and simulated more closely velocities and interactions of particles and fluids under real processing conditions and reported no significant effect on the h_{fp}. They immobilized test particles in the rotating cans, which might have caused particle-to-particle interaction different from a real processing condition. They reported a decrease in the overall heat transfer coefficient (U_o) for liquid with particles. They attributed this to the decreased relative particle-to-fluid velocity due to the drag exerted on the fluid by the particles.

Deniston et al. (1987) reported a slight increase in h_{fp} with increasing particle volume fraction, which was lowered for higher particle contents. They attributed this to the tight packing in the can (higher particle volume fraction), which restricted the particle free movement. Stoforos (1988) mentioned that mixing effect by moving particles contributes to a homogeneous temperature distribution in the can, especially for highly viscous products.

Effect of Particle Properties

Fluid-to-particle heat transfer coefficient (h_{fp}) will remain unaffected by the "type of particle" as long as the specific properties of the particle under study (such as density or surface roughness) do not affect the particle-fluid pattern. When particles are allowed to move freely in the can, their different density can influence their behavior in the fluid and thus result in different h_{fp} values. Hassan (1984) found higher h_{fp} for potato than for Teflon particles processed in water. Also, Teflon particles exhibited higher h_{fp} than aluminum particles of the same size when processed in silicone fluids. Stoforos (1988) also reported similar results. He contributed this to high thermal diffusivity of the aluminum particles, which resulted in faster heat conduction in the aluminum particles as compared to Teflon particles.

Effect of Particle Size

Hassan (1984) and Lenz and Lund (1978) studied the effect of different sizes (2.22–3.49 cm) of potato, Teflon, and aluminum spheres on heat transfer coefficients. They reported an increase in both overall (U_o) and fluid-to-particle heat transfer coefficients (h_{fp}) with an increase in particle diameter for particles heated in water. Also, Lekwauwa and Hayakawa (1986) reported higher fluid-to-particle heat transfer coefficients with an increase in particle size (from 0.89 to 2.30 cm). They also reported that temperature difference between fluid and particles increased as the particle size increased. Deniston et al. (1987) reported that potato particle size (2.22, 2.86, or 3.5 cm diameter) did not influence h_{fp}.

Conclusions

The retorting process has not evolved much since its inception by Nicholas Appert in 1800s, except for better quality retorts, temperature monitoring software and hardware, and different options for agitating cans in the retort during the heating process to get better and uniform heat transfer. However, the concept of retorting is moving toward retorting pouches versus cans for a better quality product and to overcome issues such as overheating of food products around walls in the can and longer time required to accomplish the required lethality values. In pouches, the greater surface area results in a short time to achieve the required lethality values, which results in better quality products. With commercial innovations in retort pouches, we are seeing several products in the marketplace at institutional and retail levels utilizing retort pouch technology. Although non-thermal processing technologies are also gaining interest in the commercial world, retorting still remains the most cost-effective means to achieve a shelf-stable low-acid food.

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3 Aseptic Processing

Rakesh K. Singh

Introduction

Several products, such as puddings, pastes, sauces, and beverages, using aseptic processing have been in the marketplace for three decades. Invariably, the product including raw material for aseptic processing has to be pumpable, which means that liquid foods or liquids with particulates can be processed through an aseptic processing system. This requires separate sterilization of a pumpable food, containers, and closures followed by the cooling of the food and filling and sealing in containers under a sterile environment (Clark 2004). Aseptic processing of foods is classified as acid or acidified products having pH less than 4.6 and low-acid products with pH > 4.6. The regulations for low-acids foods are more stringent than those for acid or acidified foods due to the possibility of *C. botulinum* growth. Therefore, many of the aseptic products available are acid or acidified foods (both liquid and particulates) and only some low-acid liquids (Singh and Lee 2002; Singh and Nelson 1992). However, new developments in technology, understanding of processes, and new packaging materials are being used for development of aseptic low-acid beverages in plastic bottles as well as low-acid particulate foods.

The containers for aseptic packaging have ranged from consumer-size retail packs of a few grams (60–180 g) to bulk storage containers up to more than about 4–6 m^3 (1.0–1.7 million gallons). The largest bulk containers used today carry single strength orange juice from Brazil to different countries in tanker ships. The bulk storage of orange juice is refrigerated to control the chemical reactions in the juice and not for microbiological reasons. Other uses of bulk storage have been in tomato paste storage and shipment (190 L drums of tomato paste and banana puree, rail car and truck containers) and 378,000 L (100,000 gallon) tanks for storage of soy sauce and other liquids. Particulates such as diced tomatoes, pineapple cubes, strawberry toppings, and others have been aseptically packed in 1,140 L bags, which are kept in rigid boxes for support. The recent interest is to be able to package nutraceutical or functional foods, especially beverages, in aseptic plastic containers. The heat-sensitive nature of some components in functional foods requires that the heat treatment be limited to relatively lower temperatures for shorter times. There are also opportunities for packaging semiprocessed foods for institutional use. The products for the institutional market could be in 4–20 L plastic packages.

The product characteristics important for successful operation and design of the system relate to flow and heating/cooling characteristics of the product. During processing, the product travels through a series of equipment or unit operations where specific treatments are given. Some of the important pieces of equipment are refrigerated storage tankers/tanks, booster pumps, heat exchangers (heating and cooling both), holding tubes, deaerators, valves, piping, other pumps, and a packaging system (fig. 3.1). Thus the performance of the component may affect the performance of the entire system. When considering the resulting quality of the finished product, the processor should not view the equipment as individual components to be linked together but as an entire system. The selection of heat-

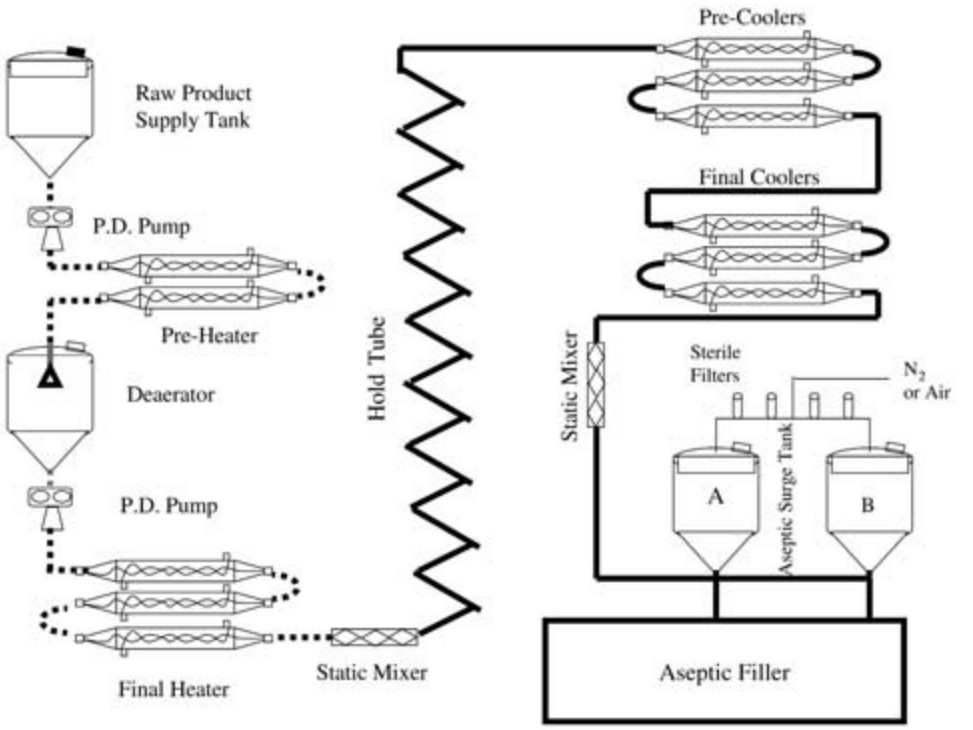

Figure 3.1. Assembled aseptic processing system.

ing and cooling systems is very important for each type of product. The surface area required for cooling is usually twice of that required for heating. Foods can be sterilized either in direct or indirect heat exchangers depending upon the nature of product. Direct heat exchangers use culinary steam in contact with the product (steam injection or steam infusion method), whereas a metal wall separates the product and heating/cooling media in the indirect heat exchangers. Those liquid foods that do not have fibers or small seeds are sterilized in plate heat exchangers. The products with small particles and moderate viscosity can be sterilized in tubular heat exchangers (double or triple tube). There are some newer plate heat exchangers with wide gaps that can handle fibers/small seeds or even moderate viscosity products. Highly viscous foods and those containing large particulates are sterilized as well as cooled in scraped surface heat exchangers. Sometimes a product may require one type of heat exchanger for heating and another type for cooling. For example, cheese sauce can be sterilized in a steam injection system but cooled by a scraped surface heat exchanger.

Before an aseptic processing system is put together, a team of experts should look at some key design elements. These include the type of process, goal, and criteria. The process used is heating of a product for a certain length of time to extend its shelf life. The goal of the process is to determine the proper time-temperature combination to produce a safe and nutritious product with a long or extended shelf life. In summary, the thermal process design requires the information on components shown in figure 3.2.

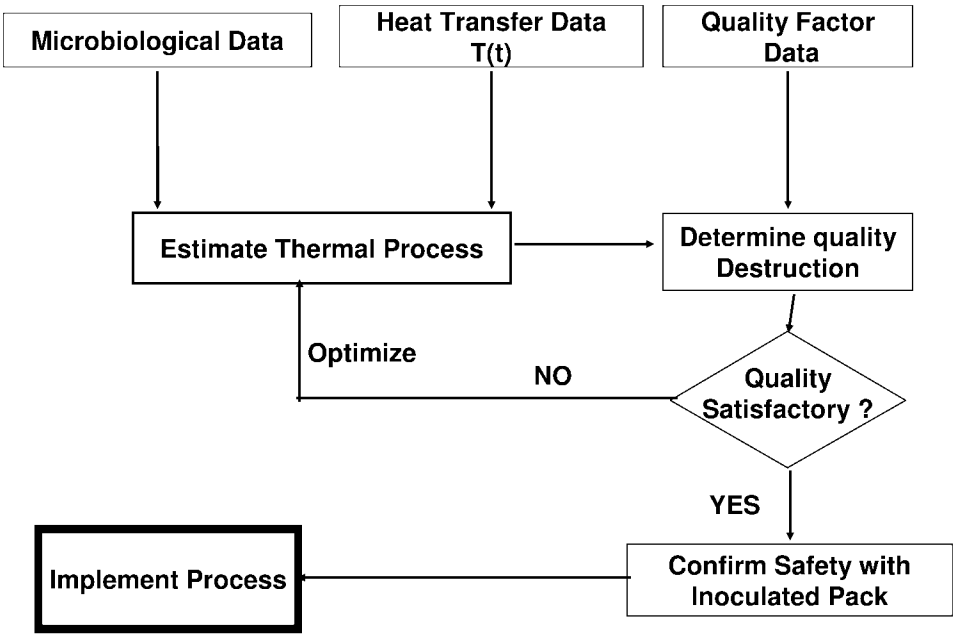

Figure 3.2. Schematic diagram of thermal process design.

Thermal Process for the Product

One of the most important product considerations is its pH. Generally, low-acid foods (pH > 4.6) require more severe heat treatment in order to attain commercial sterility than do acid or acidified food products (pH < 4.6). The fact that the processing requirements are different for high-acid versus low-acid products may simplify equipment needs because the demand for heat exchange capacity will be less for acid/acidified foods than for low-acid foods. The temperature requirement for sterilization of low-acids foods (milk, cheese sauce, puddings, etc.) must exceed 121.1°C, whereas the acid/acidified products (fruit juices and drinks) could be sterilized at temperatures below 100°C for a few seconds.

The thermal conditions needed to produce commercial sterility in the product depend on (1) nature of the food (e.g., pH and water activity); (2) storage conditions following the thermal process (refrigerated versus room temperature); (3) heat resistance of the microorganisms or spores; (4) heat transfer to the food; and (5) the initial load of microorganisms. Thermal process calculations rely on the destruction of microorganisms under a certain time and temperature profile. This simply means that if the food is subjected to a constant temperature for a longer time, there will be more destruction of microorganisms than if it was subjected to the same temperature for a shorter time. However, subjecting the food at a constant temperature for excessively long times would decrease the probability of surviving microorganisms. Furthermore, a higher temperature process will provide more microbial killing than a relatively lower temperature process for the same time period of exposure of food to the heat. In the case of fruit juices, the process is intended to inactivate native enzymes (1- to 2-log reduction), which is also sufficient for inactivation of microorganisms.

Table 3.1 Ranges of thermal resistance parameters for various entities

	D_{121} (min)	Z (°C)
Vegetative cells	0.002–0.02	4.4–6.7
Spores	0.1–5.0	6.7–12.2
Enzymes	1–10	6.7–55.6
Quality factors[a]	5–500	25–44.4
Stable vitamins	100–1,000	25–30.6

[a]Quality factors include browning reactions and flavor changes.

Quantitatively, the microbial destruction is a logarithmic decay process with respect to the processing time. The time required to reduce the microbial population by tenfold at a constant temperature is called the D-value of the process. The D-value provides a quantitative index for heat resistance of microbial cells or spores. The process given to a food material is quantified in terms of multiples of D-value at a reference temperature (i.e., a 6D process or 12D process, etc.), which is also referred as F-value at reference condition (F_0). The accepted reference temperature for sterilization is 121.1°C and for pasteurization it is 82.2°C. The D-values of each microbial species also depend on process temperatures. Higher temperatures are more lethal than lower ones, meaning that a higher temperature would give lower D-values for the same microorganism. The temperature change required to cause a tenfold change in D-value is called Z-value and is used for comparing processes at various temperatures. An example of some D- and Z-values is given in table 3.1. The lower D- and Z-values for vegetative cells imply that they can be destroyed faster than spores. Enzyme destruction takes more time than the destruction of microorganisms. Quality factors and nutrients are relatively stable under a high temperature short time thermal process.

The target for sterilizing value or F_0 is set based on the product type and its intended use (refrigerated or shelf-stable). The target for setting up the thermal process could be based on microbial kinetics or enzyme. In the case of fruit juices and several high-acid beverages, the target is enzyme inactivation (Kim et al. 1999a). Approximations of the heat process for destruction of *C. botulinum* and commercial sterility are shown in table 3.2 (McGarrahan 1982). The reported sterilization values are for actual processes in practice, but similar values can be calculated for other processes with different targets as a multiple of D-value.

The sterility values for a heating and cooling phase are calculated from the time-temperature [T(t)] profile of the product as:

$$F_0 = \int 10^{[T(t)-TR]/z}\, dt \tag{3.1}$$

where T(t) = product temperature (°C) as a function of time t and TR = reference temperature (°C). The limit for integration time is the time for that particular segment of the process where sterilization value needs to be estimated. Equation (3.1) is useful to find out overprocessing of a product, or it can be used for high-acid foods. In the case of low-acid foods, the measured temperature at the end of the hold tube is normally used for estimating the F_0 for the process, but that value is an overestimate. If the process equipment has

Table 3.2 Approximations of heat processes for destruction of *C. botulinum* and commercial sterility (modified from McGarrahan 1982)

Process	Storage	Heat/Hold	Sterilizing Value[a]
Ultrapasteurization	Refrigerated	138°C, 2 s	F_0 1.5 min
Minimum to destroy *C. botulinum* spores	Nonrefrigerated	138°C, 4 s	F_0 3.0 min[b]
Commercial sterility	Nonrefrigerated	138°C, 8 s	F_0 6.0 min
European UHT range	Nonrefrigerated	135°C, 3 s	F_0 1.22 min
		140°C, 3 s	F_0 3.87 min

[a]Sterilizing values were calculated for Z = 10°C.
[b]Minimum for public health safety varies based on product and process.

staged heating, the inlet temperature value to a heat exchanger module can be used for calculation of the sterilization value. The process sterilization value must be equal or higher than the target F_0 value, otherwise the product will be underprocessed. If the product has particulates mixed with carrier fluid, the temperature of the fluid can be used for estimation of product temperature and thus the sterility at the center of particles (Bhamidipati and Singh 1994; Lee and Singh 1990; Lee, Singh, and Larkin 1990). The verification of sterilizing value in these situations has been done by the microbial, enzymatic, or chemical indexing methods (Berry et al. 1990; Bhamidipati and Singh 1996).

Thermal processing causes change in many quality-related factors in addition to the destruction of microorganisms. Such quality changes include nutrient loss (destruction), flavor and color change, and coagulation of proteins. Quantitatively, the destruction rate of microbial spores is different from the destruction rate of vitamins. Therefore, a process can be designed to cause high destruction of spores and less destruction of vitamins. In those cases where destruction of vitamins, flavor, or other key components is of major concern, those components can be aseptically added after cooling the product prior to packaging.

Equipment for Product Sterilization

The product is sterilized by heating in a heat exchanger, followed by holding in a hold tube, and, finally, cooling before packaging. The heat exchangers used in the industry are classified as direct or indirect heat exchangers (fig. 3.3). Direct heat exchangers are steam injection and steam infusion types, whereas indirect are plate, tubular, and scraped types. Note that direct contact with steam will add water in the product as a result of condensing steam. The added water from condensing steam can be either accounted for in the product formulation or removed via flash cooling. The heating profile in a direct heat exchanger is square-shaped due to faster heating and quick flash cooling. A plate heat exchanger operating under a high-velocity regime will give a similar temperature profile, but the profiles in tubular and scraped surface heaters show some lag.

A product's flow characteristics dictate the type of heat exchanger required. For example, relatively viscous products, such as puddings or sauces, may best be processed through a scraped surface heat exchanger, while products such as fruit juices may best be processed through equipment that allows maximum retention of volatile flavor compounds, such as plate or tubular heat exchangers. In scraped surface heat exchangers (SSHE), the heat

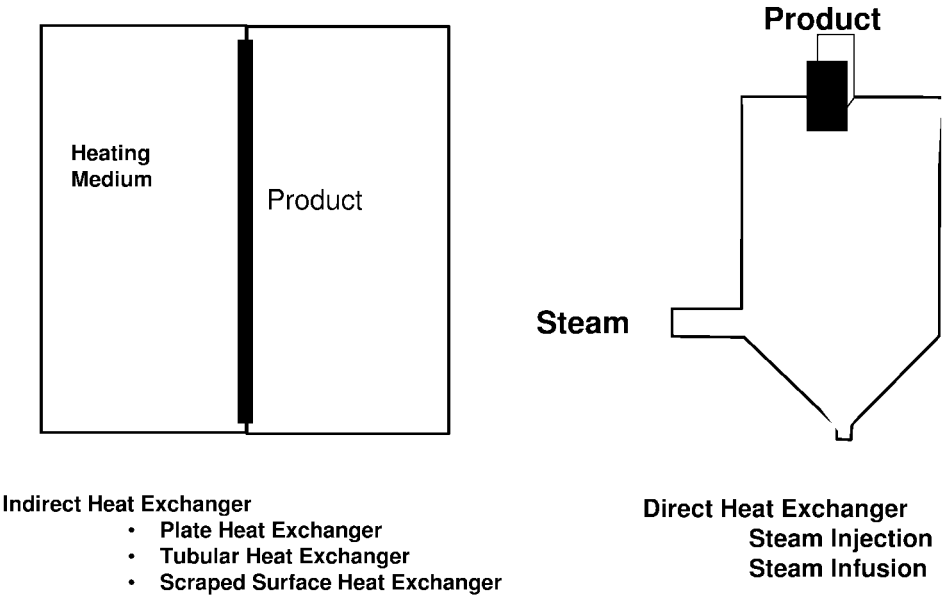

Figure 3.3. Heating units in aseptic processing.

exchanger tube is lined with specific material to minimize corrosion of the tube. This material is usually specific for a given food group. The use of this tube for different food groups may damage the heat exchanger surface. Special coatings or linings may also decrease fouling of the heat exchanger surface (Sandu and Singh 1991). Some products like cheese sauce, milk, and egg proteins are very susceptible to heat treatment and they can cause fouling in heat exchangers (Li et al. 2004). The same researchers found that the process conditions with high mean velocity, regardless of temperature, did not produce detectable fouling deposit. Maximum fouling was obtained in the holding section after the heating section of the SSHE when processing at 121.1°C and for 7 hours due to inverse solubility salts and high temperature exposure. Formation of deposit was extremely sensitive to temperature, and no fouling was found in the cooling sections (Li et al. 2004). Nonetheless, the fouled surface in the heat exchanger may cause product burn, thus causing quality defects.

Fluid Flow

As mentioned earlier, aseptic processing is a continuous process and thus the product must be pumped through a set of unit operations connected with pipes. A number of parameters are important for the flow of fluid food. These include fluid viscosity, fluid density, fluid velocity size, shape of the pipe, and surface roughness of the equipment. The flow may be broadly described as either laminar flow or turbulent flow. Laminar flow is characterized by the fluid particle moving smoothly along parallel streamlines, whereas turbulent flow is characterized by fluid particles moving in a random, tumbling, churning motion (fig. 3.4). The velocity of fluid flowing in a pipe is greatest at the center and decreases toward the

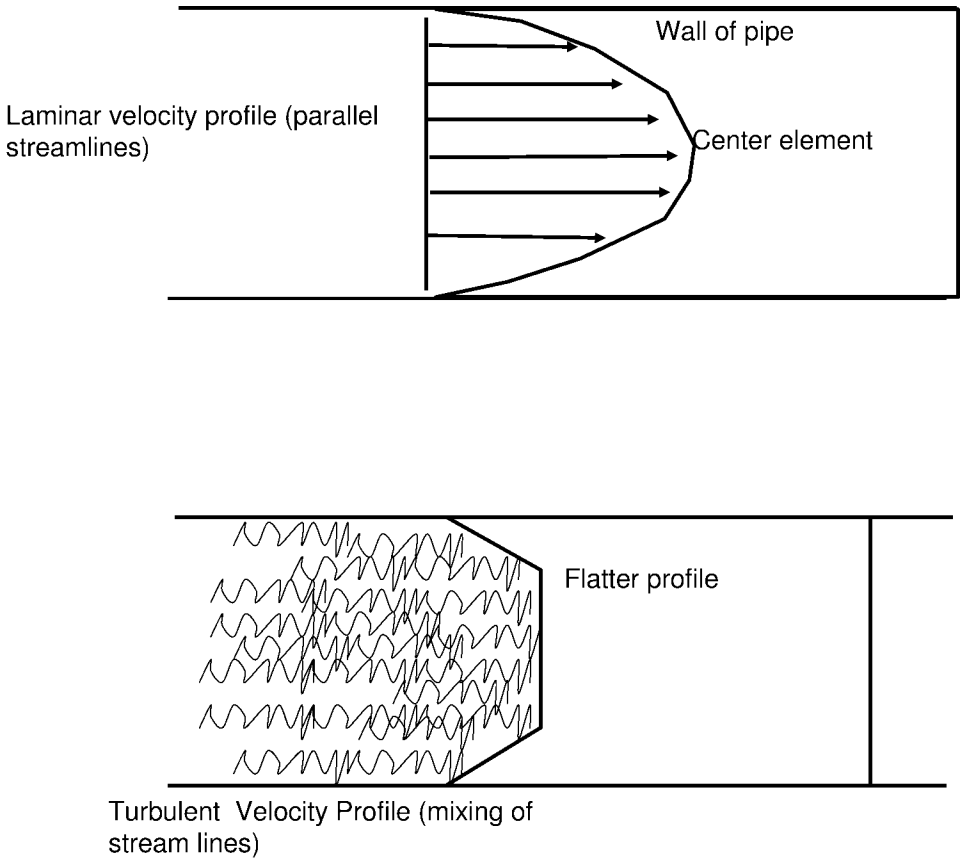

Figure 3.4. Velocity profiles in a pipe flow.

pipe wall, with the velocity of the fluid at the wall being zero. Temperature could easily distort the flow profile in a pipe flow. A constant temperature across the section of flow will not cause distortion, but a heated wall (heating section of heat exchanger) will make the flow profile flatter by causing more wall slippage (Chakrabandhu and Singh 2005b), and cooling will cause a much faster center velocity than the isothermal condition.

Viscosity is a measure of resistance of fluid to shear. Fluids are described as Newtonian or non-Newtonian based on the behavior of viscosity. The viscosity of a Newtonian fluid is not influenced by shear rate (i.e., through homogenizer, pumping, etc.), whereas viscosity of a non-Newtonian fluid can be influenced by the shear rate. Viscosity also depends on temperature of the product. Most fluids display lower viscosity with increasing temperatures unless they are heated beyond their phase transition (starch gelation or protein denaturation/aggregation). In case of phase transition the trend may reverse and the increasing temperature could cause an increase in viscosity.

The distinction between laminar and turbulent flows is based on the Reynolds number (N_{Re}), which is calculated from fluid density, fluid velocity, fluid viscosity, and the diameter of the pipe. If the flow is laminar ($N_{Re} < 2{,}300$), the fastest-moving element moves

twice as fast as the average-moving element. If the flow is turbulent (N_{Re} > 4,000), then the fastest-moving element moves only 1.22 times faster than the average.

Maximum velocity of fluid element is used for calculating the hold time as:

$$t = L/V_{max} \tag{3.2}$$

where t = time (s), V_{max} = velocity of fastest-moving particle (m/s), and L = the length of the hold tube (m). The velocity of the fastest-moving particle depends on the velocity distribution within the hold tube.

For laminar flow of Newtonian fluids, the maximum velocity is twice the average velocity, that is: $V_{max}/V_{avg} = 2$, where V_{avg} (the average velocity of fluid) can be calculated by dividing the flow rate by the cross-sectional area. In case of turbulent flow condition, the velocity ratio ranges from 1.2 to 2.

Flow properties of several food products have been characterized and used for prediction of flow in aseptic processing systems (Bhamidipati and Singh 1990; Chakrabandhu and Singh 2005a; Ditchfield et al. 2004; Son and Singh 1998). Most food fluids show non-Newtonian pseudoplastic behavior and can be modeled by the power law model:

$$\tau = K(\gamma)^n \tag{3.3}$$

where τ = shear stress (Pa), K = consistency coefficient (Pa s^n), γ = shear rate (s^{-1}), and n = power law index (dimensionless). If the value of n is less than 1, the fluid has a pseudoplastic or shear thinning behavior; if n is equal to 1, it is Newtonian; and if n is more than 1, then it is dilatant. The values of K and n are temperature dependent, with K having a higher temperature dependency than n. Bhamidipati and Singh (1990) measured the flow behavior of tomato sauce at 7° and 14° Brix with and without particulates. The values of n and K for 7° Brix sauce ranged from 0.30 to 0.86 and 0.04 to 0.34 (Pa s^n), respectively. For the 14° Brix sauce these values were 0.27–0.53 and 0.20–0.60 (Pa s^n), respectively. The values of n decreased and K increased with an increase in concentration of solids and particulates. Son and Singh (1998) modeled flow behavior of soy milk at various temperatures and solid concentrations and observed a similar trend (table 3.3).

Rheological properties of banana puree were measured at high temperatures and modeled as Herschel-Bulkley model, which is an extension of the power law model and accounts for the yield stress (Ditchfield et al. 2004). The puree presented a complex rheological behavior, which changed drastically with temperature (table 3.4). In general, the higher temperature lowers the viscosity, but the puree showed increased viscosity for the temperature range of 50–60°C and 110–120°C. Such unexpected changes were attributed to the presence of starch in banana and the interactions of polysaccharides. Therefore, it is very important to have experimental data on rheological behavior of products to be processed aseptically.

Residence Time Distribution (RTD)

In a complete system, the velocity distribution leads to a residence time distribution (Singh 1993). The RTD is measured by a salt (tracer) injection test for liquids and a slug of particles via a side loop for slurries. Quantification of RTD provides information on minimum and maximum times spent by food elements in the system. It also provides insight into

Table 3.3 Flow behavior of soybean milk as influenced by the temperature and solids content (after Son and Singh 1998)

	9% solids		13% solids		16% solids	
	25°C	65°C	25°C	65°C	25°C	65°C
n	0.77	0.77	0.67	0.68	0.58	0.60
K (Pa s^n)	0.02	0.008	0.14	0.04	0.70	0.18

n = Power law index.
K = Consistency coefficient.

Table 3.4 Parameters obtained from experimental data fitting for banana puree flow curves to the Bingham, power law, and Herschel-Bulkley models for the increasing shear rate (up) and decreasing shear rate (down) at various temperatures (after Ditchfield et al. 2004)

Temperature		Bingham Model			Power Law model			Herschel-Bulkley Model			
(°C)		S_0 (Pa)	K (Pa.s^n)	R	K (Pa.s^n)	n	R	S_0 (Pa)	K (Pa.s^n)	n	R
30	up	101.10	0.103	0.90	76.74	0.104	0.89	81.04	4.67	0.442	0.99
	down	91.14	0.129	0.92	44.40	0.198	0.98	31.91	21.44	0.281	0.99
40	up	100.36	0.081	0.92	64.94	0.116	0.83	82.20	3.15	0.457	0.96
	down	78.53	0.110	0.89	74.08	0.071	0.59	50.03	9.12	0.354	0.98
50	up	86.52	0.054	0.89	43.35	0.163	0.87	74.76	1.06	0.614	0.99
	down	68.85	0.056	0.90	79.10	0.055	0.58	58.47	1.32	0.594	0.95
60	up	83.64	0.119	0.84	81.71	0.054	0.72	68.98	4.92	0.412	0.96
	down	77.54	0.085	0.95	82.41	0.041	0.53	67.90	1.70	0.552	1.0
70	up	72.43	0.071	0.86	55.00	0.108	0.75	56.30	2.02	0.542	1.0
	down	69.44	0.046	0.83	73.77	0.054	0.61	55.38	1.79	0.556	1.0
80	up	65.21	0.063	0.90	71.68	0.046	0.75	56.35	1.71	0.523	0.97
	down	57.12	0.055	0.93	24.60	0.216	0.87	52.38	0.13	0.889	0.95
90	up	60.42	0.035	0.85	69.27	0.031	0.60	54.93	0.32	0.701	0.96
	down	46.48	0.045	0.79	45.16	0.080	0.39	44.57	0.30	0.787	0.99
100	up	59.11	0.040	0.87	71.60	0.035	0.71	50.86	0.17	0.809	0.93
	down	42.67	0.056	0.87	45.78	0.073	0.58	35.17	0.43	0.709	1.0
110	up	27.81	0.031	0.89	35.85	0.053	0.75	30.85	0.06	0.871	0.98
	down	18.21	0.035	0.93	16.81	0.149	0.83	12.21	1.13	0.539	0.99
120	up	36.21	0.031	0.80	47.27	0.054	0.70	40.94	0.03	0.966	0.95
	down	27.91	0.033	0.85	28.53	0.092	0.80	28.82	0.97	0.516	0.97

S_0 = Yield stress.
K = Consistency coefficient.
n = Power law coefficient.

channeling or dead spaces in the system. Experimental determination of RTD is required by the U.S. Food and Drug Administration during the commissioning trials for a filed process. The regulatory requirement is only for the hold tube if only the hold tube sterilization value had to be accounted for in the process filing. The measurements should be done for the actual product and the actual conditions.

In laminar flow, using coiled tubes instead of straight tubes has been suggested as a

means to achieve radial mixing of the fluids, otherwise the spot injection of tracer material will exit in the same streamline flow and will not provide the realistic distribution of flow (Levenspiel 1972). This is due to the secondary flow (Dean's effect), which occurs as a result of the imbalanced centrifugal force of the flow in the curved tube. The secondary flow pattern can be described as a double vortex circulation in the perpendicular plane to the main flow, superimposed on the velocity profile in the axial direction. In addition to the narrower RTD attained in the curved tube, it has also been suggested that the existence of the Dean effect helps enhance the heat transfer in tube flow (Kao 1987). In order to create radial mixing of fluid near the injection port and near the monitoring port, a small inline mixing section is used. However, after the commissioning trail the inline mixing section is replaced with a same-sized pipe to avoid entrapment of fibers or particles.

Several studies on RTD have been carried out in straight hold tubes (Abdelrahim et al. 1997; Alhamdan and Sastry 1997; Baptista et al. 1996; Bhimidipati and Singh 1995b; Dutta and Sastry 1990; Palmieri et al. 1992; Yang and Swartzel 1991). However, only a few studies have been reported on the RTD of food suspensions in curved tubes. Salengke and Sastry (1996) investigated residence time of particles (20–40% v/v) in tube bends and found that an increase in particle concentration or bend radius increased the residence time. Grabowski and Ramaswamy (1998) investigated the RTD of individual food particles in the curved section (180° bend) of a holding tube of an aseptic processing simulator and found that the linear velocity of food particles in the bend was reduced by approximately 8–15% in comparison to the velocity in the straight section. Particle size and shape and viscosity of the carrier liquid were found to influence the velocity of the food particle in the curved section.

RTD of liquids and particulates in SSHEs has been studied extensively for in-depth understanding of flow pattern and heat transfer (Lee and Singh 1991a, 1991b, 1991c, 1992, 1993, 1998; Singh and Lee 1992). The distribution curves mostly resembled a gamma distribution rather than the normal distribution observed for hold tube studies. The results indicated that the mean residence time of the solid phase was higher than the mean residence time of fluids. Abdelrahim et al. (1997) studied the RTD of carrot and meat cubes suspended in starch solutions in an entire aseptic processing system and pointed out that particle size, shape, density, concentration, fluid viscosity, and flow rates affect the RTD. Lee et al. (1995) also studied the RTD of particulates in a hold tube as well as in an SSHE. The results indicated that the liquid mean residence times were not significantly influenced by particle concentration up to 30% (drain weight) in a holding tube. The variation of RTD without particle loading was higher and resulted in a broader distribution. A 20% particle loading significantly reduced the liquid mean residence times in SSHE regardless of the mutator speed. The studies suggested that the thermal process calculations based on theoretical residence time will be conservative and will result in considerable overprocessing of the liquid portion of the product.

Heat Transfer to Product

Indirect heat exchangers use a heating medium to heat the wall that contacts the product. The rate of heat transfer depends on the heating medium temperature, its flow velocity, wall thickness and wall material, product flow velocity, and so forth. These resistances in the path of heat transfer should be known in order to calculate the temperature of the product. The values for heat transfer coefficients for heating medium (steam or water) are avail-

able in textbooks. However, those for the product side have to be experimentally measured. Kim et al. (1999b) experimentally measured the product side heat transfer coefficient for orange juice in a plate heat exchanger. The value was in the range of 2,000–6,300 W/m^2 °C and depended on the heating or cooling mode, temperature, and velocity. The grand mean value was 3,800 W/m^2 °C. The heat transfer coefficient was higher in the cooling section than in the heating section, and the values increased with increasing velocity of fluid.

If the product has suspended particles, then the fluid food will heat the particulates. For proper processing, the center of the fastest-moving particle should be sterile. The extent of heating at the center of the particle will not only depend on the properties of particles and the carrier fluid but also on the RTD of particles and their rotation. In order to predict the particle center temperature, several researchers have measured the heat transfer coefficient (h_{fp}) at the fluid-particle interface. The techniques to estimate h_{fp} have aspects of both the inverse heat transfer (conduction) problem and parameter estimation. A survey of the literature shown in table 3.5 indicates that there is a large variation in the values of h_{fp} determined by different researchers (18–7,870 W/m^2 °C). This is due to variations in experimental conditions and type of products. The different products used for these studies included water, starch solutions, and gum solutions. The different methods of measurement included calculation of temperature based on direct measurement of particle temperature with stationary particles and moving fluid, as well as moving particle in stationary or moving fluids, or calculations based on the destruction of imbedded bacterial spores (Heppel 1985) or enzymes. Chakrabandhu and Singh (1998) measured the h_{fp} values for rotating and stationary particles to simulate the actual conditions in a tube flow. It is obvious that the rotating particles had higher h_{fp} values than the stationary ones. In a subsequent study the same authors determined h_{fp} for multiple particles in straight and coiled tube flow situations (Chakrabandhu and Singh 2002). Significantly higher h_{fp} value was observed for particles in coiled tube as compared to that in straight tube (table 3.5).

Table 3.5 Heat transfer coefficient values between liquid and particles (h_{fp}) reported in literature (modified from Bhamidipati and Singh 1995b)

Source	Range of h_{fp} (W/m^2 °C)
Heppel 1985	930–7,870
Sastry et al. 1989	2,039–2,507
Chang and Toledo 1989	146–303
Chandarana and Gavin 1989	18.0–65.2
Sastry et al. 1990	638–3,005
Alhamdan et al. 1990	652–850
Weng et al. 1992	122.6–233.7
Astrom and Bark 1993	50–513
Mwangi et al. 1993	58.3–1,301.3
Balasubramaniam and Sastry 1994	986–2,270
Bhamidipati and Singh 1995a	108–195
Chakrabandhu and Singh 1998	120–2,400 stationary particle
	360–4,300 rotating particle
Chakrabandhu and Singh 2002	538–1,450 coiled tube
	204–1,220 straight tube

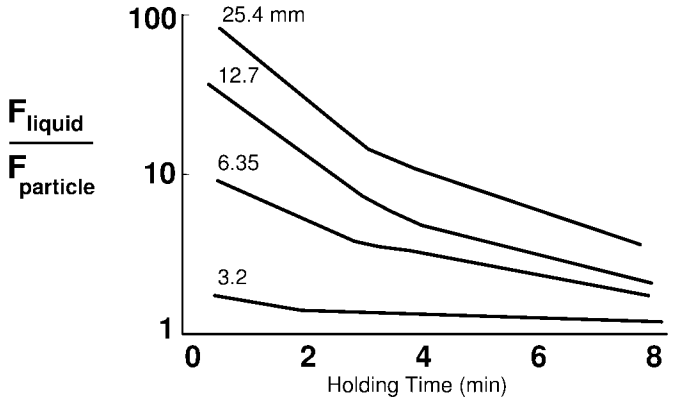

Figure 3.5. Lethality in liquid to that in particulates affected by particle size and holding time. This is on a semi-log scale, and the Y-axis indicates ratio of lethality. From de Ruyter and Brunet (1973).

Sterility or microbial lethality in liquid and particles are different because of the resistance to the heat transfer. The smaller particulates (< 3 mm) get heated at the same rate as the liquid (de Ruyter and Brunet 1973). However, larger particles may require significantly longer time to reach the intended temperature, thus causing overprocessing of the liquid (fig. 3.5). In the figure, one can notice that a 25.4 mm cube particle for a 2 minute hold time would require hundredfolds higher lethality in liquid to receive the intended lethality at the center of the particle. This overprocessing has hindered the adaptation of aseptic processing for low-acid particulates.

Once a minimum residence time is established, a process temperature can then be calculated for the liquid foods using the following:

$$T = TR + Z \log (F/t) \tag{3.4}$$

where T = process temperature (°C) measured at the end of the hold tube, TR = reference temperature (°C), Z = temperature (°C) change necessary for the D-value to change by a factor of 10 or for 1-log or 90% reduction, t = hold time (minutes or seconds) calculated from flow rate and tube diameter, and F = sterilizing value needed to achieve commercial sterility for the product. However, the product temperature in the center of particulate will be lower than the liquid temperature, and it is impractical to measure it during the process. Therefore, numerical and analytical models have been developed to predict the least lethal particle temperature and use the predicted value for process verification (Bhamidipati and Singh 1994; Lee, Singh, and Chandaranu 1990; Lee, Singh, and Larkin, 1990).

In many cases one has to combine the fluid flow and heat transfer characteristics to gain knowledge of the complex system. The complexity comes from the non-Newtonian nature of fluid, large particulates, and turbulent flow. Son and Singh (2002) developed models (a computer program) for simulation of turbulent flow and successfully verified them with known analytical solutions of non-Newtonian laminar flow and by comparing the bulk temperatures from the analytical calculation with the simulated average temperatures along the length of the heat exchanger. For the purpose of verifying simulated velocity pro-

files, the RTDs of soybean milk were measured in a helical-type tubular heat exchanger. The experimentally determined velocity profiles were flatter than those simulated under laminar and turbulent flow conditions due to the secondary flow by a helically coiled tube. To compensate for the curvature effect, the original simulation was modified by a correction factor based on the curvature ratio (heat exchanger internal diameter/diameter of coil) and Reynolds number. Temperature profiles in a heating tube were also measured by inserting thermocouples at the end of the heat exchanger. The measured temperatures of 9% soybean milk were consistent with the simulated temperatures. However, in the case of 16% soy milk, the measured temperatures were slightly higher than those of simulation. Through the successful simulation of velocity and temperature distributions under turbulent flow conditions, one can find the desirable processing conditions to maximize the quality of aseptically processed soybean milk without numerous experiments.

Determining and Controlling Product Flow Rate

As described previously, thermal inactivation of microorganisms within a food product is an integral of time and temperature. Therefore, a thermal process for a particular product will include time and temperature functions. The conventional approach to assure the proper time at the specified temperature involves the use of a "hold tube" within the system immediately following the product heaters. This term seems somewhat of a misnomer since product does not remain stationary but continues to flow through the entire system, including the hold tube. The size of the hold tube is designed to retain the fastest particle of product for a specified number of seconds at a specified flow rate. Therefore, if the temperature of the product at the end of the hold tube is at or above the minimum specified in the process and the flow rate has not exceeded the specified maximum, then every element of the food will receive at least the minimum thermal process.

Certain characteristics are essential to a specified thermal process. The product supply throughout the system must be steady and the flow rate must be controlled. Thus, an appropriate device must be included that will regulate the product flow rate through the hold tube. This is usually accomplished using a positive displacement pump designated as the timing or metering pump. This pump can be either fixed rate or variable speed (fig. 3.6). If the pump is variable speed, a means must be provided to prevent unauthorized speed changes so that the maximum product feed rate is not exceeded. The type of pumps most commonly used for this purpose are reciprocating piston-type pumps for low to medium viscosity products or interlocking lobe-type pumps as shown in figure 3.6 for medium to high-viscosity products. Centrifugal pumps should not be used as timing pumps because they are not positive displacement pumps and flow rate will be affected by system pressure.

Some means must also be developed to verify the specified product flow rate. One method is to correlate pumping rate of water through a cold system under a no-load (no backpressure) recording tachometer to provide an indirect record of the product flow rate. The presence or absence of backpressure in the system should be noted. Instruments in the form of various flow measuring devices, which give a direct indication of product flow, have been developed. However, the regulatory status of such flow meters is uncertain at present. Adequate data to verify accuracy and reliability of such instruments will be needed before these instruments can be used to satisfy regulatory requirements for flow rate documentation.

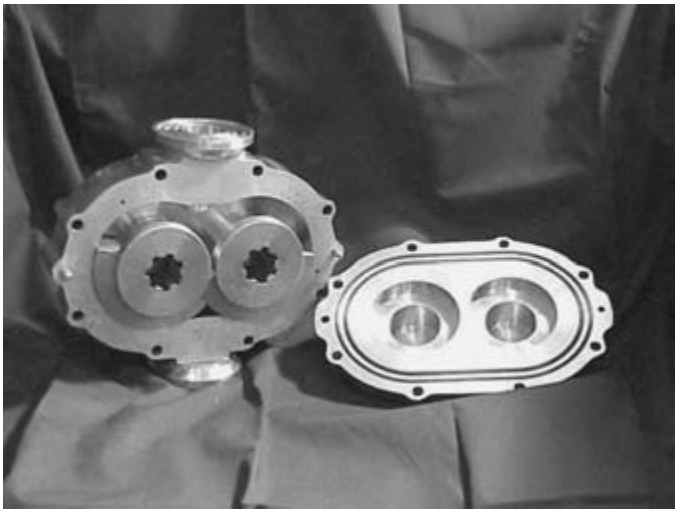

Figure 3.6. Face view of a lobe-type positive displacement pump used as a metering pump in an aseptic system.

Filling and Packaging

The containers for aseptic products are sterilized by heat or chemicals. Steam is used for sterilizing metal cans and drums, whereas heat of thermoforming sterilizes the thermoformed plastics. A combination of 30% hydrogen peroxide and heat is used in sterilizing preformed aseptic containers and closures (Clark 2004). The bulk bags are sterilized by irradiation, whereas chemicals are used for sterilizing the bulk rigid containers. The bulk containers for storage of orange juice are sterilized by flooding with 25 μg/g (25 ppm) iodophor. The calculation of sterility for packaging material is done in the same manner as that for the product.

The filling environment is sterilized by heat or chemicals. After sterilization, the environment is maintained sterile using filtered air or inert gas (Clark 2004). The filters must be sterilized and validated to assure that there is no recontamination of the sterile environment.

Monitors and Controls

In order to verify that all critical factors are met or exceeded and that equipment is functioning as intended, adequate instrumentation, recorders, and controls must be in place. Particular attention should be given to the location of sensors and to the controlling logic. As previously noted, the temperature of every particle of the product must be at or above a specific temperature for a specified time. Achieving the proper hold time depends on proper sizing of the hold tube and the flow rate of the product. For homogeneous products, temperature can be documented by indicating and recording devices properly located in the hold tube. The temperature at the inlet of the tube is monitored with a temperature recorder-controller located at the final heater outlet. An acceptable temperature-indicating

device such as an accurate thermocouple recorder or a mercury-in-glass thermometer (MIG) must be installed between the hold tube outlet and the inlet to the first cooler. In addition, there must also be an automatic recording device located in the product stream at the hold tube outlet. The product temperature should be measured at the hold tube. The temperature-recording device chart graduations must mot exceed 1°C (2°F) within a range of 6°C (10°F) of the desired product sterilization temperature.

The processor should keep in mind that the temperature-indicating device (thermocouple or MIG) is considered the reference temperature instrument by the regulatory agencies. The temperature recorder must be adjusted to agree as nearly as possible with, but in no event higher than, the known accurate temperature-indicating device. The temperature-indicating device must be tested for accuracy against a known accurate standard thermometer upon installation and at least once a year thereafter or more frequently, if necessary, to ensure its accuracy.

If regeneration is used, the pressure on the sterile product must always be higher than the pressure of the raw product. One pressure sensor is typically located at the sterile product outlet and the other at the raw, and sterile product must be recorded continuously. Other requirements and recommendations regarding temperature monitoring and recording devices are contained in the regulations. Those who will be in charge of these functions should become familiar with the appropriate regulatory requirements.

Temperature-monitoring equipment, as well as pressure sensors, timers, and so forth, will typically be interfaced with various control systems. Control systems available for both processing and packaging equipment can be adapted for complete automation. However, this adaptation may not necessarily mean that these installations will meet regulatory requirements or that the operation is being properly controlled. The logic controlling such systems must be evaluated to verify that adequate controls, interlocks, and other safety features have been incorporated and are functioning as intended. The control software and system performance should be verified at installation and routinely thereafter. The interlocks (alarms) that monitor critical functions must be tested periodically to prove that they are functioning properly and the results of these challenges recorded. Changes to the control software should only be made by authorized personnel.

At present, regulatory agencies responsible for the safety of shelf-stable, low-acid foods do not endorse exclusive use of automatic control systems to assure commercial sterility without verifying adequate operation. In addition, look for some operator intervention in the form of handwritten records and operator interaction with the control system. Thus, appropriate gauges and recording devices should also be included so that operators can observe results and record the information in the daily production log. This precaution will also serve as a check against the automatic system. Further recommendation regarding automatic control systems can be found in NFPA's bulletin 43-L, "Automatic Control Guidelines for Aseptic Systems Manufacturers and Companies Using Aseptic Processing and Packaging for Preserving Foods."

Processing System Sterilization

As required by regulatory agencies, all product contact surfaces downstream from the final product heater must be brought to a condition of commercial sterility prior to production. Most systems use saturated steam or pressurized hot water to sterilize the processing system. The system is sterilized by maintaining the temperature within all parts of

the system at or above a specified temperature by continuously circulating the sterilizing medium for a specified period of time. If steam is used as a sterilizing medium, adequate provisions must be made to remove condensate from the system. Inadequate sterilization can result from condensate collecting in low spots within the system due to excessively low temperature.

Aseptic systems may include unique items such as aseptic pumps, flash tanks, and surge tanks in addition to heat exchangers, piping, and valves. Due to the large capacity of various surge tanks, these units are usually sterilized with saturated steam rather than hot water. Surge tank sterilization may proceed separately from the sterilization cycle for the rest of the processing system but should occur simultaneously with this cycle. The surge tank may require special attention, such as documentation of uniform heat distribution during sterilization. Microbiological challenges may also be needed to confirm sterility of inaccessible areas associated with the surge tank where temperatures cannot be directly measured.

The preproduction equipment sterilization cycle is usually monitored by temperature sensors located at the coldest point(s) within the system. The sterilization time should incorporate only that portion of the cycle where all temperatures are at or above the established minimum temperature. If the temperature drops below the minimum sterilization temperature, the timer should be restarted to zero and timing should not restart until the proper temperature is reestablished. A permanent and continuous record of temperatures must be made during the sterilization period.

Maintenance of Sterility

After the system sterilization cycle is completed, a transition phase is initiated during which the system beyond the end of the hold tube is cooled and readied for the introduction of product. From the end of sterilization through the end of the production period, the system must be maintained in a sterile condition. One of the most common ways to prevent contaminants from entering the system is to maintain the product under constant positive pressure. As stated earlier, a backpressure device located after the product coolers and typically before the filler is used to maintain the product at a pressure that is usually 69–103 kPa (10–15 psig) over the pressure exerted by the product at its maximum temperature. This excess pressure not only prevents flashing of the product but also the recontamination after sterilization.

There are some areas where rotating shafts (aseptic) or reciprocating shafts (valve stems) may allow entrance of bacteria in the sterile product area (zone). An effective barrier against the entry of microorganisms needs to be provided at all potential contamination sites. Steam seals are commonly used for this purpose. Steam seals consist of an area where steam in a groove or trace in the housing forms a ring around a pump shaft or covers the total stroke of a valve stem. Steam must be continually supplied to the seal area, and the proper operation of the steam seal must be verified by the operator. Operator verification usually consists of visually checking for steam discharge from the individual seal or an indication of temperature at seal discharge points.

An automatic flow diversion device may also be utilized in an aseptic processing system to prevent a potentially nonsterile product from reaching the packaging equipment. The flow diversion device, usually located after the backpressure valve, must be designed so that it can be adequately sterilized and operated in a reliable manner. It is recommended

that the flow diversion device be activated by the control system that monitors temperature at the end of the hold tube and other devices that monitor critical factors such as differential pressures in a regenerator or positive pressure in a surge tank. Should there be a deviation in any of these critical factors, the flow diversion device should divert product flow away from the filler and prevent product from being packaged.

Process Confirmation

Once the hold tube length, process temperature, and product flow rate have been calculated and the system has successfully completed trial runs, tests of inoculated packs of product or simulated product should be conducted for confirmation of proper system operation. These inoculated pack studies are conducted by batch inoculating the product with an appropriate test organism followed by processing at the maximum flow rate (minimum residence time) specified in the scheduled thermal process. Temperatures should be varied during the inoculated pack study to yield a product processed to preselected lethality values. Generally, five temperatures should be used and the pack is run continuously, starting with the highest temperature and then adjusting the temperature downward to the next target temperature.

At least one hundred packages containing inoculated product should be collected from each temperature interval. These packages should be incubated and monitored for spoilage. Results of the inoculated pack should correlate with calculated lethality delivered by the system at each temperature and should confirm operation of the equipment.

The automatic controls and safety devices built into both the processing and packaging systems should also be challenged to verify proper function. For example, the flow diversion device should be challenged to verify that no product is packed following a temperature drop or other fault. Other interlocks, such as system shutdown in the event of a loss of sterile air pressure, or loss of proper pressure differentials, should also be verified. Agreement among sensing devices, indicators, and recorders should be checked, and the programming of automatic control devices should be verified. Calibration of all sensing and measuring devices must be completed before system start-up and at subsequent intervals as prescribed by regulations.

It is suggested that at least four small-scale production runs of uninoculated product be packed and incubated, followed by 100% examination for evidence of spoilage. Records of these commissioning trials, as well as other test results, must be retained by the packer.

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4 UHT and Aseptic Processing of Milk and Milk Products

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Introduction

Although ultra-high-temperature (UHT) technology has been used successfully in milk processing for 40 years, several aspects still present challenges for UHT processors. The key challenges include selection of optimal processing conditions, particularly the temperature-time profile, raw milk quality, and poststerilization handling. These challenges are discussed in this chapter. The physical and chemical changes occurring in UHT milk during heating and storage limit its shelf life; this aspect is also discussed, along with control mechanisms to ensure the production of high-quality UHT milk.

Ultra-high-temperature (UHT) processing involves heating milk in a continuous-flow system to a high temperature (~135–145°C) and holding it at that temperature for a short time (1–10 seconds) followed by rapid cooling. This produces a "commercially sterile" product, that is, a product in which bacterial growth is highly unlikely to occur under normal storage conditions. Since heating and cooling take place through relatively small distances in the flowing liquid in the UHT process, the heat penetration problems of in-container sterilization are avoided. This rapid heat transfer rate minimizes undesirable changes in the taste and nutritional quality of the resulting product. Since UHT is a continuous process, it produces uniform product quality that does not depend on the size of a container, unlike in-container sterilization. This attribute is especially important for products containing heat-sensitive ingredients and highly viscous products with poor heat transfer properties.

Aseptic processing involves UHT processing followed by filling the product into sterile containers in a sterile environment and sealing the containers in a sterile manner in a continuous process. In practice, UHT-processed product is usually transferred to an aseptic tank before it is aseptically packed into containers. This provides greater operational flexibility and allows the use of processing and aseptic packaging equipment with different capacities. The most popular aseptic packages are the tetrahedral-shaped paperboard cartons exemplified by Tetra Pak and Combibloc products, although multilayered plastic bottles are also now popular.

The introduction of UHT treatment of milk, coupled with aseptic packaging, made a fundamental improvement in the bacteriological safety of milk and extended its shelf life from the typical 2–3 weeks for refrigerated pasteurized milk to 6–9 months without refrigeration. No preservative is added to UHT milk for its extended shelf life at room temperature, an important point for today's additive-conscious consumer.

Although pasteurization of milk effectively removes potential pathogenic bacteria, the heat process is not sufficient to destroy heat-resistant bacterial spores. UHT processing of milk was originally developed with the objective of producing sterile milk of superior organoleptic and nutritional quality to that of its in-container-sterilized counterpart. UHT

milk is also known as long-life milk and as ultrapasteurized milk, particularly in the United States (Mehta 1980). Being a highly perishable biological material, milk is very susceptible to microbial and chemical degradation even at refrigeration temperatures. Therefore, it is a major challenge to manufacture sterile milk that will retain good sensory characteristics and physical stability during storage at room temperature for several months.

Aseptic packaging is the process that allows UHT-processed milk to have a nonrefrigerated shelf life of several months. While UHT treatment destroys all microorganisms that are likely to grow under the normal conditions of storage, aseptic packaging prevents the ingress of contaminating organisms during packaging and subsequent nonrefrigerated storage of the unopened package. Therefore, the full benefit of commercial UHT treatment cannot be realized without the use of aseptic packaging. In fact, aseptic packaging was revolutionary in taking UHT processing from the research laboratory to the grocery store.

UHT products are very appropriate in countries where environmental temperatures are high and home delivery and refrigeration is not common. UHT milk is also favored for other situations involving lack of refrigeration or requiring added convenience, for example, camping, traveling, emergency preparedness, disaster response, and space travel. In addition, UHT treatment could be beneficial for countering bio-terrorism, as it can produce bacteriologically safe product even if the raw material is contaminated with pathogenic organisms.

UHT processes are also used in some special cases where other processes are inadequate. For example, in the production of microfiltered whole milk, the cream portion, which contains fat globules that cannot pass through the microfiltration membrane, is UHT processed and finally mixed with the microfiltered skim milk to produce an extended-shelf-life (ESL) product.

Historical Development

The early development of UHT and aseptic processes has been reviewed by Hostettler (1972), Westhoff (1978), and Mitchell (1990). Table 4.1 summarizes these developments. Although UHT processing was developed as early as 1938, aseptic processing, which enabled the distribution of milk without refrigeration, commenced only in 1961.

The first continuous-flow heater, in which milk was indirectly heated at 125°C with a holding time of 6 minutes, was manufactured in 1893 (Hostettler 1972), only 11 years after the first commercial pasteurizer was used (Kelly et al. 2005). The direct heating method, in which milk is mixed with a jet of steam, or hot gas, in a continuous-flow heater to obtain temperatures of 130–140°C, was patented in 1912; this process avoided the burn-on encountered in the indirect heating (Hostettler 1972). Although various types of heating systems, for example, steam injection and infusion, were developed for UHT heating to achieve a better sterile product by 1927, the difficulty of packaging the sterile milk without recontamination into suitable containers for transport hindered the commercialization of UHT products until 1953. UHT-processed milk, produced in an innovative heater known as Uperiser® and aseptically packed in cans, was first developed in Switzerland in 1953 (Hostettler 1972; Robertson 2003) through the collaborative efforts of a dairy company and a machinery manufacturing firm. A similar heating technique was employed for long-shelf-life milk, but this was aseptically packaged into a tetrahedral paperboard carton for sale in Switzerland in 1961 (Robertson 2003). The aseptic packaging technique provided the impetus for the tremendous growth in the UHT milk and dairy products market. Now

TABLE 4.1 Early UHT and aseptic processes

Year	Product	Processing Conditions	Processing Plant	Product Quality or Significance	Reference
1938	Chocolate milk	149°C/15 sec	Heat-cool-fill (HCF) unit	Good	Ball and Olson 1957
1942	Cream	127–138°C	Direct steam injection	Excellent	Ball and Olson 1957
1951	Pea soup	140–150°C/8.8 sec	Flash heating and cooling by tubular heat exchanger (indirect heating)	Excellent	Havighorst 1951
1953	Milk	UHT-treated milk in can 150°C/2.4 sec	Direct steam injection	Excellent	Robertson 2003
1961	Milk	UHT-treated milk in paperboard carton	Direct steam injection	Excellent	Kosaric et al. 1981
1964	Ice cream mix and concentrated milk formulations	UHT-treated product in 4-liter cans	Sulzer steam injection with Dole aseptic canner	First commercial aseptic process in Australia	Zadow 1998
1969	Milk	Not available	Indirect plate heat exchanger	First UHT milk in U.S. market	Kosaric et al. 1981

in some countries, for example, Germany, France, Italy, and Spain, UHT milk and dairy products constitute a substantial share of the dairy products market. However, in other countries such as the United States, U.K., and Australia, UHT milk accounts for less than 10% of market milk sales.

UHT products now include liquid products such as white milk, flavored milks, energy and sports drinks, soy "milk," and other grain-based beverages; viscous products such as cream, yogurt, custards, and salad dressings; and foods with discrete particles such as vegetable purees, baby foods, desserts, sauces, dips, topping mixes, and stews. However, the dairy industry is the major user of UHT processing and production of UHT dairy products continues to grow. This chapter focuses on these products with particular emphasis on UHT milk.

Definitions

UHT Milk

According to IDF (1981), "UHT milk is a type of sterilized milk produced by a single uninterrupted continuous-flow heating process involving a high-temperature short-time combination of at least 130°C for a few seconds—or any other combination which will give the same results—associated with aseptic packaging, and should pass the following tests:

- keeping quality test as described in IDF standard 48 (1969), and
- give turbidity when subjected to the turbidity test by a modified Aschaffenberg Test."

In this definition, the keeping quality test is designed to establish the sterility of the product and the turbidity test is used to ensure the product has not been subjected to excessive heat treatment (Zadow 1986). According to the keeping quality test, UHT products must be microbiologically stable at room temperature, either measured after storage until the end of the desired shelf life, or after incubation at 55°C for 7 days or at 30°C for 15 days (Codex Alimentarius 2004).

The modified Aschaffenberg turbidity test is a simple test for the presence of undenatured whey protein. In the test, casein and denatured whey proteins in the UHT milk are precipitated with ammonium sulfate and the resulting solution filtered. The undenatured whey proteins remain in the filtrate and produce turbidity when the filtrate is boiled. The amount of turbidity is indicative of the amount of undenatured whey proteins. In-container-sterilized milk, having no undenatured whey proteins, gives no turbidity, while UHT milk containing residual undenatured whey proteins produces a positive turbidity test.

The Aschaffenberg turbidity test is not very satisfactory for distinguishing UHT and in-container-sterilized milk, as it often produces a negative turbidity result for UHT milk from indirect UHT plants and plants with a long preheating stage, regardless of the high heat conditions (Lewis and Heppell 2000). Despite this limitation, residual β-lactoglobulin is used in current standards for this purpose along with the concentration of lactulose. For UHT milk, the standards for β-lactoglobulin and lactulose are > 50 mg/L and between 100 and 600 mg/L, respectively, and for sterile milk, < 50 mg/L and > 600 mg/L, respectively.

The minimum temperature and time standards for UHT treatment vary between jurisdictions, for example, 135°C and ≥ 1 second in the EU and 137.8°C and ≥ 2 second in the United States (Pearce 2004).

The Codex Alimentarius Draft Code of Hygienic Practice for Milk and Milk Products states that "UHT treatment is normally in the range of 135 to 150°C in combination with appropriate holding times necessary to achieve commercial sterility" (Codex Alimentarius 2004). No minimum temperature-time condition is specified. However, it is generally recognized that this should be equivalent to an Fo (see below) of ~3, the 12-log reduction of *Clostridium botulinum* criterion used in canning. This is despite the fact that the occurrence of *C. botulinum* in milk is extremely rare (Codex Alimentarius 2004).

The nominal temperature-time combination cited for a UHT process, for example, 140°C for 4 seconds, refers to that of the holding tube that is located immediately after the high heat exchanger and maintains the same sterilization temperature as the peak temperature reached in the UHT sterilizer. However, the application of a single temperature and time oversimplifies the processing situation in UHT plants and implies two common assumptions:

1. the only times and temperatures that matter are those in the holding tube, and
2. all particles in the milk receive the same heat treatment.

The first assumption ignores the fact that there are different temperature-time profiles in UHT plants, while the second takes no account of the residence time distribution of the particles as they pass through the holding tube. These topics are discussed below in the sections "Temperature-Time Profiles of UHT Plants" and "Residence Time Distribution in UHT Processing," respectively.

Bacterial (B*, Fo) and Chemical (C*) Indices

In order to provide a measure of the effect of a particular heating regime on the bacteria in milk and on the chemical components of milk, some useful indices have been developed. The major ones are B* and Fo (bacterial) and C* (chemical).

B* is a measure of the bacteriological effect of a heat treatment relevant to treatment at a reference temperature of 135°C. A process with B* = 1 produces a 9-decimal reduction of thermophilic spores assuming a Z-value of 10.5 and is equivalent to holding the product at 135°C for 10.1 seconds. B* can be expressed as follows:

$$B^* = 10^{((T-135)/10.5)} * dt/10.1 \quad (4.1)$$

Another bacterial index with which food technologists are familiar is Fo, an index commonly used in canning. Numerically, it is the equivalent time in minutes at the reference temperature of 121.1°C (250°F) of a heat process, assuming a Z-value of 10°C. It can be expressed as follows:

$$Fo = 10^{((T-121)/10)} * dt \quad (4.2)$$

Fo is based on the destruction of spores of the anaerobic pathogen *Clostridium botulinum.* While it is a measure of the lethality of the heat treatment, it is more appropriate to heating at temperatures around 120°C than around 140°C. As indicated above, UHT processes should have an Fo $\geq$ ~3 to ensure bacteriological safety.

C* is a measure of the chemical effect of a heat treatment relevant to treatment at a reference temperature of 135°C. A process with C* = 1 reduces the concentration of thiamine by 3% assuming a Z-value of 31.4 and is equivalent to holding at 135°C for 30.5s. C* can be expressed as follows:

$$C^* = 10^{((T-135)/31.4)} * dt/30.5 \quad (4.3)$$

As a general rule, a UHT plant should have a B* of at least 1 and a C* of no greater than 1.

C* is based on the kinetics of destruction of thiamine, that is, the higher the index, the greater the destruction of this vitamin and, by inference, the greater the change in other chemical components of milk. However, not all chemical components change with temperature and time at the same rate as thiamine. A good example of this is that heating at 90°C for 30 seconds, common UHT preheating conditions, destroys less than 0.1% of thiamine but denatures up to 75% of the major whey protein, β-lactoglobulin.

Principles of UHT Processing

There are two basic principles of UHT processing that distinguish it from in-container sterilization.

UHT Principle 1: For the same bacterial destruction, a high-temperature short-time treatment results in less chemical change than a low-temperature long-time treatment.

This is illustrated in table 4.2, which shows the relative chemical effect at different temperatures for the same bacterial inactivation effect. Using typical Q_{10} values (Q_{10} is the rel-

Table 4.2 Chemical and bactericidal effects with temperature (assuming Q_{10} for spore destruction is 10 and for chemical change is 3)

Temperature (°C)	Time for Equal Bactericidal Effect	Chemical Change for Same Bactericidal Effect
115	1	100
125	.1	30
135	.01	9
145	.001	2.7

ative change in reaction rate with a 10°C change in temperature) for chemical reactions and bacterial spore inactivation, the table indicates that the amount of chemical change at 145°C is only about 2.7% of that at 115°C. Given this quite dramatic effect, the question arises as to the optimum combination of temperature and time. A partial answer to this lies in the second principle of UHT processing.

UHT Principle 2: Minimum times and temperatures are dictated by the need to inactivate thermophilic bacterial spores while the maximum times and temperatures are determined by the need to minimize undesirable chemical alterations.

This is illustrated in the (log) time-temperature graph in figure 4.1 showing the line corresponding to a 9-log reduction of thermophilic spores (the B* = 1 line) and a line representing 3% reduction in thiamine (the C* = 1 line). The B* = 1 specification has now been generally accepted as the desired lower limit and represents a higher heat treatment than that required for the previously accepted 9-log reduction of mesophilic spores.

However, since the discovery of the extremely heat-resistant mesophilic sporeformer *Bacillus sporothermodurans* in UHT milk (IDF 2000), an even higher level heat treatment denoted by the dotted line in figure 4.1 is recommended for reduction of such highly heat-resistant sporeformers by 9 logs.

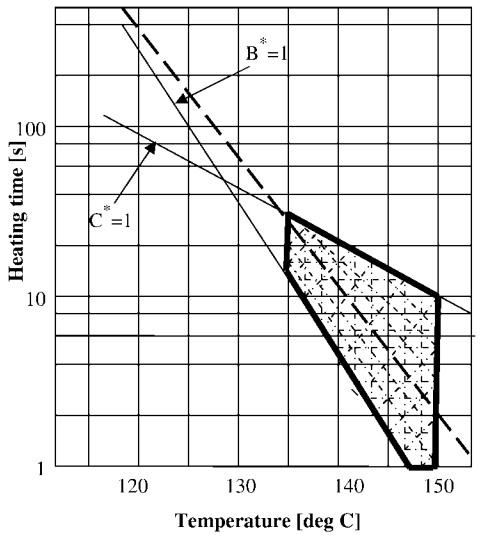

Figure 4.1. Lines for 9-log reduction of thermophilic spores (B* = 1), 9-log reduction of highly heat resistant spores (dotted line), and 3% destruction of thiamine (C* = 1).

Treatments with C* ≤ 1 show little destruction of nutrients, minimal flavor change, and no detectable color change due to Maillard browning. More intense treatments result in greater destruction of thiamine and other vitamins and greater flavor and color change. The upper temperature at which a plant can operate at a C* ≤ 1 is limited by the minimum holding time that the plant's physical configuration will allow.

UHT Heating Systems

There are two main ways in which heat can be transferred from the heating medium to the product, for example, milk, in UHT heating; these are classified as "direct" and "indirect." In *direct* heating, superheated steam is mixed directly with the product, while *indirect* heating involves a heat exchanger, either tubular or plate, that transfers the heat across a partition between the product and the heating medium, which can be either superheated steam or hot water. Each heating mode has its advantages and commercial plants featuring combinations of both modes have been introduced to take advantage of the benefits of both types. A summary comparison of direct and indirect systems is given in table 4.3.

Table 4.3 Comparison of direct and indirect UHT heating systems (Datta et al. 2002)

Parameter	Direct System	Indirect System
Processing characteristics and parameters		
Preheating (at ~90°C) "protein stabilization" step	Uncommon	Widely used
Sterilizing temperature for equal sterilization effect	3–4°C higher than in indirect systems	3–4°C lower than in direct systems
Homogenizer placement	After sterilization (requires aseptic homogenizer)	Before or after sterilization
Heating velocity (kcal/h/m^2/°C)	100,000–120,000 (film heat transfer coefficient)	3,000–4,000 (overall heat transfer coefficient)
Ability to process viscous product	Reasonable, especially with infusion	Little with plate but some capability with tubular
Fouling/burn-on	Minimal	A major problem especially with plate heat exchangers
Run time	Long (twice the length for plate-type indirect systems)	Short (tubular longer than plate type)
Heat regeneration	~50%	≥90%
Operating cost	Higher than indirect due to lower heat regeneration	More cleaning reduces advantage of high heat recovery
Plant cost	Higher than indirect	
High-quality steam requirement	Yes	No
Electric power requirement	Higher	Lower
Ability to reach very high temperature (i.e., > 145°C)	Capable	Limited
Ability to destroy heat-resistant sporeformers without excessive chemical damage	High	Low

(*continued*)

Table 4.3 Comparison of direct and indirect UHT heating systems (Datta et al. 2002) (*continued*)

Parameter	Direct System	Indirect System
Process control issues	Careful control of water removal after high heat treatment required to prevent concentration or dilution	Need to control pressure increase and temperature differential between product and heating tube or plate as fouling layer builds up
Possibility of contamination from heating medium through pinholes	Nil for sterilizing section. Possible in regeneration and other indirect heating and cooling if pressure differential not maintained	More significant than direct especially with plate heat exchanger
Water requirement	Greater (~1,500 L water per 1,000 L product) than for indirect system	Less than for direct systems
Other process features	Steam injection causes some homogenization	Tubular is most common UHT heating system. Corrugated tubes used to increase turbulence
Product (UHT milk) characteristics		
Flavor	Less cooked	More cooked
Oxygen level at packaging (see Table 4.5)	Low (< 1 ppm) unless milk becomes saturated with air in the aseptic tank	High (7–9 ppm)
Sediment formation during storage	High (2× indirect)	Low
Susceptibility to age gelation	High	Low
Plasmin and plasminogen level	Neither completely inactivated	Plasmin generally inactivated but some residual plasminogen
Fat separation	Low, especially for steam injection	More than for direct
Heat indices—HMF, lactulose, furosine	Low	High
Heat index—undenatured ß-lactoglobulin	Medium	Low
Folic acid and vitamin C retention	High if oxygen level is low	Low

In direct systems, the superheated steam is either "injected" into a stream of the product (steam into product) or the product is sprayed as a thin film or fine streams into superheated steam in an "infusion" chamber (product into steam). These two systems are known as *steam injection* and *steam infusion,* respectively. It should be noted that direct heating is only used for the high-temperature section of a so-called direct plant; indirect heating is used for preheating and final cooling. In indirect plants, preheating uses hot product to heat incoming cold product, thus regenerating some of the heat used and reducing overall energy usage.

In both direct processes, the temperature of milk is raised almost instantaneously by transferring the latent heat of vaporization of the steam to the product. In direct heating, steam is condensed and the milk is diluted by about 1% per 5.6°C rise in temperature; for an increase in temperature of 60°C (e.g., from 80°C to 140°C) by direct heating, the increase in volume of the liquid is approximately 11% (Lewis and Heppell 2000). This extra water is removed when the heated milk is flash cooled in the vacuum chamber. In order to

ensure that there is no dilution of the final milk, its temperature on leaving the flash vessel should be approximately the same as the preheated milk temperature.

An alternative means of indirect heating in UHT processing is electrical heating using electrically heated tubes (Deeth 1999). It is usually used for the high-temperature section only with the preheating section using the hot product to heat incoming cold product. The technology has been used in several plants in Europe for pasteurization and sterilization of foods including milk, sweetened milk, cream, and other dairy products.

Scraped surface heat exchangers are used for UHT processing of viscous products and products containing particulate matter. The temperature differential between the product and the heating surface is relatively high, which indicates a high potential for some parts of the product to be overheated and for burn-on to occur on the heating surface. This system is incapable of regeneration of heat and consequently has relatively low energy efficiency compared with other indirect heating systems (Deeth and Datta 2003). Overheating and burn-on in scraped surface heat exchangers can be overcome by the use of a direct steam injection system using multiple injectors. Condensation of the injected steam forms a film of water on the walls of the heating chamber and prevents local overheating and burn-on. Equipment using this principle is used commercially for UHT processing of viscous products (e.g., the Rototherm®, Bell 2004–2005).

Commercial and pilot-scale UHT plants are produced by several manufacturers. In the UHT literature, these are often referred to by their brand names, for example, VTIS, AROVAC, and Ultramatic, and the mode of heating is not always obvious. To assist in understanding such literature, a list of some commercial UHT sterilizers, compiled according to their heating mode, is presented in table 4.4.

Temperature-Time Profiles of UHT Plants

To address assumption 1 from the "Definitions" section that *the only times and temperatures that matter are those in the holding tube*, it is necessary to consider the temperature-time profile of UHT plants. Figures 4.2 and 4.3 illustrate simplified profiles of plants using direct high-temperature heating (injection of infusion) and indirect heating (with plate or tubular heat exchangers), respectively. Since temperatures below ~70°C have little or no effect on bacterial spores or chemical components in milk, the assumption holds reasonably well for direct processes where the times of heating from ~70°C to ~140°C and subsequent cooling to ~70°C are very short (less than 1 second). However, it does not hold well for the indirect process, where the time taken to heat the milk from ~70°C to ~140°C and to subsequently cool to the same temperature is substantial. This time usually includes a preheating section in which the temperature is raised from ~5°C to ~90°C and a preheat holding section at ~90°C for ~30 seconds known as a "protein stabilization" step. This heat load adds significantly to both the chemical and bacterial effects, but because of the first principle of UHT processing discussed above, it adds substantially more to the chemical effect than the bacterial effect. This is illustrated in figure 4.4, which shows the relative contributions of the heating, holding, and cooling sections to C* and B* in an indirect UHT plant with the temperature-time profile depicted in figure 4.3. In this example, the holding tube contributes 66% to the total B* (6.75) but only 39% to total C* (1.83). The heating and cooling sections account for the balance. The corresponding figures for the holding tube contribution in a direct plant with the temperature-time profile in figure 4.2 are 98% and 95%, respectively; the total B* and C* for such a plant are 2.3 and 0.3, respectively.

Table 4.4 Commercial UHT systems

Commercial Name of UHT Sterilizer	Name of Manufacturer	Heating Mode
VTIS	Alfa-Laval (U.S.)	Steam injection (D)
ARO-VAC Process	Cherry-Burrell Corp., USA	Steam injection (D)
Uperiser	Alpura Co (Switzerland)	Steam injection (D)
Grindrod	Smith, Kline and French of USA	Steam injection (D)
Languilharre System	Ets Laguilharre of Court voie (France)	Steam infusion (D)
Thermovac	Breil and Martel (France)	Steam infusion (D)
Palarisator	Paasch & Silkeborg (Denmark)	Steam infusion (D)
Steritwin UHT Sterilizer	Stork	Steam infusion (D)
Ultra Therm	Creamery Package Divisions, USA	Steam infusion (D)
Da-Si Sterilizer	Da-Si Industries	Steam infusion (D)
Spiratherm	Cherry-Burrell Corp., USA	Indirect spiral tubes (Ind)
Gerbig	Gerbig of Germany	Indirect tubes (Ind)
Sterideal System	Gebr. Stork & Co (Amsterdam)	Indirect tubes (Ind)
Ultramatic	APV	Indirect plate (Ind)
Ahlborn Process	Ed Ahlborn of Hildesh (Germany)	Indirect plate (Ind)
Sordi Sterilizer	M. Sordi of Lodi (Italy)	Indirect plate (Ind)
UHT Steriplak-R	M. Sordi of Lodi (Italy)	Indirect plate (Ind)
Dual Purpose Sterilizer	Alfa-Laval, Sweden	Indirect plate (Ind)
High heat infusion	APV	Combined
Tetra Therm Aseptic Plus Two	Tetra Laval	Combined
Votator Scraped Surface heater	Votator Division, USA	Scraped surface (Ind)
Thermutator heater	Cherry-Burrell Corp., USA	Scraped surface (Ind)
Rotatherm	Gold Peg, Australia	Scraped surface (D)
Actijoule	Actini	Electrically heated (Ind)

Sources: Bell 2004–2005; Mehta 1980; Kosaric et al. 1981; Zadow 1993.
D = direct heat treatment; Ind = Indirect heat treatment.

The temperature-time profile has a major effect on the characteristics of the product as well as on plant operation. While differences occur between plants of the same mode of heating, for example, different indirect plants, the most marked differences are between the direct and indirect plants, as shown in table 4.3 (Datta et al. 2002).

The different temperature-time profiles and their consequences present several challenges for the UHT processor. Some of these are outlined below.

Maximizing Spore Destruction while Minimizing Flavor Impairment

A major challenge of UHT treatment of milk is heating the milk sufficiently to inactivate heat-resistant bacterial spores without causing excessive changes to the flavor. The solution to this apparent dilemma lies in the first principle of UHT processing. Direct heating processes, *injection* or *infusion*, operate predominantly at a high temperature for a short time and effectively destroy bacteria while causing minimal chemical change, including flavor change. Under direct heating, the short time taken to raise the temperature of the milk from preheat to sterilization allows little chemical change to occur. Consequently, milk produced by direct processes has very good flavor characteristics (Jensen 1996). In fact, some milks produced by direct heating with very short holding times have flavors al-

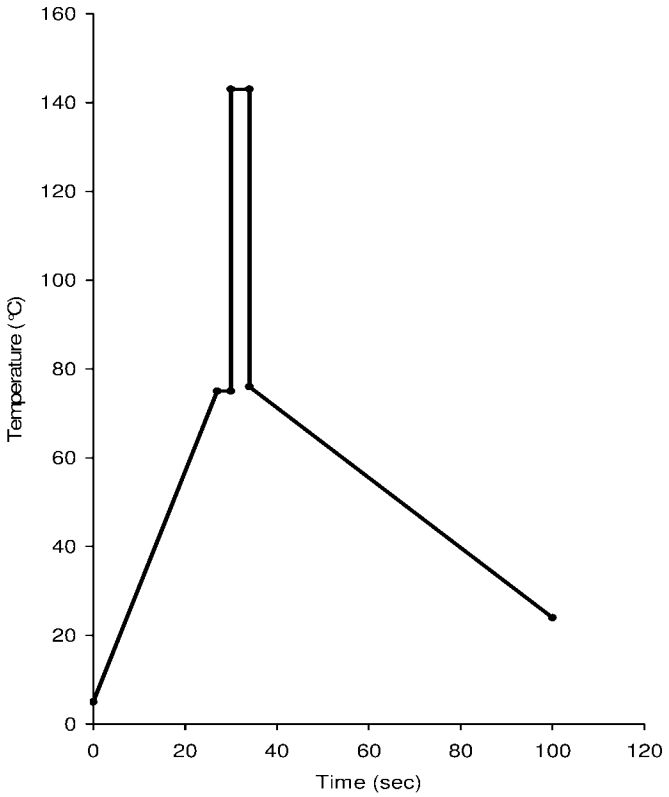

Figure 4.2. Temperature-time profile of a direct UHT plant.

most indistinguishable from HTST pasteurized milks (heated at ~72°C for 15 seconds). It should be noted, however, that for a 2-second holding time, a minimum temperature of 142°C is required to achieve a B* of 1. Direct processes also minimize destruction of vitamins, especially the water-soluble vitamins, and hence preserve the nutritive value of the milk.

Maximizing Energy Recovery while Minimizing Flavor Impairment

Adoption of a direct heating process may be beneficial in relation to flavor and nutrient change, but it comes at a price. It is very difficult to recover the heat used in classical direct processes because the hot milk is flash cooled in a vacuum chamber, which also removes the water condensed from the steam used during heating. This loss of energy efficiency has been partially overcome in commercial plants in two ways: (1) removal of water from preheated milk in a vacuum chamber prior to the direct heating step followed by indirect cooling of the hot milk by incoming cold milk (the APV High-Heat Infusion® process); and (2) placing the vacuum chamber after an initial cooling/regeneration step (the Tetra Pak Tetra Therm Aseptic Plus® process). Temperature-time profiles for these processes are shown in figures 4.5 and 4.6, respectively. Heat regeneration and the extent

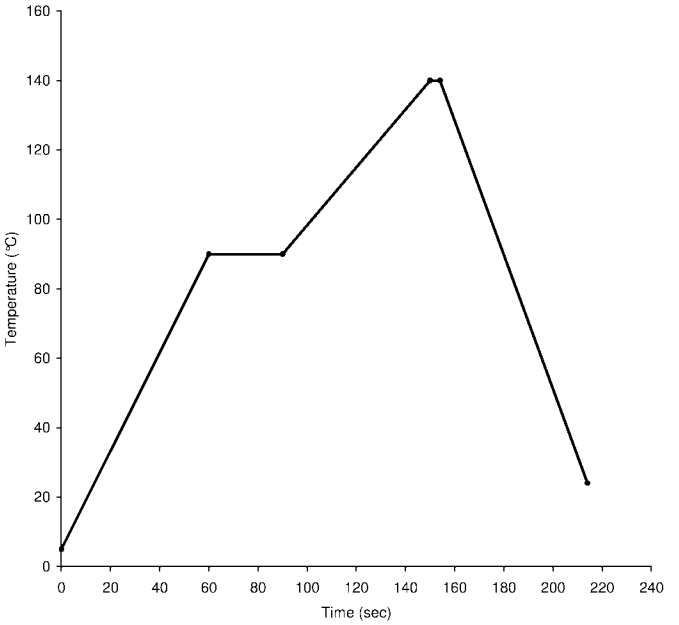

Figure 4.3. Temperature-time profile of an indirect UHT plant.

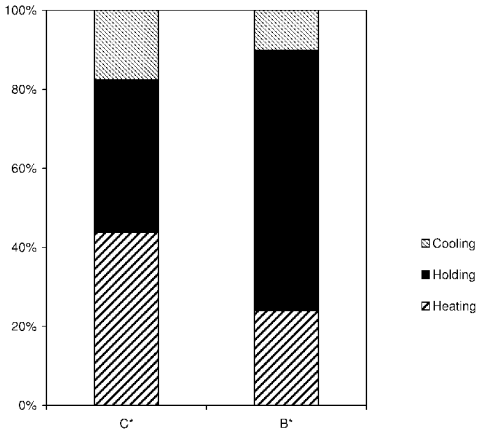

Figure 4.4. Percentage contribution of the heating, holding, and cooling sections to total C* and B* of an indirect UHT plant with a temperature-time profile as shown in figure 4.3. Total C* = 1.83; total B* = 6.75.

of chemical changes for such processes are intermediate between the classic direct and indirect processes.

Minimizing Age Gelation while Minimizing Flavor Impairment

Another challenge related to the use of direct processes to minimize the production of cooked flavor is that directly processed UHT milk is much more prone to age gelation than indirectly heated milk (Manji et al. 1986). Age gelation, which occurs in most UHT milk

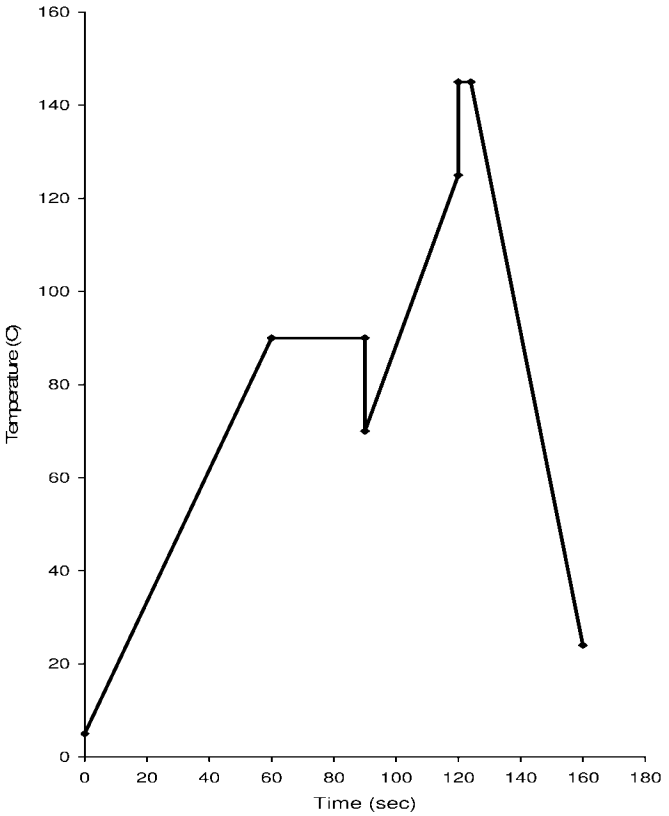

Figure 4.5. Temperature-time profile of the APV High-Heat Infusion® process.

over time, is caused by destabilization of the casein micelle and its colloidal suspension in milk. A major initiating factor is proteolysis of caseins caused by either the native milk proteinase, plasmin, or proteinases originating from contaminating psychrotrophic bacteria in the raw milk before heat treatment. Both of these enzyme types have considerable heat stability and can survive UHT treatments. However, they survive direct heat treatments better than indirect heat treatments and hence direct milks tend to gel at an earlier time than do indirect milks (Datta and Deeth 2001).

In a recent development by Dutch scientists, a rapid direct steam injection heating system capable of heating a product at 160–200°C for 0.1 seconds was developed. While milk treated in this system had little cooked flavor, the residual plasmin activity caused the development of bitter flavors during storage (Huijs et al. 2004).

Finding the Ideal Chemical Indicator of Heat Treatment

A challenge addressed by several researchers is the development of a chemical indicator of heat treatment to provide an indication of the amount of heat applied to a milk. Such an index would give a processor a measure of the amount of chemical "damage" the milk may

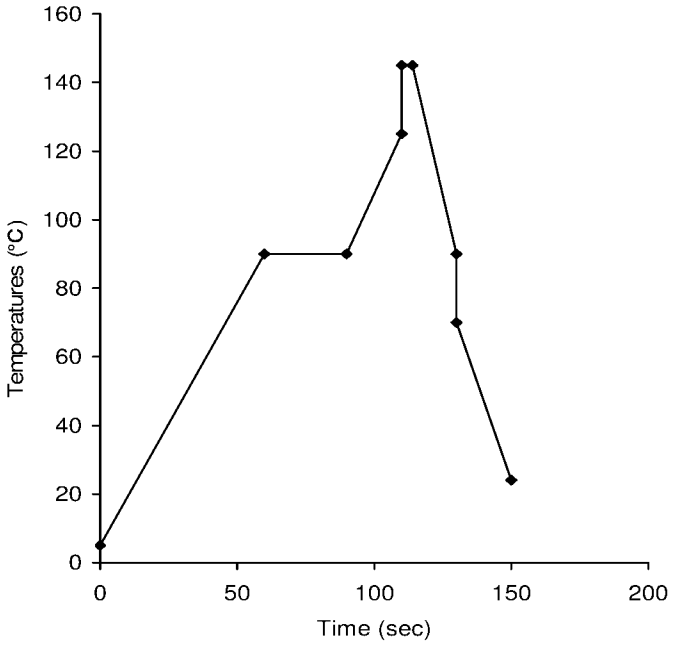

Figure 4.6. Temperature-time profile of the Tetra Pak Tetra Therm Aseptic Plus® process.

suffer and its likely susceptibility to gelation. Common chemical indicators used are furosine, lactulose, hydroxymethylfurfural, and denatured whey proteins; the first three relate to new compounds formed during heating, whereas the last relates to loss of native components.

In order to understand the significance of these indicators, it is necessary to know the nature of the reactions causing their formation/destruction. For example, whey proteins, particularly β-lactoglobulin, begin to denature at ~70°C whereas lactulose production only commences at ~95°C. The implication of this is that for a temperature-time profile such as that depicted in figure 4.3, ~85% of β-lactoglobulin is denatured by the end of the preheat holding time (based on the kinetic data of Dannenberg and Kessler 1988), but a negligible amount of lactulose is formed (based on the kinetic data of Rombaut et al. 2002). In other words, it is possible to produce milks with a high level of β-lactoglobulin denaturation but very low lactulose levels. Furthermore, during storage the lactulose content remains reasonably constant but the other three indices change due to reactions, such as the Maillard reaction, that continue, albeit at a lower rate than during high-heat processing (Elliott et al. 2003).

It is interesting to relate this back to the relationship between the severity of heat treatment and the susceptibility of milk to age gelation. It is believed that a major factor involved in the increased resistance of indirect UHT milk to age gelation is the amount of denaturation of β-lactoglobulin, which interacts with the κ-casein on the outside of the casein micelle and causes interference to proteinase attack on the caseins in the micelle (Snoeren and Both 1981). A further effect is the role of denaturation of β-lactoglobulin in inhibition of proteinase, especially milk plasmin (Kelly and Foley 1997).

Predicting the Effect of Particular Temperature-Time Profiles

Because of the risk of some highly heat-resistant spores (HHRS) not being destroyed and of age gelation occurring when milk is given a minimal heat treatment, some processors increase the intensity of the heat treatment, "just to be sure." While they may achieve their aim, it is possible that the amount of heat used is excessive and causes undue flavor and vitamin change and even color change (browning). In addition, the increased energy consumption is a further consideration. Predictions of these changes based on the known kinetics of the reactions can assist processors in assessing the implications of changing the plant operating conditions—temperatures, times, and flow rates. Computing such changes for direct plants is quite simple but for indirect plants it becomes much more complex. Computer programs have been developed for determining the process parameters and the extent of chemical changes during processing (Browning et al. 2001); one developed by NIZO Food Research in the Netherlands, NIZO Premia, is available commercially.

Residence Time Distribution in UHT Processing

Assumption 2 in the "Definitions" section above, that *all particles in the milk receive the same heat treatment,* assumes that all particles move through the holding tube at the same rate. This is a theoretical ideal situation that is never achieved in reality. In practice, the particles in a fluid (e.g., milk) take a range of times to pass through the holding time; the spread of these times is known as the residence time distribution (RTD). In UHT plants, the residence time of the fastest particle in the holding tube can be much less than (e.g., half) that of the average time, the nominal time cited.

The residence time of a particle of fluid is defined as the time period between its entry into and its exit from the system. In continuous processing, such as UHT processing, the distribution of these times, the RTD, can be described by the function E(t), the exit age distribution. This function was developed in classic work by Danckwerts in 1953. RTD can be expressed as the relationship between E(t), defined as the fraction of fluid particles leaving the system at a given time, and time (t). A typical RTD, obtained using the pulse tracer technique (Lewis and Heppell 2000), is shown in figure 4.7.

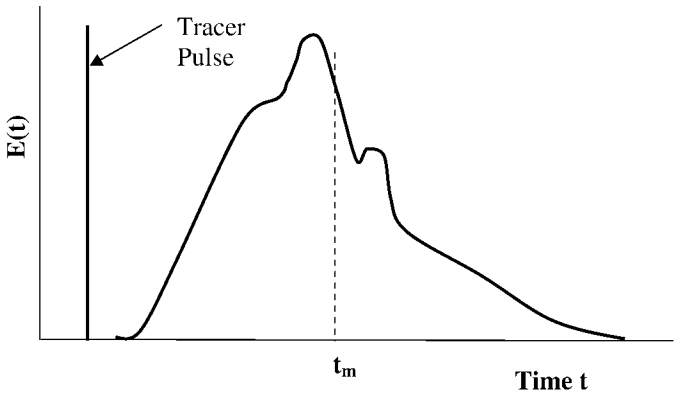

Figure 4.7. A residence time distribution curve as obtained using a tracer pulse technique. "Tracer pulse" indicates the point of injection of tracer. (Redrawn from Lewis and Heppell 2000. With kind permission of Springer Science and Business Media.)

E(t), whose unit is time^{-1}, can be expressed as follows:

$$E(t) = \frac{C(t)}{\int_0^\infty C(t)dt} \tag{4.4}$$

where C(t) is the exit concentration of a particle at a certain time *t*.

The *mean residence time* of a fluid particle can be derived in two different ways: one is based on the flow characteristics of the fluid particle while the other depends on the configuration and operation of the UHT plant.

Based on the particles' flow characteristics, the mean residence time (tm) can be defined as follows (Levenspiel 1999):

$$t_m = \frac{\int_0^\infty t. \times C(t).dt}{\int_0^\infty C(t).dt} \tag{4.5}$$

When the curve in figure 4.7 is normalized so the total area under the curve is equal to one, the integral on the denominator in the right-hand side of equation (4.5) becomes equal to one and becomes:

$$t_m = \int_0^\infty t \cdot \times \cdot C(t) \cdot dt \tag{4.6}$$

This t_m can be determined from tracer concentration values over discrete time intervals using Simpson's rule for integration (Heppell 1985).

Based on the configuration and operation of the UHT plant, the mean residence time (now designated τ) of a fluid particle in a particular section of the UHT plant can be defined by the equation:

$$\tau = \frac{V}{Q} \tag{4.7}$$

where V is the volume of the section and Q is the product flow rate under steady state conditions.

Ideally, t_m should be equal to τ, that is, the efficiency ϵ, as given by equation (4.8), should be 1:

$$\varepsilon = \frac{t_m}{\tau} \tag{4.8}$$

Possible causes of a low value of t_m, and hence a low efficiency, are channeling of the flow, areas of dead flow, and experimental error due to unstable tracer (Lewis and Heppell 2000). The flow characteristics can be improved by features in the holding tube that disrupt the streamline flow such as bends or baffles in the tube, special tube designs such as

the spiral design of Spiroflo® tubes and the helical coiled design of the HydroCoil® (Carlson 1998), and entrance and exit effects. These induce a swirling motion and create turbulence resulting in a higher mean residence time, t_m, of particles in the holding tube and a consequent increase in efficiency, ϵ.

The difference between t_m and τ is reflected in the width of the RTD. The RTD and the t_m depend on the nature of the flow of the product through the holding tube, which can be described by the Reynolds number, *Re*, a dimensionless number given by the formula:

$$Re = \rho v d/\mu \tag{4.9}$$

where ρ = density, *v*= velocity, *d*= diameter and μ = viscosity. A low Reynolds number is associated with low-density, high-viscosity products moving slowly through narrow tubes. Such conditions give rise to laminar flow and a broad RTD. Conversely, a high Reynolds number is associated with turbulent flow and a narrow RTD.

A practical consequence of the RTD is that some bacterial spores may traverse the holding tube very quickly. When the nominal (mean) residence time, τ, is close to the minimum holding time required for a B* of 1, these spores may not be inactivated and hence the product may not be commercially sterile. This is unlikely to be a safety issue for milk, as the heat resistance of vegetative pathogens is much lower than a B* = 1 heat treatment, and the presence of the major sporeforming pathogen, *C. botulinum,* is extremely unlikely (Codex Alimentarius 2004). However, it may be a cause of spoilage. In fact, Cerf and Davey (2001) suggested that it may account for a large proportion of the "failures," that is, nonsterile packs, often attributed to "leaky cartons." They stated that the incidence of these from UHT plants around the world is 1–4 per 10,000; Robertson (2003) considered that < 1 per 10,000 is common commercially.

Therefore, in determining the B* value of a process, the Reynolds number should be taken into account. For example, if the Reynolds number is 10,000, the ratio of mean residence time (nominal holding time) to the minimum residence time is ~0.78. Consequently, the effective B* value, that is, that of the fastest particle, is ~0.78 B* determined on the basis of the nominal holding time, τ. The practical implication is that if a plant is set up to operate at ~B* = 1 without taking the RTD in the holding tube into account, the effective B*, ~0.78, will be below the recommended value of 1.

Another consequence of the RTD is that the slowest particles will be overheated with the consequence of excessive chemical change. Dairy products with higher viscosity, for example, chocolate milk, puddings, and custard, may encounter this difficulty. In laminar flow, the velocity profile of the particles shows the slowest-moving particles closest to the wall of the tube, which facilitates burn-on or fouling of the equipment. This in turn leads to narrowing of the flow passage and a decrease in the overall heat transfer coefficient, which causes the temperature of the heating fluid to be increased to maintain the product temperature. These consequences exacerbate the situation by reducing the Reynolds number and increasing the temperature differential between the heating medium and the product, which increases the fouling rate. High flow rates through the plant can reduce the RTD and reduce the amount of deposit formation (Belmar-Beiny et al. 1993); however, in some plants it may not be possible to significantly vary the velocity.

The discussion of RTD above focused on the holding tube since that is the section of the UHT plant to which the nominal temperature-time combination normally refers. However, it applies equally well to the heating and the cooling sections, particularly in in-

direct plants. In fact, the flow through these sections of a tubular plant will be associated with a lower Reynolds number than that through the holding tube because of the narrower tubes used in these sections to facilitate heat transfer.

Aseptic Packaging Systems

The aseptic packaging system involves filling the sterile product in an aseptic environment into a sterile container and sealing the container hermetically so that sterility is maintained throughout the handling and distribution processes. This has proved to be an effective system as it has been estimated that the contributions of the milk and the surface of the package to total contamination rate were 1 in 1,000 and 1 in 1,000,000, respectively, showing that the milk is 1,000 times more likely to cause contamination of the final packed product than the surface of the package (Kosaric et al. 1981).

Two main aseptic packaging systems are used commercially for UHT-sterilized products: filling into preformed sterile containers and the aseptic form-fill-seal system (von Bockelmann and von Bockelmann 1998). The prerequisites of these aseptic packaging operations include sterilization of the packaging materials and establishment and maintenance of a sterile packaging environment.

Sterilization of Packaging Material

Three sterilization techniques are used, either alone or in combination, for packaging materials in aseptic processing. These are chemical treatment, irradiation, and heat treatment. The combination of hydrogen peroxide and heat has proved to be a very effective chemical sterilant for the surface of carton packaging material. Two main procedures are used for the application of H_2O_2 on the packaging materials: a wetting system and an immersion bath system.

The wetting system comprises formation of a thin film of H_2O_2 solution (15–35% concentration), containing polyoxyethylene-sorbitan-monolaurate (0.2–0.3%) as a wetting agent, on the inner part of the packaging materials. H_2O_2 is evaporated from the inner surface by heating at 120°C.

In the immersion system, the packaging material is immersed in a bath of 35% H_2O_2 at 70°C for 6 seconds. Hot air at 125°C is used to evaporate the residual H_2O_2. Paperboard is widely used as a packaging material in aseptic processing. The form-fill-seal system, which forms the carton in situ from a continuous sheet, includes sterilization of the paperboard sheet as it emerges from the roll, using the immersion system. The sterilized paperboard is then formed into a tube, filled with UHT product, and sealed below the level of the product.

An alternative to H_2O_2 alone is a mixture of peracetic acid and H_2O_2 (4%). Known as Oxonia, it is used at 60°C for sterilizing filling machines as well as PET bottles. It is noncorrosive to metal and is particularly useful for sterilizing packaging machines and screw caps that have surfaces that are difficult for sterilants to access. It also produces a low level of harmless residuals (Carlson 1998).

Gamma radiation at room temperature is used to sterilize heat-sensitive packaging materials, for example, plastics and laminates such as flexible bags used in Intasept® aseptic packaging machines. Aluminium foil used in closures on aseptic packages can be sterilized by gamma radiation followed by UV ray irradiation (Brody 2000).

Sterile Environment

A sterile environment is of paramount importance during aseptic packaging to provide sterile conditions for sterilization of the packaging materials and to ensure the sterility of the product is maintained from the processing line to the sterile container. Two techniques are used for sterilization of the air in aseptic filling machines, incineration and filtration, alone or in combination. In incineration, air is heated to 340°C and subsequently cooled by a water cooler or a regenerative heat-exchange system. Filtration using HEPA (high-efficiency purified air) filters is used to eliminate bacteria and dust particles from air. The recommendations for HEPA-filtered air systems according to von Bockelmann and von Bockelmann (1998) are

- 99.99% removal of all particles larger than 0.3 μm, and
- low-velocity and low-pressure air supply to maintain a laminar flow.

Laminar flow across the filling chamber prevents microorganisms from a contaminated package or packaging area to cause further contamination. By controlling the direction of flow of the air, the microbial contaminants are swept away from the surface and made unavailable for contamination (Carlson 1998). A positive pressure of about 0.5 bar should be maintained in the filling chamber.

Raw Material Quality

The quality of the raw material used in UHT processing is of utmost importance. It is arguably more important than for pasteurized products because of the long periods of storage of UHT products at ambient temperature when even very slow development of defects may lead to a defective product. In practice, some manufacturers select milk of the highest quality to use in UHT process in order to minimize processing difficulties and the incidence of storage-related defects.

Heat-Resistant Proteinases and Lipases

The UHT process destroys all vegetative bacteria and most sporeformers but does not inactivate some of the enzymes produced by psychrotrophic bacteria such as *Pseudomonas* species, the most common bacterial contaminants of raw milk. Such enzymes are typically produced when the bacterial count exceeds ~10^6 per mL. If milk with such a bacterial count is UHT processed, these enzymes, particularly proteinases and lipases, can remain active in the UHT milk. Since UHT milk is usually kept at room temperature and may be stored for several months, even traces of these enzymes can produce noticeable changes and result in bitter flavor and gelation (from proteinases) and rancid flavors (from lipases).

Detection of such low levels of these enzymes is a challenge. Several methods have been proposed for proteinases (Chen et al. 2003; Fairbairn 1989) and lipases (Deeth and Touch 2000) but to date there is no universally accepted method for either enzyme. Such methods have to be specific for the bacterial enzymes and correlate well with the effect of the enzyme in milk. The latter condition is significant, as some assay procedures employ nonnatural substrates and the action of the enzymes on these does not correlate well with their action on the natural substrates (casein for proteinase and triacylglycerols for lipase).

Heat-Resistant Amylases

UHT-sterilized desserts such as custard, thickened with modified starch, can undergo a dramatic decrease in viscosity during storage (Barefoot and Adams 1980). Immediately after manufacture the starch granules are clearly visible, but when the product has thinned the granules are no longer detectable. These authors attributed the decrease to the action of heat-stable amylases and showed that a concentrate produced from the pudding had measurable amylase activity against a starch substrate. The amylase appeared to be a bacterial α-amylase as it was metal (especially Ca^{++}) dependent and quite heat stable. Furthermore, the defect could be reproduced by the addition of *Bacillus stearothermophilus* α-amylase to unspoiled pudding.

Anderson et al. (1983) demonstrated that *B. stearothermophilus* α-amylase had considerable heat stability in pudding, with 26% surviving 143°C for 22.2 seconds. These authors also reported that the amylase of *Xanthomonas campestris*, the organism used to produce xanthan gum, exhibited similar thermal inactivation characteristics to *B. stearothermophilus* α-amylase. They commented that some processors had overcome the problem of thinning by eliminating xanthan gum from their formulations, apparently because it contained a contaminating amylase. However, other manufacturers have implicated the nonfat dry milk as the source of the enzyme. It has been shown that up to 15% of the psychrotrophic bacterial contaminants in raw milk can produce amylase (Barefoot 1979) and these may be the source of the amylase in the milk powder.

Thermoduric Bacteria

Spoilage of UHT milk and dairy products by thermoduric bacteria that survive the high heat process is not common. This is largely because the recommended conditions of UHT processing, that is, equivalent to B* of ≥ 1, are sufficient to destroy virtually all spoilage bacteria. However, there are situations where even these conditions may be inadequate.

As mentioned above, a comparatively recent challenge related to raw milk quality is the occurrence of the HHRS *B. sporothermodurans* (IDF 2000). A nominal heat treatment of 144°C for 4 seconds produces only a 1-log inactivation of its spores (Pearce 2004). This organism has the unfortunate combination of properties of high heat resistance and mesophilic growth; that is, its optimum growth temperature is around room temperature. *B. sporothermodurans* does not appear to cause spoilage other than a slight discoloration of the milk and seldom reaches counts of greater than 10^5 per mL. However, it is extremely difficult to remove from contaminated equipment and has caused the closure of some UHT plants. A practice that has been shown to spread this organism is reprocessing out-of-date UHT milk. Such a practice should not be permitted.

Milk powders that are used in large quantity for production of reconstituted and recombined UHT milk may contain heat-resistant spores of *Geobacillus thermoleovorans,* one of two species found in milk powders and often referred to as *B. stearothermophilus;* the other is *B. flavothermus,* which is less heat-resistant than *G. thermoleovorans* (Pearce 2004). These spores can be present in raw milk and germinate, grow, and resporulate during milk powder production. If they are present in high numbers in milk powder used for production of recombined UHT milk, normal UHT heat treatment may be insufficient to reduce the spore counts to acceptable levels (Pearce 2004).

Effect on Fouling

UHT processing often causes milk solids to attach tenaciously to the heat exchanger surface, a phenomenon known as fouling or burn-on. As discussed below, it is a major concern during UHT processing. Several factors affect the rate of fouling, one of which is the quality of the raw milk. Some manufacturers use an alcohol stability test on the raw milk as a guide to its propensity to foul during heat processing. For good stability, the raw milk should be stable in at least 74% alcohol (IDF 1981). The pH has a major effect on both the alcohol stability and the rate of fouling; the former decreases and the latter increases as the pH of the milk decreases. This may be due, at least in part, to an increase in ionic calcium that is known to be related to fouling; the higher the ionic calcium, the greater the propensity for fouling. Goats' milk has higher ionic calcium than cows' milk and a much greater tendency to fouling.

A decrease in pH often accompanies bacterial growth in raw milk so milk with a high bacterial count may foul more during processing. High bacterial counts in raw milk occur after prolonged storage or storage at an elevated temperature. Kastanas et al. (1995) found that good quality raw cows' milk could be stored for at least 7 days at 2°C prior to UHT processing at 140°C before unacceptable fouling was observed.

Seasonal variation in the composition of milk also affects the rate of fouling during UHT processing. Grandison (1988) observed a twofold range in processing run times over a 12-month period. Since the variation in the amount of deposit occurred almost entirely in the high-heat section where the deposit consists largely of mineral, he suggested that the variation may be due to variation in the mineral components and that a decrease in mineral content may reduce fouling. However, Burton (1967) found a similar seasonal variation in the amount of deposit was strongly positively correlated with fat content and not the mineral or protein contents of the milk.

Burn-On or Fouling during UHT Processing

Fouling is the cause of one of the most important problems in the dairy industry, the need for constant cleaning of heating equipment, that affects the economic efficiency of the plant (due to plant down time, increased fuel and cleaning materials costs, and increased capital cost). It also affects the thermal efficiency of the plant (the deposit on the surface acts as an insulator reducing the rate of heat transfer to the milk) and the quality of milk (the milk may contain detached pieces of the deposits, which may lead to sensory defects).

There are two distinct types of fouling depending on the temperature to which the milk is heated—Type A and Type B (Burton 1988). Type A deposit, which is generally soft, voluminous, and curd-like, is produced when the heating temperature is below 110°C and is called protein fouling since the major component (50–70%) of the deposit is protein, the remainder being mostly mineral salts. In contrast, Type B deposit is brittle and gritty in nature and occurs when the heating temperature is > 110°C; it is called mineral fouling as 70–80% of the deposit is mineral and the remainder mostly protein. In general, the deposit formed in the preheating section is of Type A while in the high-heat section it is Type B.

Fouling is most pronounced in indirect UHT plants. Much less occurs in direct plants; however, some fouling occurs downstream of the steam injector, in areas such as the holding tube or restriction valve (Grandison 1996; Truong et al. 2000). In plate heat exchang-

ers, which contain narrower flow channels than tubular heat exchangers, the deposit restricts the flow of product and causes an increase in the backpressure that may at times exceed the safety limit of the gaskets if left unattended. Tubular heat exchangers are not as susceptible to such pressure increases due to their ability to accommodate a greater thickness of deposit before the product flow is seriously disrupted.

Fouling can be reduced in the following ways:

- Forewarming/preheating the milk: Preheating of milk greatly reduces the amount of deposit formation. The decrease in deposit formation is due to an increase in the whey protein denaturation. This results in the reduction of the Type A deposit that interferes with the flow of milk, thereby decreasing the run time of the plant (Jelen 1982).
- Use of additives: This is a possible way of reducing deposit formation but is not legal in some countries. Since low pH of milk is associated with an increase in deposit formation, substances that raise the pH of the milk can be effective in reducing fouling.

Addition of sodium pyrophosphate decahydrate ($Na_4P_2O_7 \cdot 10H_2O$) reduces the amount of deposit formed (Burdett 1974; Burton 1965, 1966, 1968). The reduction is attributed to phosphate stabilization of the casein micelles, which decreases release of calcium phosphate from the micelles during high-temperature heating. Calcium phosphate, the major contributor to Type B fouling, deposits at high temperatures because of its reverse solubility, that is, it becomes less soluble with increased temperature.

Poststerilization Contamination

Contamination by Thermophilic Sporeformers

A major concern in the handling of milk after high-temperature sterilization is recontamination. Heat-resistant thermophilic sporeformers such as *B. stearothermophilus* and *B. licheniformis* are the most commonly encountered poststerilization contaminants in UHT milk and milk products. They can cause the "flat sour" defect, characterized by acid production but no gas. However, these thermophiles do not grow in milk under "normal" storage conditions (< 30°C) and have a growth optimum of ~55°C. They have been known to cause problems if the milk reaches a high temperature during transportation.

Poststerilization contamination may result from several sources but an important one is the seals in the homogenizer (if downstream). Kessler (1994) showed that spores trapped under seals had enhanced heat stability, largely attributable to very low water activity in their microenvironment, and could act as a reservoir of contaminating spores. Flat sour defects due to contamination by *B. stearothermophilus* can arise in this way. Frequent seal changes have been found to be an effective, although expensive, way of minimizing such contamination.

Contamination by Fusarium oxysporum

Another microbial problem that has affected several companies in recent years is the filamentous fungus *Fusarium oxysporum.* This organism can cause a flavor similar to that of blue-vein cheese in UHT milk within a few weeks. It also produces gas and is often detected when packages become swollen or "blown." It is a common fungus of plants and soils and can enter UHT milk packages through contaminated air in the filling machine. Negative air

Table 4.5 Amount of dissolved oxygen content in direct- and indirect-heated UHT milks (Datta et al. 2002)

Processing Conditions	Dissolved Oxygen (ppm)	Reference
Indirect (141°C/3.6 s)	9	Burton et al. 1970
Indirect (141°C/3.6 s with de-aeration)	<1	
Indirect (141°C/3.6 s)	6.3–7.3	Thomas et al. 1975
Direct (143°C/3.0 s)	0.7	
Indirect (141°C/3.6 s)	9.0	Thomas et al. 1975
Indirect (141°C/3.6 s with de-aeration)	1.0	
Direct (143°C/3.0 s)	< 1.0	
Direct (132–143°C/12 s)	0.32–0.47	Rerkrai et al. 1987
Direct (149°C/3.4 s)	1.5	Earley and Hansen 1982
Indirect (140°C/3 s)	7.8	Adhikari and Singhal 1991
Indirect (time/temperature unknown)	4.50–5.80	Fink and Kessler 1986
Indirect (138°C/2 s)	8.3	Ford 1969
Indirect (138°C/2 s with de-aeration)	5.7	
Direct (145°C/3–4 s)	< 0.1	
Indirect (140°C/4 s)	8.4	Adhikari and Singhal 1992
Indirect (140°C/3 s)	6.2[a]	Zadow and Birtwistle 1973
Direct (140°C/3 s)	1.2–1.7[a]	

[a] Calculated from partial pressure data.

pressures in aseptic filling areas may facilitate contamination of the packaging equipment if there is a source of the fungus nearby. Once the fungus has contaminated a filling machine, it is difficult to eliminate (K. Scrimshaw, pers. commun. 2004).

Storage-Related Defects

Development of Stale/Oxidized Flavor

The flavor of UHT milk continues to be one of the major impediments to its greater consumer acceptance (Perkins and Deeth 2001). It is a complex mixture of different flavors described as cooked, sterilized, and stale/oxidized. The typical "cooked" flavor, characterized by sulphur-containing volatiles, usually dissipates within the first few days due to oxidation by air in the package. The sterilized flavor is related to the intensity of the heat treatment and remains fairly constant during storage. The stale or oxidized flavor is due to oxidation of lipids by dissolved air and may be detectable after about 4 weeks of storage. It is, however, very dependent on the amount of dissolved oxygen in the milk, which in turn is dependent to a large degree on the headspace in the package. Table 4.5 summarizes the amount of dissolved oxygen present in directly and indirectly heated UHT milk.

In a recent study of stale flavor, Perkins et al. (2005) compared the headspace in 1-liter laminated plastic bottles and in 1-liter paperboard cartons. The volume of headspace was 7–8 mL in cartons filled by the form-fill-seal method, up to 40 mL in filled individually preformed cartons, and up to 63 mL in the plastic bottles. The volume in the latter ensured the milk remained saturated with air during storage, while in the cartons with the least headspace, the dissolved oxygen content decreased during storage and the milk showed the lowest level of stale volatiles, mainly aliphatic aldehydes and methyl ketones. It is of inter-

est that direct UHT processing involves a flash cooling step in a chamber under vacuum, which effectively removes a large proportion of the dissolved air and is often cited as a benefit of such processing. If such milk is packaged immediately it commences storage with a low oxygen content. However, the benefit of the vacuum chamber is quickly negated if the milk is stored in an aseptic tank for some time before filling and/or the package contains a significant headspace.

Age Gelation

The shelf life of UHT milk is sometimes limited by age gelation, which is characterized by an increase in viscosity during storage and ultimately formation of a gel, similar to that of custard. The gel is a three-dimensional network of whey proteins and caseins that binds water and engulfs casein micelles and fat globules. The skeleton of the matrix is a protein complex formed between heat-denatured β-lactoglobulin and κ-casein.

While several mechanisms have been suggested, one proposed by McMahon (1996) explains many of the aspects of the phenomenon. β-Lactoglobulin, denatured by the high-heat treatment, covalently bonds with κ-casein via disulphide bonds to form large polymeric βκ-complexes. This subsequently weakens the ionic bonds that anchor κ-casein to the micelles. During storage, proteolytic cleavage by either plasmin or bacterial proteases, or nonenzymic actions, releases the βκ-complex from the micelles. The βκ-complexes accumulate in the milk serum between micelles and when their concentration reaches a critical level, viscosity markedly increases and a semirigid gel is produced.

Many factors affect the rate at which gelation occurs but the major ones are proteolysis, severity of heat treatment, storage temperature, bacteriological quality of milk, and milk solids content.

- *Proteolysis:* A widely held view is that gelation is caused by proteolysis of casein caused by either the natural milk proteinase, plasmin, and/or heat-stable proteinases produced by psychrotrophic bacterial contaminants in the raw milk before processing. Milks containing these bacterial proteases are known to be particularly susceptible to gelation; however, milks without these enzymes but containing plasmin can also gel. Plasmin is one of the few natural milk enzymes that are resistant to heat and may withstand UHT conditions. Furthermore, it can increase during storage as its inactive precursor, plasminogen, which is also present and heat-stable, is converted to the active plasmin by the plasminogen activator. Mastitic milk and milk from cows in late lactation have elevated levels of plasmin.

  Plasmin mostly attacks β- and α_{s2}-casein but is almost inactive against κ-casein. However, the heat-stable bacterial proteases degrade κ-casein (to a peptide similar to para-κ-casein released by the action of rennet) faster than β- and α_{s1}-casein. Thus the type of protease action can be determined by HPLC analysis of the peptides (Datta and Deeth 2003; Lopez-Fandino et al. 1993).
- *Severity of heat treatment:* The more severe the heat treatment, the longer age gelation is delayed. Thus milks sterilized by indirect heating methods are less susceptible to gelation than milks treated by the direct steam injection and infusion methods. Furthermore, retort-sterilized milk is more stable than UHT milk. As discussed above, possible effects of increased heating severity are increased inactivation of the proteases and more denaturation of the whey proteins.

- *Storage temperature:* The rate of gelation of UHT milk is markedly influenced by the temperature of storage. Storage at refrigeration temperatures (~4°C) and "high" temperatures (35–40°C) delays gelation, while intermediate storage temperatures enhance the rate of gelation. Gelation occurs at a maximum rate at ~25–28°C. The reduced gelation at 35–40°C may be due to a high degree of proteolysis by proteases, which prevents the degraded proteins from forming a strong gel matrix. It has also been suggested that gelation may be inhibited if existing regions of the casein that could take part in protein-protein interactions are blocked by casein-lactose interactions involving lysine residues, which precede browning in UHT milk stored at temperature above 30°C.
- *Bacteriological quality of milk:* Milk of poor bacteriological quality at the time of processing is much more susceptible to gelling than good quality milk. The enhanced gelation milk is largely attributable to the production of heat-stable bacterial proteases in the milk. Poor-quality milk may also have a lowered pH, which also increases the milk's potential to gel.
- *Milk composition:* Milks with high solids contents such as concentrated milks are much more susceptible to gelation than normal milks. Furthermore, gelation can occur in the absence of proteolysis, suggesting that the mechanisms of gelation in the two types of milk are different.

UHT skim milks are more susceptible to gelation than whole milks. This is attributable to the reduced rate of proteolysis in whole milk caused by partial masking of the protein by fat, which prevents access to the caseins by the proteases.

The two main practical ways of preventing gelation or reducing its incidence are to use high-quality raw milk and to use an indirect heating system for the high-heat treatment. Suitable high-quality raw milk has a low somatic cell count and low bacterial count. It should be processed as soon as possible after milking and milk more than 48 hours old should be avoided whenever possible.

Addition of sodium hexametaphosphate (SHMP) (0.05% and 0.1%) to milk before heat treatment is effective in retarding gelation. SHMP does not inhibit proteolysis but prevents proteolysed milks from gelling. Its effect appears to be through interaction with calcium ions within the casein micelles, which stabilize the micelles and prevent dissociation of the βκ-complexes.

A method proposed for reducing the activity of heat-resistant proteases in the milk is low-temperature inactivation (LTI), that is, heating at ~55°C for 30–60 minutes (Barach et al. 1976). The treatment is effective either before or after the high-heat treatment. Inclusion of an LTI step with UHT sterilization has been reported to significantly prolong the shelf life of UHT skim milk. It has been shown by some researchers to considerably extend the shelf life of the milk before age gelation occurs. It appears to have an inhibitory effect on proteases and prevents proteolysis, although its mechanism has not been conclusively determined. Autodigestion of the enzymes has been proposed as a possible mechanism for LTI.

Conclusions

Treatment of milk and milk products at ultra-high-temperatures for a few seconds, combined with aseptic packaging, is a widely used and very successful technology. However, several aspects of the technology present challenges for the manufacturer. These include

the use of high temperatures well above the boiling point of water; the necessity to maintain the treated product in a sterile condition while packaging and during storage; and the requirement for the product to be of acceptable quality after storage at ambient temperatures for several months. These challenges occur at every stage of the process from the raw materials, through the heating and aseptic packaging operations, to storage of the product. Poor-quality raw materials lead to problems during processing and inevitably lead to poor-quality UHT products. The temperature-time profile during processing has a marked effect on final product quality and should always be considered rather than a single temperature-time description of the process. Postprocessing microbiological contamination is a constant concern and can occur in the plant after the sterilization section, particularly in downstream homogenizers, and during packaging. Finally, flavor and texture changes occur during storage that limit the shelf life of the product. A sound knowledge of the chemical, microbiological, and engineering aspects of the process and the products is essential for optimizing UHT processing and aseptic packaging of milk and dairy products, and for ensuring UHT products remain safe and of high quality during a period of storage at ambient temperature.

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5 Microwave and Radio-Frequency Heating

Gaurav Tewari

Introduction

In microwave and radio-frequency processing, the electromagnetic waves of certain frequency are used to heat food products. Generally two frequencies (915 and 2,450 MHz) for microwave food processing and three frequencies (13.56, 27.12, and 40.68 MHz) for radio-frequency processing are used.

Heat is generated by dielectric and ionic mechanisms. Dielectric heating is due to the oscillations of the water molecules in the food. Oscillatory migration of ions in the food also generates heat under the influence of the oscillating electric field. These provide rapid heating and thus require less time to bring the product to the desired temperature. These also provide relatively more uniform heating compared to conventional hot air heating. Equilibration of the product following heating can help to level the temperature distribution and improve uniformity.

Microwave heating of foods was first envisioned by Dr. Percy Spencer, who patented the idea in 1945. By 1991, microwave heating had become so popular that in North American homes alone, there were over sixty million microwave ovens (Owusu-Ansah 1991). Microwave techniques were developed originally for military requirements. The objective was to design and manufacture microwave radar, navigation, and communications during the Second World War. After the war, many peacetime uses of microwaves were developed, including microwave processing systems as well as domestic and commercial microwave ovens.

In food heating operations, microwave heating offers several distinct advantages when compared to conventional heating methods. The advantages include speed of heating, energy saving, precise process control, and faster start-up and shutdown times (Decareau 1985). Other advantages include higher quality product in terms of taste, texture, and nutritional content.

Theory and Characteristics

Microwaves are nonionizing, time varying electromagnetic waves of radiant energy with frequencies ranging from 300 MHz to 300 Ghz; that is, wavelengths ranging from 1 mm to 1 m in free space. Due to possible interferences with TV and radio waves, microwave ovens operate at 915 and 2,450 MHz, allocated by the International Telecommunications Union. Domestic ovens operate at 2,450 MHz. Microwaves travel similarly to light waves; they are reflected by large metallic objects, absorbed by some dielectric materials and small strips of metal, and transmitted through other dielectric materials. For example, water and carbon absorb microwaves well; on the other hand, glass, ceramics, and most thermoplastics allow microwaves to pass through with little or no absorption. They can also be refracted when travelling from one dielectric material to the next, analogous to the way light waves bend when passing from air into water.

How Microwaves Produce Heat

There are two main mechanisms by which microwaves product heat in dielectric materials: ionic polarization and dipole rotation. Ionic polarization occurs when ions in solution move in response to an electric field. Kinetic energy is given up to the ions by the electric field. These ions collide with other ions, converting kinetic energy into heat. When the electric field is rotating at 2.45×10^9 Hz, numerous collisions occur, generating a great deal of heat.

However, the dipole rotation mechanism is more important. It is dependent on the existence of polar molecules. The most common polar material found in foods is water. Water molecules are randomly oriented under normal conditions. In the presence of an electric field, the polar molecules line up with the field. As mentioned above, the electric field of a microwave system alternates at 2.45×10^9 Hz, so that while the molecules try to align themselves with this changing field, heat is generated. When the field is removed, the molecules return to their random orientation.

In capacitive or radio-frequency heating, the material is usually placed between electrodes, whereas in microwave heating a closed cavity or oven is used. When microwaves interact with polar or polarizable molecules in foods, the polar or polarizable (p/p) molecules try to reorient themselves to follow the field. This results in heat generation by the p/p molecules if the time for the establishment and decay of their polarization is comparable to the period of the high oscillation provided by the microwave frequency. The classical view of microwave heating is to consider the heating process as due to the rotation of a dipolar molecule in a viscous medium dominated by friction (Stuchley and Stuchley 1983). Heating may also result from the movement of electrically charged ions within foods (Giese 1992). In essence, microwave heating of foods results from interaction of the microwaves with ionic and/or dipolar content of the food. Water, proteins, and carbohydrates are among the dipolar ingredients in food. In foods, these are volumetrically distributed within the food material. Consequently, microwave heating results in volumetric heating. The effectiveness of this volumetric heating and the depth to which it occurs is determined by the dielectric properties of the material and the frequency of the microwave. The dielectric properties of the material determine the amount of incident microwaves reflected, transmitted, or absorbed by the material.

For low-moisture hygroscopic foods (such as egg white powder), Adu and Otten (1993) suggested that the increase in product hygroscopicity due to moisture loss that increases the latent heat of vaporization during heating will require heating equations that account for changes in product hygroscopicity to accurately predict the microwave heat and mass transfer characteristics of foods during mathematical modelling of microwave heating for dry foods.

Dielectric Properties of Foods

The dielectric property most important in the microwave heating of foods is the ratio of the dielectric properties expressed as ratios of the dielectric properties of free space. This gives the relative dielectric constant and relative dielectric loss factor. The relative dielectric constant governs the amount of incident power absorbed or reflected while dielectric loss factor measures the amount of absorbed energy dissipated or transmitted within the food.

The dielectric properties of foods at microwave frequencies are related to their chemi-

cal composition. They are also highly dependent on the frequency of the applied electric field, the moisture content, temperature, and bulk density (Decareau 1985; Metaxas and Meredith 1983). The dielectric constant at any given frequency increases with moisture content. The loss factor may increase with moisture content depending on temperature, moisture content, and frequency. The loss factor remains approximately constant for moisture contents below the critical moisture content of the material. At constant temperatures, the loss factor increases with increasing moisture content for most solid foods with moisture content greater than their critical moisture content. The loss factor may, however, increase or decrease with temperature. In materials where the loss factor increases with increase in temperature, uneven heating intensifies and thermal runaway may result.

The power supply is a significant part of the capital cost of a microwave oven. A magnetron requires anywhere from 4 to 10,000 V to operate. Therefore the generator must step up the voltage from the electrical outlet (110 V) to the operating requirements. The magnetron creates the microwave signal, which is fed into the cavity (in the case of an industrial process, this might be a conveyer belt).

A microwave oven, compared to a conventional oven, is more efficient. For example, if you want to bake in an oven, you must first heat about 15 kg of steel and the air in it before the hot air is transferred to the potato. By the time the potato is cooked, you only get about 2% efficiency. In a microwave oven, there is about 50% efficiency in the oven cavity, and almost all of it will be used to cook the potato (Buffler 1986).

Microwave Food Processing

Microwave food processing activities that have shown great promise include thawing and tempering, reheating, drying, cooking, baking, sterilization and pasteurization, and blanching.

The first industrial-scale microwave process was introduced in the early 1960s. This process was for the drying of fried potato chips. The first lightweight countertop oven was designed in 1965. Some of the first oven sales were to research laboratories of food manufacturers, universities, and government laboratories, where studies were conducted on blanching of vegetables, coffee roasting, freeze-drying, and so forth.

In general, microwave equipment is more expensive to purchase than conventional technology. However, the use of microwaves industrially is more economical than conventional methods. In addition, capital costs of industrial microwave ovens have been reduced due to recent advances in equipment design. Cooking times of microwaves are one-quarter of the time or less than conventional methods. Presently, microwaves are used to achieve quick internal heating, and conventional heat sources are used to produce the desired surface browning or crispness.

On top of being flexible, microwave processing offers the user a high degree of control; for instance, no-lag start-up heating, flatter temperature profiles in the finished product, and rapid response to the removal of heat. Microwaves have the ability to heat products while they are still in sealed packages even if the packaging acts as an insulator, and they are capable of volume expansion within a closed container as well as generating pressure (Sanio and Michelussi 1986).

Some commercially proven applications include dehydration of low-moisture solids, precooking of meat products, and tempering of frozen foods. Other microwave operations such as vacuum drying, pasteurization, sterilization, baking, blanching, and rendering are less significant.

Thawing and Tempering

Tempering of foods requires raising the temperature of the food item from a solidly frozen condition to about −2°C, where it is still in a firm state so that it can be easily sliced or separated. Conventional tempering of frozen foods is a long and arduous process taking from several hours to a few days to complete depending on the size, type, and initial temperature of the food product. Disadvantages of conventional thawing and tempering include large cold-storage areas, large inventories of frozen products, bacterial growth resulting from the long durations of thawing, large amounts of drip loss, adverse color changes, surface oxidation, and high consumption of fresh water (in cases requiring the use of water as the thawing liquids) (Rosenberg and Bogl 1987). Microwave tempering eliminates many of these disadvantages. Thus thawing and tempering has become one of the most important applications of microwave technology in the food industry.

Microwave tempering is defined as taking a product from freezer temperature to a condition (between −4 to −2°C) in which the product is not frozen but is still firm. Tempering at this temperature avoids overheating and results in minimal quality deterioration and tremendous energy savings. The lower microwave tempering temperatures and shorter tempering durations eliminate the conditions for microbial growth. In addition, the shorter time for microwave tempering eliminates the need for large temperature-controlled storage areas and large inventories of frozen product. Drip loss reduction of up to 10% adds to the advantages (IFT 1989). An additional benefit is the ability to process products while they are still in their original container.

Most microwave tempering units operate at 915 MHz due to the higher penetration depth that this frequency provides over 2,450 MHz frequency. The high penetration depths are of extreme importance since the target materials are usually fairly large, for example, frozen meat blocks or blocks of butter (Richardson 1992).

Reheating

Reheating is the process of increasing the temperature of previously cooked or heat-processed food from ambient temperature to a higher temperature. The ability of microwave heating to provide faster volumetric heating without the need to heat the container or package material of the foods has made reheating one of the most frequent microwave processing applications. It is this advantage that has resulted in the tremendous success of microwave energy use in domestic and food retail service centers.

Drying

Drying refers to processes that result in the reduction or removal of moisture from a material up to the point where it attains moisture equilibrium with its environment. Microwave drying has been shown to be possible for a number of food products, but the cost of such systems makes it impractical for total drying operations. Consequently, they are mostly applied in combination with hot air or other conventional heating techniques.

Microwave drying is mainly employed during the falling rate period for finish drying or precise control of final moisture content. This is because during the falling rate period, the rate limiting step results from the resistance provided by the solid material to the supply of moisture to the surface of the product for evaporation. During the falling rate period

of drying, conventional drying becomes a slow process, as heat must first be conducted through already dried material to the evaporating front inside the material before moisture can migrate to the surface. Microwaves operate directly on the polar water molecules. Consequently, microwave drying is able to speed up the moisture migration process. Moreover, the selective heating of wet portions of the food reduces case hardening and surface browning.

Pasta drying by microwave energy has now become an industry standard (Giese 1992). The process is a combination of conventional hot air drying followed by a microwave and hot air stage. Freshly extruded pasta with a moisture content of approximately 30% is hot air dried at 71–82°C to around 18%. Combined microwave and hot air drying then lowers the moisture level to about 13%. Microwave vacuum has been used for the production of fruit juice concentrates, and microwave freeze-drying has been tried for various food products including fruits, coffee and tea, mushrooms, and meat and fish proteins.

Other microwave drying applications include systems for soybean drying and dehusking as a further step toward oil extraction (Decareau 1986). When soybeans are conventionally dried they have to be kept from 3 to 5 days for the moisture within the seed to equilibrate. Moreover, the seed coat of the dried beans needs to be split for effective dehulling before oil extraction. The volumetric heating resulting from microwaves eliminates the undesirable moisture gradient and thus eliminates the need for the moisture equilibration. Microwaves effectively split the seed coat, resulting in effective dehulling.

Cooking

Microwave cooking has been found to result in yield increases of 25–38% of bacon because none of the product is lost by overcooking (IFT 1989). In addition, the process produces high-quality rendered fat as a by-product. Two alternative systems for bacon cooking are available. The bacon may first be preheated with hot air before cooking with microwave energy. The second alternative uses microwave energy alone for the whole process. The bacon so processed is mainly supplied as precooked bacon to food service operations.

Blanching

The heat treatment applied to food material to inactivate cell activity and enzymes is known as blanching. In food processing, this treatment is commonly applied to fruits and vegetables. Blanching serves to prevent development of off-flavors and off-colors in frozen fruits and vegetables. For most fruit and vegetables, microwave blanching does not produce significant improvements over conventional steam blanching (IFT 1989). However, a more successful application may be found for the enzyme inactivation of selected products like tomatoes or whole soybeans (Klinger and Decker 1989). Metaxas and Meredith (1983) reported that microwave energy effectively inactivates the growth inhibitor enzyme antitrypsin at a temperature of 105°C in 2 minutes. The minimum time to achieve the same result was 30 minutes when conventional heating methods were used.

Baking

Microwave baking has been successful for several flour-based foods. As mentioned earlier, the main disadvantage of microwave baking is the lack of crust formation and surface

TABLE 5.1 Microwave food processing applications (IFT 1989)

Application	Frequency (MHz)	Power (kW)	Products
Tempering, batch or continuous	915	30–70	Meat, fish, poultry
Drying, vacuum or freeze-drying	915 or 2,450	30–50	Pasta, onions, snack foods, fruit juices
Precooking	915	50–240	Bacon, poultry, sausages, meat patties, sardines
Pasteurization/sterilization	2,450	10–30	Fresh pasta, milk, semisolid foods, pouch-packaged foods
Baking	915	2–10	Bread, doughnut proofing

browning in baked foods for which crust formation and browning are important. Consequently, in most applications, combined microwave and hot air baking reduced oven time for baking by up to 66% (Richardson 1992). Decareau (1985) reported that microwave baking resulted in a good retention of the yeasty flavor and attributed this to the lower ambient temperature conditions.

Another application of microwave energy in the baking industry is in enhancing or speeding up the proofing of yeast-raised dough products. Microwave proofing has reduced the total proofing time for yeast-raised doughnuts to an average of 4 minutes as compared to the conventional 25–35 minutes (IFT 1989).

Sterilization and Pasteurization

Sterilization refers to the complete destruction of microbial organisms. Commercially, sterility means that all pathogenic, toxic-producing organisms and spoilage organisms have been destroyed or reduced to safe levels. Microwave sterilization operates in the temperature range of 110–130°C. Pasteurization is a gentle heat treatment usually at temperatures between 60 and 82°C. Microwave sterilization/pasteurization has been applied to several foods including fresh pasta, bread, granola, milk, and prepared meals. The main advantage of microwave sterilization or pasteurization is the effective reduction in the time required for the heat to penetrate to the food center. Table 5.1 gives a summary of microwave food processing applications.

Precooking

Precooking combines microwaves with steam and/or hot air techniques in the commercial cooking of beef patties, bacon, poultry, battered and breaded fish, and so forth. Some of the advantages of microwave precooking are reduction of production time, floor space, and shipping and refrigeration costs; and increased yield that provides less product waste (sanitation problems) and labor needs.

Microwave Food Process Design

In designing microwave food processes and packaging, various factors that affect microwave heating of foods should be taken into consideration if the effect of uneven heat-

ing associated with the use of microwaves is to be kept under control. These factors fall into two broad categories. The first one is thermo-physical properties of the food. The second is factors associated with the dielectric characteristics of food and the field intensity distributions provided by various microwave energy applicators and heating systems.

Physical Factors

The thermo-physical factors that require serious consideration in the design of microwave food processes and packaging systems are

Size and shape of food: The physical size and shape of foods affect the temperature distribution within the food. This results from the fact that the intensity of the wave decreases with depth as it penetrates the food. If the physical dimensions of the food are greater than twice the penetration depth of the wave, portions of the food nearer the surface can have very high temperatures while the mid-portions are still cold. On the other hand, if the dimensions of the food are much lower than the penetration depth of the wave, the center temperature can be far higher than the temperature at the surface. This situation normally results in "the focusing effect," which results from the combined intensity of the wave (in three space dimensions) being higher at the inner portions than the outer portions of the product.

Some shapes reflect more microwaves than others. In addition, some shapes prevent increasing amounts of the waves from leaving the material by reflecting them back into the interior. For most spherical and cylindrical foods, wave focusing occurs for product diameters between 20 and 60 mm. In rectangular foods, focusing causes the overheating of corners. Thus in package design, sharp corners are avoided and tube-shaped pans have been suggested (Giese 1992). Moreover, in foods with corners, packages are designed using metals or aluminum foils to reflect microwave energy away from corners and thus selectively heat some portions more.

Surface area: In microwave heating, the product temperature rises above its ambient temperature due to volumetric heating. Higher product surface area therefore results in higher surface heat loss rate and more rapid surface cooling. During microwave heating, the highest temperature is not at the surface of the product (despite the higher intensity of power absorbed there) but somewhere in the interior.

Specific heat: How much a food product will heat given a specific amount of energy depends on its heat capacity. The implication of this for microwave heating is that different food products heated together have different temperature histories. To control this, some microwave food packages are sealed tight to allow heat transfer between hotter and colder foods, thus giving similar temperature history for different foods in the same package.

Dielectric Properties

The dielectric behavior of foods affects their heating characteristics. As stated earlier, the dielectric properties of some foods increase with temperature but decrease for others. When foods with opposite dielectric behavior are heated together, temperature differences between them intensify with time. Thus foods with similar dielectric characteristics are put together or special packages are designed to facilitate heat transfer between dissimilar foods.

Radio-frequency (RF) heating also has great potential for rapid and uniform heating patterns in foods, providing safe, high-quality foods. In RF heating, the wavelength of the chosen frequency is large compared to the dimensions of the sample being heated, while in microwave heating, the wavelength is comparable to, or even smaller than, the sample dimensions. Thus, RF offers the advantages of providing more uniform heating over the sample geometry due to deeper wave penetration into the sample and more uniform field patterns compared to microwave heating (Zhao et al. 2000). RF heating has also been used for heating packaged bread, blanching vegetables, thawing frozen foods, postbaking snack foods, and pasteurization.

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6 Novel Thermal Processing Technologies

Antonio Vicente and Inês Alexandra Castro

Introduction

Heating is probably the oldest means of processing foods and has been used by mankind for millennia. However, the technology used to heat foods in order to process them has had a spectacular evolution during the twentieth century that has continued until the present time. Technologies such as ohmic heating, dielectric heating (which includes microwave heating and radio-frequency heating), and inductive heating have been developed that can replace, at least partially, the traditional heating methods that rely essentially on conductive, convective, and radiative heat transfer. They all have a common feature: heat is generated directly inside the food and this has direct implications in terms of both energetic and heating efficiency. Also, infrared heating has been developed as a means of heat processing of foods, and the main differences between dielectric, ohmic, and infrared energy can be summarized as follows (Fellows 2000):

- Dielectric energy induces molecular friction in water molecules to produce heat, whereas ohmic heating is due to the electrical resistance of a food and infrared energy is simply absorbed and converted to heat.
- Dielectric heating is determined in part by the moisture content of the food, whereas the extent of heating by radiant energy depends on the surface characteristics and color of the food and ohmic heating depends on the electrical resistance of the food.
- Dielectric and ohmic heating are used to preserve foods, whereas infrared radiation is mostly used to alter the eating qualities by changing the surface color, flavor, and aroma.
- Commercially, microwaves and radio-frequency energy are produced at specified frequency bands that are allocated to prevent interference with radio transmissions, whereas radiant heat is less controlled and has a wider range of frequencies. Ohmic heating uses normal electrical supply frequency (50–60 Hz) electricity.
- The depth of penetration into a food is directly related to frequency; the lower frequency dielectric energy penetrates more deeply than radiant energy. In contrast, ohmic heating penetrates throughout the food instantly.
- The thermal conductivity of the food is a limiting factor in infrared heating, whereas it is not so important in dielectric and ohmic heating, where electrical conductivity plays a key role instead.

These are called novel *thermal* processing technologies, meaning that the change in temperature is the main processing factor, as opposed to the novel *non-thermal* processing technologies such as pulsed electric fields, high pressure, pulsed light, ultrasound, and gamma radiation, among others, where temperature may also change but is not the major parameter responsible for food processing.

The case of inductive heating is very special because the existing information is very incomplete. Apart from a project at the Technical University of Munich (unpublished data),

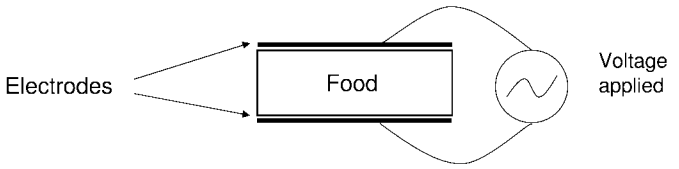

Figure 6.1. The basic principle of functioning of an ohmic heater.

performed during the 1990s, it has not been possible to find other works where the application of inductive heating to food processing has been studied. Inductive heating is based on the generation of an electric current through the food, aiming at heating it, which is induced by the vicinity of electromagnetic fields. Such fields may be generated by different methods, including the use of electric coils and the use of flowing food material as the secondary coil of a transformer (Piyasena et al. 2003; Sastry and Barach 2000).

Ohmic Heating

In ohmic heating (OH) (fig. 6.1), heat is internally generated due to the electrical resistance of foods (de Alwis and Fryer, 1990). Other electrical heating methods can be distinguished from OH as the latter

- needs electrodes contacting the foods (e.g., in microwave electrodes are absent),
- uses an unrestricted frequency (except for the specially assigned radio or microwave frequency range), and
- uses an unrestricted waveform (although typically sinusoidal).

OH was successfully applied in food processing in the nineteenth century to pasteurize milk (Getchel 1935). In 1938 the so-called "Electropure Process" was already used in approximately fifty milk pasteurizers in five U.S. states and served about 50,000 consumers (Moses 1938). Applications such as this were abandoned due to high processing costs (de Alwis and Fryer 1990) and due to the short supply of inert materials needed for the electrodes (Mizrahi et al. 1975).

Despite these early drawbacks, research on ohmic applications in fruits, vegetables, meat products, and surimi has kept on, mainly in the last two decades (Castro et al. 2003; Palaniappan and Sastry 1991a, 1991b; Wang and Sastry 1997).

Aseptic processing is considerably developed in the food industry, especially for liquid foods that are processed predominantly by means of heat exchangers. Their application to particulate foods is limited by the time required to ensure the correct processing of the center of larger particles, often causing overcooking of the surrounding volume. Consequently, product safety is achieved at the expense of quality.

The renewed interest in OH technology is due to the fact that products are of a superior quality to those processed by conventional technologies (Castro et al. 2003; Kim et al. 1996; Parrot 1992). The main advantages claimed for this technology are uniformity of heating and improvements in quality with minimal structural, nutritional, or organoleptic changes (Skudder 1989). Possible applications include most of the heat treatments such as

blanching, evaporation, dehydration, and fermentation (Cho et al. 1996) as well as pasteurization and sterilization.

A consortium of food processors, equipment manufacturers, ingredient suppliers, academia (food science, engineering, microbiology, and economics), and government was formed in 1992 to evaluate a 5 kW pilot-scale continuous-flow ohmic system (APV Baker, Ltd., Crawley, UK) and to develop a wide variety of shelf-stable products, including broccoli and cheese, shrimp gumbo, strawberries in glaze, oriental chicken and pasta primavera. Such products were found to have equal to, or higher than, texture, color, and nutrient retention compared to those processed by traditional methods such as freezing, retorting, and aseptic processing. The consortium concluded that the technology was technically and economically viable, having the following as main advantages (Parrot 1992):

- continuous production without heat-transfer surfaces;
- rapid and uniform treatment of liquid and solid phases with minimal heat damages and nutrient losses (e.g., unlike microwave heating, which has a finite penetration depth into solid materials);
- ideal process for shear-sensitive products because of low flow velocity;
- optimization of capital investment and product safety as a result of high solids loading;
- reduced fouling when compared to conventional heating;
- better and simpler process control with reduced maintenance costs;
- environmentally friendly system.

OH also features some disadvantages, namely those related to the high initial operational costs and the lack of generalized information or validation procedures.

Actually, OH is used industrially in North America, Europe, and Asia to produce a variety of high-quality, low- and high-acid products containing particulates. A considerable number of additional applications are being developed for this technology as shall be seen further in this chapter.

The Basics

As in other heat-processing technologies, OH is essentially used to increase the temperature of food materials to a point at which the food is considered adequately processed. In order to ensure that proper treatment is being given to the foods, the so-called cold spot must be identified and its temperature known. Once the cold spot is known, its temperature can either be directly measured or determined by means of an adequate model (de Alwis and Fryer 1990; de Alwis et al. 1989; Fu and Hsieh 1999; Orangi et al. 1998; Sastry and Palaniappan 1992a; Sastry and Salengke 1998).

Heating is achieved through the application of an electric field to a food with a given electrical conductivity (σ) by means of direct contact with electrodes.

Electric Field

The electric field (voltage distribution) is a function of the electrode, system geometry, electrical conductivity, and also of the applied voltage (de Alwis and Fryer 1990) The electric field is determined by the solution of Laplace's equation:

$$\nabla\left(\sigma \cdot \nabla V\right) = 0 \tag{6.1}$$

where σ is the electrical conductivity and ∇V is the voltage gradient. This equation results from the combination of Ohm's law with the continuity equation for electric current (Hayt 1981).

The solution of equation (6.1) has been obtained by de Alwis and Fryer (1990) for a static ohmic heater containing a single particle. For the general case of many different particles flowing in a fluid composed of several liquid phases (e.g., vegetable soup with solid particles), the mathematical solution for equation (6.1) is, to our knowledge, still unknown. In these cases, the prediction of the electric field has been based on semiempirical models such as those presented by Sastry and Palaniappan (1992a) or Sastry (1992).

Heat Generation

The heat ($\dot{Q}$) generated in a food by the current that flows through it is proportional to the square of its intensity (I), the proportionality constant being the electrical resistance (R), thus yielding:

$$Q = R \cdot I^2 \tag{6.2}$$

If both electrical conductivity (σ) and voltage gradient (∇V) are known, equation (6.2) becomes:

$$Q = \left|\nabla V\right|^2 \cdot \sigma \tag{6.3}$$

where σ is a function of both temperature and position. The dependence of temperature is normally described by a straight line (Palaniappan and Sastry 1991a):

$$\sigma_T = \sigma_{ref} \cdot \left[1 + m \cdot \left(T - T_{ref}\right)\right] \tag{6.4}$$

where σ_T is the electrical conductivity at temperature T, σ_{ref} is the electrical conductivity at a reference temperature, T_{ref}, and m is the temperature coefficient. Although this is the rule, there are some exceptions (Castro, Teixeira et al. 2004; Wang and Sastry 1993;).

The dependence of position is important mainly in those cases in which the food is not a homogeneous material (e.g., seafood soup).

Electrical Conductivity

The most important parameter in OH is the food electrical conductivity, σ (Fryer and Li 1993), the main characteristics of which are

- σ can vary in different directions (i.e., can be anisotropic);
- changes in the matrix structure will impose changes in the value of σ (e.g., during starch gelatinization or cell lysis);
- if σ displays a value that is not suitable for ohmic processing, it can be modified, for example, by blanching.

However, as mentioned before, the most remarkable feature of σ is its dependence on temperature (see equation (6.4)). Ions are responsible for conducting electricity in food

materials; at higher temperatures, there is a lower opposition to the movement of ions than at lower temperatures and this is reflected in the value of σ (Anonymous 1982). The behavior of σ with temperature is different, however, for solid and liquid foods.

It has been shown that for most solid foods σ increases sharply with temperature at around 60°C and this has been attributed to the breakdown of cell wall materials (Sastry and Palaniappan 1992b), which release ionic compounds to the bulk medium. It has been shown that the relationship between σ and T becomes linear for increasing values of the electric field (Palaniappan and Sastry 1991a), which is in accordance with the relationship between σ and T represented by equation (6.4).

For liquid foods, σ always has a linear relationship with T; however, the value of σ decreases if nonpolar (thus nonconductive) constituents are present. These constituents can be considered as "dissolved solids" and a relationship has been established in this case for σ with T (Palaniappan and Sastry 1991b):

$$\sigma_T = \sigma_{ref} \cdot \left[1 + K_1 \cdot \left(T - T_{ref}\right)\right] - K_2 \cdot S \tag{6.5}$$

where K_1 and K_2 are constants and S is the dissolved solids content.

Even when particles of a significant size are suspended in a fluid, an analogous influence of solids concentration on σ has also been reported (Castro et al. 2003; Zareifard et al. 2003). In this case, however, the value of σ is not that of the fluid but a combined value for the mixture fluid + particles, the so-called effective electrical conductivity (σ_{eff}), which is also influenced by solid size.

The increase of the ionic content of a food provokes an increase of σ (Sastry and Barach 2000) and this has been demonstrated by several authors (Castro et al. 2002; Palaniappan and Sastry 1991a, 1991c; Wang and Sastry 1993).

Particle Orientation and Geometry

In OH, other parameters are known to directly influence the heating rate of the different constituents of a food, such as particle orientation and geometry.

Particle geometry is important only when the aspect ratio of the solid particle is far from unity (Larkin and Spinak 1996). According to de Alwis et al. (1989), for a static OH system containing a fluid and a single elongated particle where $\sigma_{particle} < \sigma_{fluid}$, the particle will heat slower than the fluid if it is placed with its longer axis parallel to the electric field (fig. 6.2a), and it will heat faster than the fluid if it is placed with its longer axis perpendicular to the electric field (fig. 6.2b). The reverse heating rate will occur if $\sigma_{particle} > \sigma_{fluid}$ (fig. 6.2c and 6.2d).

These findings were confirmed by mathematical modeling (de Alwis and Fryer 1990; Fu and Hsieh 1999). The examples of figure 6.2a and 6.2d are considered worst-case scenarios by Sastry and Salengke (1998), as the particle will be underheated as compared to the fluid and this may result in underprocessing. In the case of figure 6.2a, the major part of the current will bypass the particle, thus heating more of the surrounding fluid and underheating the particle itself. Alternatively (fig. 6.2d), underheating may result from the particle transmitting most of the current, thus creating a low field gradient within the particle and low current densities in its vicinity. More information on the effects of particle orientation and geometry can be found in Vicente et al. (2005).

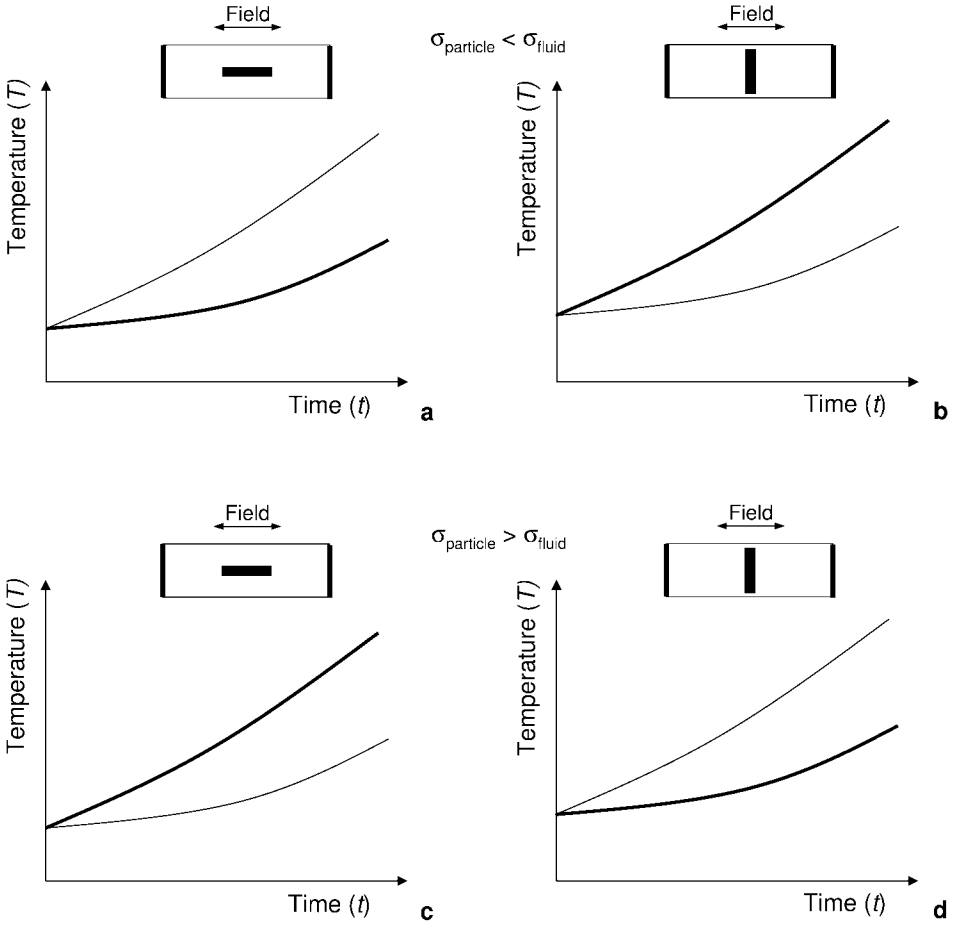

Figure 6.2. Heating curves of a single solid particle suspended in a fluid contained in a static ohmic heater (thick line = particle; thin line = fluid): (a) and (b) when the particle has a lower electrical conductivity than the fluid and its longer axis is parallel to the electric field, (a), or perpendicular to the electric field, (b); (c) and (d) when the particle has a higher electrical conductivity than the fluid and its longer axis is parallel to the electric field, (c), or perpendicular to the electric field, (d) (from Vicente et al. 2005, with permission).

The Effects of Ohmic Heating

The effects of OH on food components (e.g., enzymes and vitamins) and on microbes have been the target of research efforts for several years.

Microbial Inactivation

There are some studies involving microorganisms and OH, such as an interesting application of OH in a fermentation by *Lactobacillus acidophilus* (Cho et al. 1996). This

study concluded that the fermentation lag phase was significantly reduced (eighteen-fold) under low-voltage ohmic conditions as compared with a conventional fermentation. According to the authors, this effect may be due to the improvement of absorption of nutrients and minimization of the inhibitory action of fresh medium. The application of an electrical field may induce pore formation in membranes (similar to the electroporation mechanism used to transform cells in molecular biology studies), allowing a faster and efficient transport of the nutrients into the cells and thus decreasing lag phase. This study provides evidence that OH in the food industry may be useful to shorten the time for processing yogurt and cheese production (Cho et al. 1996), among other applications.

The kinetics of inactivation of *Bacillus subtilis* spores by continuous or intermittent ohmic and conventional heating were studied by Cho et al. (1999) to determine if electricity had an additional effect on the killing of this microorganism. Spores heated at 92.3°C had significantly lower decimal reduction time (*D*) values when using ohmic rather than conventional heating. These results indicate that electricity has an additional killing effect against bacterial spores. In another work, Palaniappan et al. (1992) indicated that electricity did not influence inactivation kinetics but the application of a nonlethal electric field reduced the intensity of the subsequent thermal treatment; this means that the electric field lowers the heat resistance of microorganisms.

The effect of an electric field on the thermal inactivation kinetics of a thermo-tolerant, ascospore-producing, filamentous fungus, *Byssochlamys fulva*, has been studied (Castro et al., pers. commun., 2003). This fungus may also produce patulin, an important mycotoxin. The death kinetics of *B. fulva* in strawberry pulp were determined and the *D* values obtained under OH (3.27 minutes) were half of those obtained under conventional heating (7.23 minutes). Both the non-thermal inactivation mechanism and the effect of OH on the production and degradation of patulin need to be studied in more detail.

Enzyme Degradation

The use of enzymes in the food industry for processing reasons is very widespread (Somogyi et al. 1996; van Loey et al. 2002). Enzymes may also have negative effects on food quality such as production of off-odors, tastes, and altering texture. While for other technologies studies on the degradation kinetics of enzymes have been performed (Barbosa-Canovas et al. 1998; Castro et al. 2001; Denys et al. 2000; Grahl and Markl 1996; Ho et al. 1997), the effects of OH on enzyme activity have not been investigated extensively. There is a recent study by Castro, Macedo et al. (2004) where the effects of OH on the degradation kinetics of lipoxigenase (LOX), polyphenoloxidase (PPO), pectinase (PEC), alkaline phosphatase (ALP), and ß-galactosidase (ß-GAL) have been determined. This study demonstrated that the electric field has an additional effect on LOX and PPO inactivation, where much lower *D* values were found as compared to conventional heating, meaning that a shorter treatment is needed to achieve the same inactivation degree, thus reducing negative thermal effects in the other food components. In the case of PEC, ALP, and ß-GAL, the electric field seems not to have an influence in enzyme inactivation kinetics as both conventional heating and OH *D* and *z* values are identical. The authors hypothesize that the presence of the electric field may disturb the metallic prosthetic groups pres-

Table 6.1. Kinetic constants and thermal inactivation parameters for the degradation of ascorbic acid in several fruits

Product	Temperature Range (°C)	pH	°Brix	E_a (kJ.mol^{-1})	k_0 (s^{-1})	z (°C)	$D_{75°C}$ (min)	Source
Strawberry pulp	60–97 (conventional heating)	4.0	14.5	21.36	0.15	46.7	175	Castro et al. 2004a
Strawberry pulp	60–97 (OH)	4.0	14.5	21.05	0.14	46.7	169	Castro et al. 2004a
Grapefruit juice	61–96 (conventional heating)	3.05	11.2	21.0	3.90×10^{-2}	49.3	1354	Saguy et al. 1978
Grapefrui juice	60–91 (conventional heating)	3.05	31.2	22.0	6.10×10^{-2}	45.0	1228	Saguy et al. 1978
Lime	20–92 (conventional heating)	5.92	6.3	58.1	1.55×10^{4}	35.8	1186	Alvarado and Viteri 1989
Lemon	20–92 (conventional heating)	2.94	6.0	46.5	3.59×10^{2}	44.6	949	Alvarado and Viteri 1989
Tangerine	20–92 (conventional heating)	4.10	13.4	44.6	2.25×10^{2}	46.5	771	Alvarado and Viteri 1989
Grapefruit	20–92 (conventional heating)	3.54	11.2	56.9	9.29×10^{3}	36.5	1276	Alvarado and Viteri 1989
Orange juice	70.3–97.6 (conventional heating)	3.60	12.5	128.3	3.23×10^{13}	19.0	24110	Johnson et al. 1995
Orange juice	70.3–97.6 (conventional heating)	3.60	36.7	97.4	1.62×10^{9}	24.9	10447	Johnson et al. 1995
Orange juice	65–90 (conventional heating)	(a)	(a)	12.6	3.30×10^{4}	(a)	(a)	Lima 1996
Orange juice	65–90 (OH)	(a)	(a)	12.5	3.26×10^{4}	(a)	(a)	Lima 1996

Source: Vicente et al. 2005, with permission.
(a) = Not reported.

ent on LOX and PPO (and absent in PEC, ALP, and ß-GAL), thus causing the enhancement of activity loss. However, this hypothesis needs to be verified.

Vitamin Degradation—Ascorbic Acid

The degradation mechanism of ascorbic acid is specific to the particular system in which it is integrated. Several studies have been made to determine the kinetic parameters of thermal degradation of ascorbic acid in food systems under conventional and OH conditions (table 6.1).

One of these studies, using industrial strawberry pulps (Castro, Teixeira et al. 2004), concluded that the reaction follows first-order kinetics for both conventional and OH treatments, being the kinetic parameters identical for both heating processes. The conclusion was that the presence of low-intensity electric fields (< 20 $V.cm^{-1}$) does not affect the ascorbic acid degradation. Similar conclusions were obtained by Lima (1996) for orange juice systems.

Equipment

In simple terms, an ohmic heater can be built with a pair of electrodes, a container for the food to be processed, and an alternating power supply. Such a heater can be integrated into a batch or continuous process. As a rule of thumb, batch processes are used to cook, for example, meat products (Piette and Brodeur 2001), while continuous processes are more appropriate for viscous fluids or fluids containing particles.

The typical batch ohmic heater is a horizontal cylinder with one electrode placed in each extremity (fig. 6.3a).

Continuous processing calls for more flexibility and different designs can be found, depending on the manufacturer (fig. 6.3b to 6.3d). For the configurations in figures 6.3b and 6.3c, the electric field is perpendicular to the flow; these configurations are often called cross-field. When the electric field is parallel to the flow (as in fig. 6.3d) the configuration obtained is termed in-field. The best configuration for each case will depend primarily on the food system to be processed and also on the type of operation (e.g., blanching, cooking, pasteurization).

Applications

The application of OH technology has been reported in the thermal processing of high-acid food products (e.g., tomato-based sauces), and in the pasteurization of whole liquid egg (Parrot 1992), fish pastes, and on the processing of meat products as an alternative means of cooking.

Wang et al. (2002) applied OH to frozen meat samples and the results demonstrated a uniform and quicker thawing process, where meat properties such as color and pH were not changed significantly and the final products achieved a good thawing quality. Piette and Brodeur (2001) used OH to cook meat products (sausages and ham) with promising results in batch operations. Such products were found to have extremely reduced cooking times (e.g., a ham weighing one kilogram was cooked in less than 2 minutes). However, these cooking times were insufficient to reduce the bacterial load to safe levels.

In a study by Castro et al. (2003), the suitability of several strawberry-based products to be processed by OH was tested. The results showed that, for most of the products, high heating rates could be achieved despite the significant differences of σ between the tested products. In another study, Wang and Sastry (1997) evaluated the effects of an ohmic pretreatment to vegetables and found no significant changes in the moisture content of the final products. This technology might thus be an alternative to conventional blanching treatments. Also, in the case of texture maintenance, OH can be an alternative. Eliot et al. (1999a) studied the influence of precooking by OH on the firmness of cauliflower and concluded that OH combined with low-temperature precooking in saline solutions offers a viable solution to HTST sterilization of cauliflower florets. A similar study was also per-

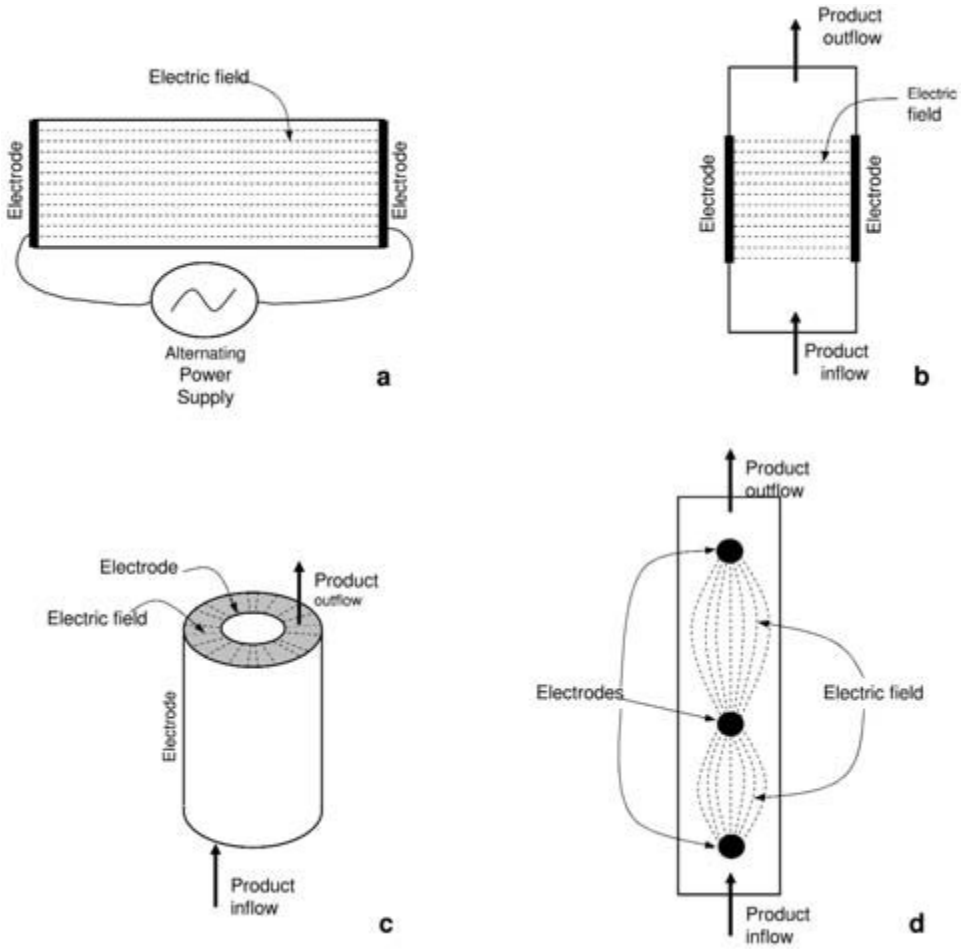

Figure 6.3. Basic configurations for ohmic heaters: (a) batch; (b–d) continuous. (b) and (c) correspond to the cross-field configuration while (d) corresponds to the in-field configuration, depending on if the electric field lines are perpendicular or parallel to the direction of the flow of the food, respectively (from Vicente et al. 2005, with permission).

formed with potato cubes (Eliot and Goullieux 2000; Eliot et al. 1999b) and concluded that an ohmic pretreatment prevented loss of firmness when compared to a conventional pretreatment (up to 50%, in some cases).

In the processing of surimi, OH has been found to improve the textural properties of the products as compared to those heated in a 90°C water bath, and a superior gel quality was achieved (Yongsawatdigul et al. 1995). In fact, higher heating rates are not beneficial to surimi manufacture (Yongsawatdigul and Park 1996), as slow heating rates produce stronger gels. OH allows a wide range of linear heating rates and therefore permits a much closer control of the process of surimi gelation.

The continuous research and development of new equipment and electrodes guarantees that other foods may soon start to be processed using OH.

Conclusions and Future Perspectives

The application of OH for food processing is still not well characterized and not all its potentialities have been fully exploited due to the complexity of the phenomena occurring during OH processing and the complexity of food materials (Vicente et al. 2005).

It has been stressed here that OH's main characteristic, that is, internal heat generation, eliminates the problems associated with heat conduction in food materials, thus preventing the overcooking typical of conventional thermal food processing. OH is therefore specially indicated for the processing of foods containing particulate materials and foods with non-Newtonian rheological properties.

Novel applications and a deeper knowledge of current applications must be obtained and further research and development work on OH in these areas is essential (Vicente et al. 2005).

Microwave Heating

Microwave (MW) technology dates back to the beginning of the twentieth century, but its industrial application has been facing new developments in the past 3 decades.

The first continuous magnetron was built by Randall and Boot, who tried to produce an MW source to power radar sets for the British military during World War II (Reynolds 1989). In 1945 Spencer filed a patent application, which was issued in 1950, after popping some corn in front of a waveguide horn. The first commercial MW oven for institutional and restaurant use was released in 1947.

The first patent describing an industrial conveyor belt MW heating system was issued in 1952 (Spencer 1952). However, the first conveyor belt MW application only started in 1962 due to the slow development of high-power MW generators. Its first major applications were the finish drying of potato chips, precooking of poultry and bacon, tempering of frozen food, and drying of pasta (Decareau 1985).

In the 1990s annual sales in the European Union exceeded ten million units, which translates to a penetration rate in households of over 80%. The European market presents similar trends.

The Basics

Microwaves, a form of electromagnetic radiation, are characterized by wavelength and frequency. The frequencies allocated by the Federal Communications Commission (FCC) for the purposes of heating are listed in table 6.2. Typically, MW food processing uses the frequencies of 2,450 and 915 MHz. Of these two, the 2,450 MHz frequency is used for home

Table 6.2 Frequencies assigned by the FCC for industrial, scientific, and medical use

	Frequency
Microwaves	915 MHz ± 13 MHz 2,450 MHz ± 50 MHz 5,800 MHz ± 75 MHz 24,125 MHz ± 125 MHz

ovens, and both are used in industrial heating. It is worthwhile to note that outside of the United States, frequencies of 433.92, 896, and 2,375 MHz are also used.

MW heating has a number of quantitative and qualitative advantages over conventional heating techniques, namely:

- *Speed:* The time required to come up to the desired process temperature can be accomplished in one-quarter of the time that conventional heating requires. This is particularly true for particulate foods that depend on the slow thermal diffusion process in conventional heating. Being so, bacterial destruction is achieved, but thermal degradation of the desired components is reduced.
- *Uniformity of heating:* The heat is generated in the product, which means that small heat conductivities or heat transfer coefficients do not play an important role in the heating process. Being so, temperature distribution can be more uniform than conventional heating. However, in some cases this uniformity may be reduced, as shall be explained further in the text.
- *Selective heating:* Microwaves couple selectively into materials that are more absorptive of the energy. Although greater efficiency can be achieved, temperature profiles can develop in multicomponent food systems.

Other advantages of MW heating systems, some depending on the application, are that it can be turned on or off instantly, the product can be pasteurized after being packaged, the systems are space saving, or they provide reduced noise levels. These advantages often yield an increased productivity and/or an improved product quality.

The main disadvantages claimed for this technology are the lack of experimental data needed to model MW heating and the need of engineering intelligence to understand and minimize uneven heating or thermal runaway. These two effects will be addressed further in this chapter.

The lack of predictive models relating the electrical properties of foods to transient time-temperature profiles (that determine product quality and microbial safety) has been one of the major obstacles to the development of new industrial MW applications.

Heat Generation

MW heating involves two distinct mechanisms: dielectric and ionic.

When microwaves (or any other radiation) are directed toward a material, part of the energy is reflected and the other part is transmitted through the surface of the material. A fraction of the transmitted energy is absorbed by the body. The dielectric properties have been defined in terms of the proportions of energy that are reflected, transmitted, or absorbed.

MW properties of basic interest in food processing are described in terms of complex relative permittivity for biological materials and can be mathematically expressed as:

$$\varepsilon^* = \varepsilon' - j\varepsilon'' \tag{6.6}$$

where ϵ^* is the complex relative permittivity, ϵ' the dielectric constant, and ϵ'' the dielectric loss factor.

The dielectric constant is a measure of a material's ability to store energy and the loss factor is a measure of its ability to dissipate electrical energy.

The dielectric heating is mainly due to the presence of water. Due to their dipolar nature, water molecules try to follow the electric field associated with electromagnetic radiation as it oscillates at the very high frequencies listed and such oscillations produce heat.

The dielectric properties dictate, to a large extent, the behavior of materials when submitted to MW field and it is, therefore, fundamental to characterize it.

The second major mechanism of heating with microwaves is through the oscillatory migration of ions in the food that generate heat under the influence of the oscillating electric field. The migration of ions toward oppositely charged regions results in multiple collisions and disruption of hydrogen bonds with water, both generating heat.

The rate of heat generation per unit volume at a particular location in the food during MW heating can be expressed by (Buffler 1993; Datta 2001):

$$Q = 2 \cdot \pi \cdot f \cdot \varepsilon_0 \cdot \varepsilon'' \cdot \left|\Delta V\right|^2 \tag{6.7}$$

where ΔV is the strength of electric field of the wave at that location, f is the frequency of the microwaves, ϵ_0 is the permittivity in a vacuum (a physical constant), and ϵ'' (is the dielectric loss factor.

This equation is only valid for a specific location, which, for practical purposes, is not adequate since the electric field distribution in a real situation is very complex (Buffler 1993) and depends on several factors, namely dielectric properties, geometry, and oven configuration.

Factors Affecting Microwave Heating

The MW heating process is affected by a number of parameters, either from the equipment or from the product to be heated, that have to be considered when designing a new product/process. Some of these critical process factors are MW frequency, moisture content, temperature, product parameters (including mass, density, geometry), and specific heat.

The spatial distribution of MW absorption is affected by those parameters, meaning that different heating rates (uneven heating) will be observed. Being so, different microbial inactivation extents will occur within the food product and may jeopardize food safety.

In this section these parameters will be briefly addressed.

Frequency

When microwaves strike the surface of a material a fraction of the energy will be reflected ($\dot{Q}_r$) and the other will be transmitted to the material ($\dot{Q}_{trans}$). Considering an incidence angle of 90°, the reflected power is related to the dielectric constant (ϵ') by equation (6.8):

$$Q_r\left(90\right) = \frac{\left(\sqrt{\varepsilon'} - 1\right)^2}{\left(\sqrt{\varepsilon'} + 1\right)^2} \tag{6.8}$$

and $\dot{Q}_{trans}$ is, in turn:

$$Q_{trans} = 1 - Q_r \tag{6.9}$$

However, only a fraction of the transmitted power is generated into heat $\dot{Q}_{abs}$. The absorbed power can be mathematically expressed as:

$$Q_{abs} = 5.56 \times 10^{-4} \cdot f \cdot \varepsilon'' \cdot \left|\Delta V\right|^2 \tag{6.10}$$

being the variables defined as above.

The penetration depth, *d*, can be defined as the depth into a sample at which the energy (MW power) has decreased to 1/*e* (about 36.8%) of its transmitted value. The penetration depth may also be expressed as the half power depth (i.e., the depth from the surface at which the power has been reduced to 50% of the incident power). It can be expressed as a function of ϵ' and ϵ'':

$$d = \frac{\lambda_0 \cdot \sqrt{\varepsilon'}}{2 \cdot \pi \cdot \varepsilon''} \tag{6.11}$$

where λ_0 is the vacuum MW wavelength (for 2.5 GHz, λ_0 = 12.2 cm).

Therefore, the selection of the MW frequency is important because it relates to the size of the object to be heated. For example, *d* for water is approximately 2.3 cm at 2,450 MHz and 20.0 cm at 915 MHz. Being so, large food blocks would be better processed at lower frequencies because a higher MW penetration could be achieved. For most common food products *d* varies from 0.6 to 1.0 cm at 2,450 MHz.

Frequency also affects dielectric properties (and consequently MW heating), although in complex food systems this effect is almost negligible.

Moisture Content

MW heating is greatly affected by the presence of water in foods (Mudgett 1985; Nelson and Kraszewski 1990; von Hippel 1954). Water is the major absorber of MW energy in the foods and the higher the water content the higher the dielectric loss factor, thus the better heating. Due to their dipolar nature, water molecules try to follow the electric field associated with electromagnetic radiation as it oscillates at very high frequencies and such oscillations of the water molecules produce heat. Hasted et al. (1948) and Kaatze (1989) determined dielectric properties of the water as a function of frequency for 20 and 50°C. A summary of this data is presented in table 6.3.

Table 6.3. Dielectric properties of water as a function of frequency (adapted from Kaatze 1989)

	Dielectric Constant (ϵ') Temperature (°C)		Dielectric Loss Factor (ϵ'') Temperature (°C)	
Frequency (GHz)	20	50	20	50
0.6	80.3	69.9	2.75	1.25
3.0	77.4	68.4	13.0	5.8
4.6	74.0	68.5	18.8	9.4
9.1	63.0	65.5	31.5	16.5
12.5	53.6	61.5	35.5	21.4
26.8	26.5	44.2	33.9	32.0

However, in food samples water does not appear in its pure form and, depending on the material structure, it may be bound to the other food components in various forms differing in terms of energy binding and dielectric properties. Thus, the prediction of the dielectric behavior of a food product as a function of moisture content, frequency, or temperature is an extremely complex subject that represents an obstacle (or a slowdown) to the industrial use of MW technology for processing foods.

Generally, for a 20–30% moisture level, the higher the moisture content the higher its dielectric constant and dielectric loss. Moreover, low water content foods can also heat well due to the decrease of specific heat. The influence of this parameter in MW heating will be discussed further later in this chapter.

Temperature

Temperature and moisture levels change during heating and both parameters influence both the dielectric constant and the dielectric loss factor and, consequently, MW heating rate. These parameters may increase or decrease with temperature. Freezing has a major effect on physical and electrical properties of foods. Ohlsson et al. (1974) found that dielectric properties increased sharply with temperature in the temperature range of −10 to 0°C for most of the food products tested. Moreover, the initial temperature of the food system should be known in order to be able to control (by adjusting the power) the final temperature of the system.

Product Parameters

The parameters of the product to be processed influence the heating process, namely:

- *Mass:* There is a direct relation between the MW power input and the total mass to be heated.
- *Density:* The higher the density the higher the dielectric constant is, and the higher the heating rate of the material. Porous materials act like insulators because air is completely transparent (does not heat) at the industrial frequencies.
- *Size:* The optimal size is obtained when the size of the pieces to be heated is comparable to the size of the wavelength, otherwise uneven heating will occur.
- *Shape:* A torus shape is the ideal geometry to allow uniform heating, and sharp edges or corners should be avoided since they tend to overheat. Using selected frequencies and reduced power to achieve reduced heating rates, allowing thermal conductivity to assist in the temperature distribution, is the best way to MW nonuniform food products.
- *Conductivity:* The ionic conduction is one of the mechanisms of heat generation and the addition of salt (which increases conductivity) may increase the heating rate. However, it will also affect the penetration depth, causing increased heating rates in the surface and thus increasing temperature gradients.
- *Thermal conductivity:* This is an important parameter when the penetration depth of the microwaves is not sufficient to guarantee a uniform heating or when the heating time is long.
- *Specific heat:* Materials with low dielectric loss and low specific heat can heat well under MW radiation; in fact, oil can heat faster than water (Ohlsson 1983). This prop-

erty may be helpful in obtaining uniform heating because with higher specific heat the product heats slower, which results in more uniform heating.

Changes in properties during heating have a more pronounced effect in MW heating when compared to conventional heating. For example, the determination of the coldest spot is not straightforward and can change during the heating process. The MW absorption increases with temperature, which in turn increases the heating rate further, increasing the rate of MW absorption. Such coupling could lead to runaway heating (Zhang and Datta 2000; Zhang et al. 2001). At lower temperatures, MW absorption is lower, so the waves are able to penetrate a lot further into the material. As the material heats up, it absorbs microwaves more readily and the waves are not able to penetrate as far. Especially in foods with high ionic concentrations, the surface at higher temperatures can act as a shield and uneven heating takes place.

Microbial Inactivation

Several studies report the inactivation kinetics of microorganisms in food systems when using MW and conventional heating methods. However, the comparison of the two methods is not very accurate either by lack of details of the methodology or due to the different techniques used for temperature monitoring (Heddleson and Doores 1994). MW heating is often reported to cause nonuniform heating. This fact leads to survival of foodborne pathogens, including *Salmonella* and *L. monocytogenes* in cold spots that, in this case, are not the center of the food. Being so, monitoring the internal temperature that would normally be lethal may not be sufficient to ensure microbial food safety (Schnepf and Barbeau 1989).

The inactivation curves for microorganisms using MW heating are similar to those obtained using conventional heating methods. Despite this fact, an additional killing factor due to non-thermal effects was also discussed. Four major theories were proposed to explain non-thermal inactivation by microwaves: selective heating, electroporation, cell membrane rupture, and magnetic field coupling (Kozempel et al. 1998). The non-thermal effect is still not well established, but it is currently accepted that MW energy may complement or magnify thermal effects by causing nonlethal injuries to the cells. Khalil and Villota (1989) demonstrated that while both conventional and MW heating destroyed the 16S subunit of RNA of sublethally heated *S. aureus* FRI-100, only MW heating affected the integral structure of the 23S subunit. Ramaswamy et al. (2000) reported that the non-thermal effect of MW energy at sublethal temperatures is insignificant. However, they determined that, for equivalent heat treatments, microwaves enhanced inactivation. Kozempel et al. (2000) concluded that, in the absence of other stress sources such as pH or heat, MW energy did not inactivate microorganisms; but they suggested that MW energy may enhance thermal inactivation.

The major drawbacks of MW heating are its nonuniform heating and unpredictability of cold spots, which may put at risk safety of the food.

Equipment

A high-voltage system to generate MW energy exists in every MW oven. Its main component is a magnetron tube. The magnetron is a diode-type electron and its basic internal

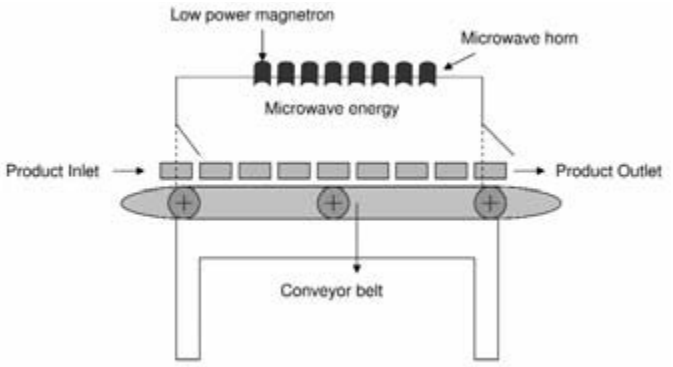

Figure 6.4. Schematic representation of a continuous microwave oven, using several low-power magnetrons as the power source.

structure includes the anode, the filament/cathode, the antenna, and the magnets. The generated energy is then transmitted to a wave guide (a hollow metal piece).

Nowadays, the industrial MW equipment can be of two types, according to the power source used: high-power single magnetron and low-power multimagnetron devices (fig. 6.4). One of the main difficulties in continuous MW systems is the feeding of the product because radiation leakage must be avoided. When the product has large dimensions, in and out gates with complete closure have to be used. In the case of fluids or granular products with small dimensions it is possible to place dielectric loads in front of the openings (in and out) that, together with the absorption of the entering product, can keep radiation at low levels.

Applications

The main applications of MW heating in food processing are (re)heating, baking and (pre)cooking, tempering, blanching, pasteurization and sterilization, drying, and freeze-drying.

Baking and Cooking

In this industry microwaves are often combined with conventional heating or infrared surface baking. The use of combined MW baking allows the reduction of baking time and makes it possible to use flour with high α-amylase and low protein content. This method inactivates α-amylase fast enough to prevent the starch from extensive breakdown and develops sufficient CO_2 and steam to produce a high porous good dough (Decareau 1986). Currently microwaves are used in end baking, where the low heat conductivity would lead to considerably higher baking times. A MW assisted doughnut-frying operation is another possible application resulting in a shorter frying time and a lower fat uptake.

One of the main obstacles to the use of MW baking processes was to find a microwavable baking pan, but in 1981 and 1982 patents were issued for the use of metal baking pans in MW ovens (Schiffmann 1982; Schiffmann et al. 1981).

Tempering

A very successful application is meat tempering, usually a time-consuming process that, by the use of microwaves, can be considerably speeded up. Due to their higher penetration depths, tempering systems usually operate at 915 MHz. In this process it is possible to obtain uniform heating and to control the end temperature to avoid localized melting, which would be coupled to a thermal runaway effect. The best homogeneity in this application is reached in a multisource, multimode cavity, equipped with mode stirrers (Metaxas 1996). Tempering tunnels developed by LMI in France are used for beef, lamb, and ham. They operate at 2.45 GHz with an output power of 2.5 or 5 kW.

Drying

The most common industrial processes are a combination between microwaves and hot air. Successful applications are pasta drying (Decareau 1985) and the production of dried onions (Metaxas and Meredith 1983).

Puffing (combination of pre-air-intermittent and MW vacuum) and drying of snack foods is another popular application, and some products have been developed specifically for MW processing (e.g., puffed rice cake and seaweeds). Puffing vegetables and fruits with improved dehydration properties is also a new and successful application. In China, MW power is used to dry chocolate powder and milk cake and to age wine and spirits (Chen et al. 1982).

Pasteurization and Sterilization

Industrial and experimental applications of MW pasteurization or sterilization include yogurt and pouch-packed meals, as well as the continuous pasteurization of fluids like milk (Decareau 1985; Rosenberg and Bögl 1987). For the packed food systems, conveyor belt systems were employed (e.g., Harlfinger 1992). Some continuous applications were reported (Sale 1976). This seems to be an advantageous procedure because, as previously stated, microwaves may enhance thermal inactivation of microorganisms, reducing the time of thermal treatment and thus obtaining higher-quality products. Vikram et al. (2005) examined the thermal degradation kinetics of vitamin C using MW, infrared, ohmic, and conventional heating. Ohmic heating was the method that allowed better vitamin retention at all temperatures tested.

Conclusions and Future Perspectives

The main obstacles to industrial setup of MW heating processes are the difficulties in controlling the process and the high energy costs associated with this technology. The changes of dielectric properties of food products during the heating processes are not yet fully understood or modeled and, consequently, the validation of the processes has to be done almost individually for each food product, slowing down the dissemination of MW industrial lines.

Moreover, the food industry and the consumer are very conservative about the use of new technologies, and research departments have to work with reduced budgets usually directed to the development of new products. Being so, research opportunities in the technological areas are far from being exhausted.

Radio-Frequency Heating

Radio frequency (RF) heating heating involves the transfer of electromagnetic energy directly into the product, therefore inducing volumetric heating due to frictional interaction between molecules (Piyasena et al. 2003). In RF heating the food is placed between two capacitor plates, where it plays the role of a *dielectric* to which a high-frequency alternating electric field is applied. Such field will force polar molecules (e.g., water) to constantly realign themselves with the electric field. This molecular movement is very fast due to the high frequency of the field and will provoke generation of heat within the food by energy dissipation caused by molecular friction.

RF waves (as well as microwaves) are both within the radar range and this strongly limits the frequencies that can be used for applications other than communications. The allowed frequencies for RF applications are 13.56, 27.12, and 40.68 Mhz (Piyasena et al. 2003).

The fact that the wavelength at radio frequencies (e.g., 11 m at 27.12 MHz) is substantially greater than at microwave frequencies (e.g., 12 cm at 2,450 MHz) justifies the significant advantages of RF over microwaves, especially in the case of food processing applications (Rowley 2001).

As compared with conventional heat-processing technologies, RF heating presents similar advantages to ohmic and microwave heating that are essentially due to the generation of heat throughout the volume of the material to be processed. However, there are some specific advantages of RF over those alternative volumetric technologies, namely (Rowley 2001):

- there is no need for electrodes contacting the food (in contrast with, e.g., ohmic heating), allowing RF to be easily applied to both solid and liquid foods;
- due to the longer wavelength of RF, its power will penetrate more deeply in the foods as compared to microwave power;
- the construction of large RF heating systems is simpler than their microwave counterparts, and their application to continuous processes is straightforward; it is a technology particularly suited to large industrial applications.

RF's main disadvantages are (Ohlsson 1999; Rowley 2001):

- the higher equipment and operating costs for an equivalent power output when compared to conventional heating systems and also to ohmic heating;
- the reduced power density when compared to microwave heating, meaning that larger RF heating systems are needed for the same power rating and also that slower heating rates are achieved with RF as compared to microwaves;
- the so far limited research efforts regarding, for example, the determination of food RF dielectric properties.

The Basics

Not willing to enter into a deep study of the electrical properties and variables that are involved in RF heating, it is however necessary to introduce a few fundamental concepts (for a greater detail see, e.g., Piyasena et al. 2003).

The first assumption to be made is that when a food is placed between the plates of a capacitor, such food will act as a dielectric characterized by a relative dielectric constant (ϵ_r'), which is a measure of the capacity of a material to store electric energy, and a relative dielectric loss factor (ϵ_r''), which measures the dissipation of electric energy into heat, much in the same way as in microwave heating (see equation (6.6)). Both constants are relative to the permittivity in vacuum (ϵ_0). In the absence of food, such capacitor will have an effective capacitance, $C_{eff,0}$, which will be altered to C_{eff} when food is present by a factor of ϵ_r', which is, by definition, always greater than one. The presence of food will also generate a resistance across the capacitor, R, which is proportional to $1/(C_{eff,0} \cdot \epsilon_r'')$. It is the existence of this resistance that will allow the heat generation inside the food.

Heat Generation

Being able to calculate the amount of energy (in the form of heat) that is dissipated in a dielectric body when it is subjected to RF (or microwave) heating is essential for design and operation control. This calculation can be performed once the values of some of the previously mentioned properties are known.

If the heat (power) dissipated in the resistance generated by the food in the capacitor can be given by equation (6.2), then the heat dissipation per unit volume is given by (Rowley 2001):

$$Q = 2 \cdot \pi \cdot f \cdot \varepsilon_0 \cdot \varepsilon_r'' \cdot \left|\nabla V\right|^2 \tag{6.12}$$

where f is the frequency of the field, ϵ_0 is the permittivity in vacuum (a constant), and $|\nabla V|$ is the voltage gradient (field strength) in the dielectric.

For foods, the dielectric loss is mainly a consequence of the electrical conductivity, σ, case in which the dielectric loss factor, ϵ_r'', is $\epsilon_r'' = \sigma/(2 \cdot \pi \cdot f \cdot \epsilon_0)$, therefore reducing equation (6.12) to equation (6.3), which has been presented for ohmic heating.

Factors Affecting Radio-Frequency Heating

The relevant properties in RF heating are the relative dielectric constant (ϵ_r'), the relative dielectric loss factor (ϵ_r''), and the electrical conductivity (σ), which are the so-called *dielectric properties*. The first two can be combined to yield the loss tangent (tan *d*):

$$\tan\delta = \varepsilon_r'' \big/ \varepsilon_r' \tag{6.13}$$

Electrical conductivity affects RF heating due to its close relation with the dielectric loss factor.

These properties affect RF heating, for example, in terms of their influence on temperature increase (Orfeuil 1987):

$$\Delta T = \frac{2 \cdot \pi \cdot f \cdot \varepsilon_0 \cdot \varepsilon_r' \cdot \tan\delta \cdot \left|\nabla V\right|^2}{C_p \cdot \rho} \tag{6.14}$$

where T is the temperature, C_p is the specific heat at constant pressure, and ρ is the density of the substance being heated.

RF is also influenced by means of the penetration depth (d), which is defined, according to Bengtsson and Risman quoted by Piyasena et al. (2003), as the depth in a material where the energy of a plane wave propagating perpendicular to the surface has decreased to $1/e$ of the surface value and is represented by:

$$d = \frac{c}{2 \cdot \pi \cdot f \cdot \sqrt{2} \cdot \sqrt{\varepsilon_r^{'}} \cdot \sqrt{\sqrt{1 + \left(\tan \delta\right)^2} - 1}} \tag{6.15}$$

where c is the (constant) speed of propagation of waves in vacuum.

Both equations (6.14) and (6.15) demonstrate the effects of the pertinent properties and variables on RF heating. Besides those already mentioned (ϵ_r', ϵ_r'', tan δ), other properties such as the specific heat and the density also play a role as they affect, for example, the heating rate of the food.

For the particular case of temperature, and in order to determine the degree of uniformity of heating achieved with RF heating, Zhong et al. (2004) have used an infrared camera to record the temperature distribution within particulates of carrots and potatoes. Although dependent on the particles' size, the heating achieved with RF was rather uniform when compared to other heating technologies and therefore it was concluded that RF is a promising heating source in thermal processing.

The dielectric properties of foods are influenced mainly by frequency, temperature, water content, and chemical composition (Piyasena et al. 2003) and can be determined by several types of methods (Ryynänen 1995): lumped circuit methods, resonator methods, transmission line methods, and free space methods. These methods differ much in the frequency range in which they can be applied and the choice of the method is partially determined by that parameter. There are a considerable number of studies concerning the measurement of dielectric properties in various foods (see, e.g., Al-Holy et al. 2005; Ede and Haddow 1951; Nelson 1994; Tran and Stuchly 1987), but only a few have performed the measurements at RF frequencies.

The Effects of RF Heating

Much in the same way as with the determination of dielectric properties, the effects of RF heating in microorganisms and food constituents (e.g., vitamins) have been scarcely studied (in comparison, for microwave heating there is much more information available) (Oberndorfer et al. 2000).

The possibility of a non-thermal effect of RF on the death kinetics of microorganisms has been discussed for about 60 years (Geveke et al. 2002), and while some authors claim inactivation of microorganisms due to the non-thermal effect of RF (see, e.g., Fleming 1944; Nyrop 1946), others claim precisely the contrary (Ingram and Page 1953; Ponne et al. 1996; Geveke et al. 2002). Although no consensual results have been published so far, the clear trend is to consider that if a non-thermal effect of RF on microorganisms exists, it is negligible for the most usual operation conditions.

The effects of RF over the overall quality of the treated products are scarcely documented in the literature. No significant differences were found in moisture, protein, fat, ash, and NaCl content of turkey breast rolls when RF and steam cooked (Tang et al. 2005); equally, the contents of vitamins (thiamine and riboflavin) were the same in both cases and

the texture profile analysis gave similar results. However, the rate of lipid oxidation was significantly lower in RF- than in steam-cooked meat, particularly in the superficial layers of the meat, indicating that in the latter method the meat has been subjected to higher temperatures generated by the steam that have thermally damaged the membrane phospholipids, leading to the detected increased oxidation. In all, the main conclusion was that if there was an effect of RF, such effect was due essentially to the volumetric heating characteristics of this technology. However, it is noteworthy to mention that RF meat was cooked four times faster than steam-cooked meat.

Equipment

The equipment needed for RF heating consists of two fundamental elements: the one responsible for the generation of the RF waves (the *generator*) and the one responsible for the application of the RF power to the food (the *applicator*, the main part of which is the electrodes). There are basically two groups of systems used to produce and apply RF power to foods: the conventional RF heating equipment, where the RF applicator is part of the RF generator circuit, and the so-called 50 Ω RF equipment, which is more recent and is characterized by the fact that the RF applicator and RF generator are physically separated, with connection made by a high-power coaxial cable (Rowley 2001).

In any case, the RF applicator (electrodes) must comply with the needs of the product and of the process. This means that its design must be adapted and, in general, there are three main configurations for the electrodes: throughfield, fringefield, and staggered throughfield (see fig. 6.5).

The throughfield configuration (fig. 6.5a) consists of two electrodes between which the food is placed and to which a high-frequency voltage is applied. Given its design, this system is used mostly when the products to be treated are thick, and it is suitable for both batch and continuous processes.

The fringefield configuration consists of a set of electrodes (which can assume various shapes) that are alternately connected to the RF generator (fig. 6.5b). This design reduces the electric field that is needed to generate a given power density inside the product. This system is most appropriate for thin products to avoid the risk of significant electric field variations through the products' volume.

The staggered throughfield configuration (fig. 6.5c) is a modification of the throughfield configuration suitable for intermediate thickness products. The electric field needed in this case is intermediate between the throughfield and the fringefield configurations.

Applications

At this stage, it should be clear that RF heating shows promising potential to replace conventional heating methods in the food industry. There is a significant amount of publications, some dating back from the 1940s, where the use of RF is described for various applications, including blanching, pasteurization/sterilization, thawing, drying, heating of bread/baking, and meat processing, among others. Table 6.4 is a summary of examples of RF heating applications for food processing. Most of the works cited refer to improvements achieved in the quality of the foods subjected to RF treatment when compared to those processed using conventional heating technologies.

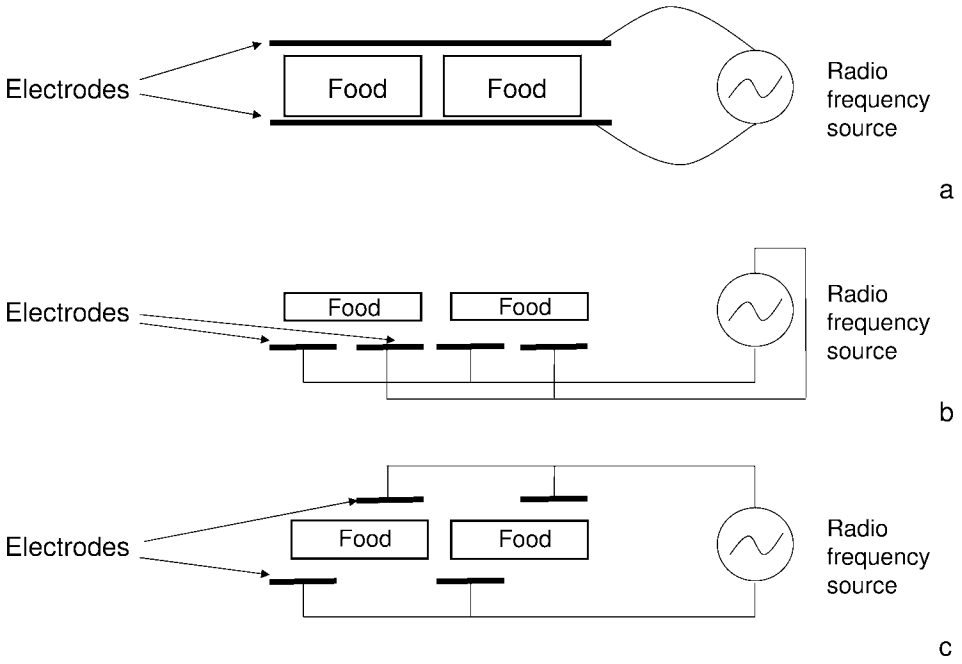

Figure 6.5. Possible electrode configurations for RF heating: (a) throughfield, (b) fringefield, and (c) staggered throughfield.

Conclusions and Future Perspectives

RF heating offers a potential alternative to conventional heating for liquid and particulate foods due to its rapid and uniform heating patterns and large penetration depth. The use of RF heating may lead to savings in energy consumption when compared to the traditional methods of heating. However, taking into account the most recent research efforts, there is no evidence that RF energy has non-thermal effects over microorganisms or food constituents (e.g., vitamins) or even that there is a synergistic effect of RF energy and heat.

Due to the lack of data on RF dielectric properties of foods, their determination is one important field for future work. Also, the potential applications of RF heating have not been totally considered and there is the need for further developments that take into account the most recent technological progress, together with a proper evaluation of its real economical potential (see, e.g., Rowley 2001). All this work must be done keeping in mind that if this technology is to be applied by the industry, it is absolutely fundamental to clearly prove its advantages over the existing alternatives.

Infrared Heating

The applications for infrared (IR) radiation are very wide and include medical, dye, automobile, and paper industries, among others. The discovery of this type of radiation dates back to 1800 when Sir William Herschel was attempting to determine the part of the visible spectrum, with the minimum associated heat, during astronomical observations.

Table 6.4. Review of some RF heating applications for the processing of foods

Process	Food	Frequency (MHz)	Source
Heating	Bread	14–17	Cathcart et al. 1947
Blanching	Vegetables	15	Moyer and Stotz 1947
Thawing	Frozen eggs, fruits, vegetables, fish	14–17	Cathcart et al. 1947
Thawing	Herring and white fish	36–40	Jason and Sanders 1962
Thawing	Meat, sausages, pies, and bacon	36–40	Sanders 1966
Cooking	Turkey breast rolls	27.1	Tang et al. 2005
Drying (roasting)	Cocoa beans	60	Cresko and Anantheswaran 1998
Sterilization	Boned ham	9	Pircon et al. 1953
Pasteurization	Cured ham	35–60	Bengtsson and Green 1970
Pasteurization (continuous)	Sausage emulsion	27	Houben et al. 1991, 1994
Enzyme inactivation	Mustard seed	13.5	Cserhalmi et al. 2001
Heating (continuous)	Carrots, potatoes	40.68	Zhong et al. 2004
Pasteurization	Ham	50–60 RF	Orsat et al. 1999

The use of this technology in the food industry was first reported in the 1950s for drying processes and, like many other processes, it was transferred from other industries (table 6.5). During the early 1970s there were many discussions about finding new methods for industrial frying/cooking of meat products (Skjöldebrand 1986), and the use of near infrared (NIR) techniques started to be discussed. During the 1970s and 1980s most of the research work on food was carried out at SIK, the Swedish Institute for Food and Biotechnology, and a new set of knowledge was gained.

The Basics

IR heating is the transfer of thermal energy in the form of electromagnetic waves. Within the IR spectra three different regions can be distinguished, depending on the wavelength: short waves (0.7–2.0 μm) that appear when temperatures are above 1,000°C; medium waves (2.0–4.0 μm) when temperatures range from 400 to 1,000°C; and long waves (4.0 μm–1 mm), when temperatures are below 400°C. The medium- to long-range wavelengths appeared to be the most advantageous to industrial applications since almost all materials to be heated or dried provide maximum absorption in the 3–10 mm region. However, new applications using short waves have been arising.

IR heating presents several advantages over conventional heating methods, some of them similar to the other types of electromagnetic heating, namely:

- *Instant heat:* There is no need for heat buildup because electric IR systems produce heat instantly.
- *Reduced operating costs:* Depending on the insulation, type of construction, and other factors, the energy savings can reach 50%. Moreover, maintenance operations are restricted to reflector cleaning and changing of the heat source.

Table 6.5. Examples of infrared industrial applications

Industry	Process
Agricultural	Incubating, warming
Bottling	Drying
Car washing	Drying
Environmental chambers	Heating
Food	Cooking
	Food warming
	Dehydrating
	Broiling
	Toasting
	Melting
Glass	Curing the varnish or paint on back—mirrors
	Tempering layers
	Drying
Medical	Incubating
	Warming
Metal treatment	Preheating—aluminum; steel
Paint	Primer, top coat alkyd, epoxy, acrylic—steel panels
	Baking lacquer finishes—steel boxes
	Drying—bicycles; car bodies; aluminum panels
Pharmaceutical	Drying water from powder—tablets
Paper	Laminating
	Calendaring—rolls
	Adhesive—labels
	Drying water from—towels
Plastics	Laminating
	Annealing
	Forming
	Embossing
Textiles	Moisture removal—carpets
	Latex & PVC backing
	Moisture removal from dyes

- *Clean and safe:* There is no production of by-products and operating the IR equipment is a low-risk task.
- *Zone control:* The IR energy does not propagate; it is absorbed only at the area it is directed into. This allows differentiating heating in nearby zones. Moreover, IR is not absorbed by the air so the surrounding air does not heat up.

Heat Generation

IR radiation is electromagnetic radiation that is generated in a hot source (quartz lamp, quartz tube, or metal rod) by vibration and rotation of molecules. Heat is generated by the absorption of the radiating energy.

An object is called a "blackbody" if it absorbs (or emits) 100% of incident IR radiation. The amount of heat emitted from a perfect radiator (blackbody) is calculated using the Stefan-Boltzmann equation:

$$Q = \sigma_{SB} \cdot A \cdot T^4 \tag{6.16}$$

where $\dot{Q}$ is the rate of heat emission, σ_{SB} is the Stefan-Boltzmann constant, A is the surface area, and T is the absolute temperature.

Considering that radiant heaters are not perfect absorbers and food products are not perfect absorbers, the Stefan-Boltzmann equation was modified and the concept of "greybody" was introduced:

$$Q = \sigma_{SB} \cdot \varepsilon \cdot A \cdot T^4 \tag{6.17}$$

where ϵ is the emissivity of the greybody (varying from 0 to 1). This property varies with the temperature of the greybody and also with the wavelength of the radiation emitted.

The degree of heating depends on the absorbed energy, which, in turn, depends on the food composition as well as on the frequency of the radiation. The factors affecting heating were explained in detail in the microwave section and are similar for IR heating. The heat transfer rate to a food can be mathematically expressed as:

$$Q = \sigma_{SB} \cdot \varepsilon \cdot A \cdot \left(T_1^4 - T_2^4\right) \tag{6.18}$$

where T_1 is the temperature of the emitter and T_2 the temperature of the absorber.

For optimization procedures, it is important to consider the penetration depth (as previously defined) of IR radiation. For short waves the penetration depth is ten times higher than for long waves.

There are some critical factors in IR heating that must be considered. These are (Ginzburg 1969; Hallström et al. 1988) radiator temperature, radiator efficiency, IR reflection/absorption properties, and IR penetration properties. Being so, the rate of heat transfer depends on

- the surface temperature of the heating and receiving materials;
- the surface properties of the two materials;
- the shape of the emitting and receiving bodies.

Equipment

Some of the types of industrial electric IR heaters are ceramic elements, quartz tubes and lamps, quartz emitters, flat-faced quartz, glass and metal panel heaters, metal-sheathed tubular (calrods), and open coil wire elements. When comparing all the different types of heaters on efficiency, life expectancy, zoning ability, and other factors, ceramic elements and quartz tubes are the preferred heaters. Metal-sheathed tubulars have a low initial cost but rate low in all areas except durability. Ceramic elements and quartz tubes are approximately 96% and 86% efficient in converting electricity into IR heat, respectively.

IR equipment may be either of batch or continuous type. Custom batch IR ovens vary in design as well as size. Different designs within the batch category include top-load, bench, cabinet, tip-up, and bell, as well as the standard front-load. For conveyor type (continuous) the oven can be divided into distinct zones with different effects of IR radiators or other combined heating technology. Hebbar and Ramesh (2002) developed a combined IR–hot air dryer for the drying of vegetables. The dryer has a capacity of 16 $kg.h^{-1}$ of raw vegetables with an IR power input of 17 kW, using a quartz medium-wave heat source.

The simplest way to control IR heating processes is to use pure on/off systems where the number of radiators/tubes operating at any one time is controlled by switches and also allows changing the speed of the conveyor.

IR heaters of all types, styles, and sizes can be added to these various oven configurations, based on the products being processed. They can be arranged to heat from the top, the bottom, the sides, or any combination thereof, depending on the application.

Applications

The applications of this technique in the food processing industry are relatively new and information is rather scarce. Several applications are still of experimental nature. Present interest in industrial heating applications centers on short-wave IR (wavelengths around 1 μm) and intermediate IR (wavelengths around 10 μm), since these wavelengths make it possible to start up and reach working temperatures in seconds, while also offering rapid transfer of high amounts of energy and excellent process control.

Skjöldebrand et al. (1994) stated that short-wave IR is a promising technology for baking, once the baking time is reduced 25%, and the final product had a comparable quality to traditional baking ovens. The combination of IR and microwave can reduce by 75% the baking time and simultaneously increase baked bread volume and decrease weight loss (Sumnu et al. 2004).

IR heating has been used for heating and cooking of soybeans, cereal grains, cocoa beans, nuts, and some ready-to-eat products; braising meat; and frying and drying apple slices (Nowak and Lewicki 2004; Ratti and Mujumdar 1995). Drying of seaweed, vegetables, fish flakes, and pasta is also done in tunnel IR dryers.

Studies on color development during IR roasting of hazelnuts were reported by Ozdemir and Devres (2000). The application of combined electromagnetic radiation and hot air was considered to be more efficient than oven radiation or hot air heating alone due to a synergistic effect. Datta and Ni (2002) also reported the application of combined IR, microwave, and hot air to control surface moisture.

The use of IR surface pasteurization to inactivate *L. monocytogenes* in turkey frankfurters was reported by Huang (2004). The results suggested that this technique could be used for surface pasteurization immediately prior to packaging, reducing the risks of foodborne listeriosis, frequently caused by this product.

The main advantage of IR heating claimed in the existing studies is the drastic reduction of process time (up to 70% reduction), which, in turn, increases productivity while maintaining product quality.

Conclusions and Future Perspectives

The use of IR technology in the food industry is still in the first stages of development and future trends will certainly be focused on

- process control and equipment development;
- understanding the interactions between heating process and product properties (organoleptic and nutritional);
- expanding the areas of application of IR heating.

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7 Radio-Frequency Heating: Commercial Developments

Gaurav Tewari

Introduction

Radio-frequency (RF) heating systems have the capability of providing heat uniformly throughout the target product. A technical description of RF heating is given in chapter 5.

This chapter deals with innovative commercial applications of RF heating. At present, the technology has been greatly applied to the lumber industry and some commercial developments are happening for the baking industry. Any additional or new applications in the food industry will be in the value-added domain (driven by the high cost of the technology) or in volume applications, where potential savings in energy and/or capital footprint offset capital costs.

Therefore, the objectives of the work were (1) to identify suitable target industries where RF processing may have applications, and (2) to conduct a selected applications review of RF heating with respect to different unit operations in the food industry.

Heating—Pasteurization and Sterilization

Scientific Review

Application of RF for pasteurization was done as early as the 1950s. Pircon et al. (1953) described a process for diathermal (energy generated by combination of the ionic and molecular mechanism) sterilization of boned ham at 9 MHz. They used an industrial model of 15 kW oscillator with 9 kV across the plates. They achieved energy conversion efficiency of 56.6% and reported that ~2.7 kg pieces of ham could be sterilized (80°C) in 10 minutes. The process had not been used commercially, probably because of high capital costs and problems with designing processing cells or cans suitable for commercial scale operation in the 1950s.

In the late 1960s, Bengstsson and Green (1970) developed continuous RF pasteurization of cured hams packaged in Cryovac casings at 60 MHz. They obtained power efficiency of about 25% at 60 MHz. With the knowledge of dielectric properties of the material, power efficiency could have been improved by proper design of the generator. The results showed that for lean 0.91 kg hams, process time to reach an internal temperature of 80°C could be reduced to one-third by heating in a condenser tunnel. Results also showed substantial reduction of juice losses and improved sensory quality compared with hot water processing.

Proctor Strayfield at Horsham, Pennsylvania, developed a "Magnatube" pasteurization system (Koral 1990) that demonstrated success in the cooking + sterilization of scrambled eggs as well as in the creation of a "skinless" meat loaf from a pumped slurry using a vertical 100-mm diameter tube system. An extremely uniform temperature distribution across the heated product was achieved, with promising yield. Prospective applications of this

technology include baby food, cooked rice, applesauce, tomato sauce, fruit purees, and particulated meat products (Morris 1993).

Houben et al. (1991) chose RF heating from different available methods as the most promising technique for a continuous and flexible pasteurization of sausage emulsions. RF heating at 27 MHz was selected based on the dielectric properties of sausage emulsions and the required penetration depths. Tests were conducted in a specially designed continuous heating line. Overall energy efficiency was around 30%. Sausage products heated in the continuous line had a good appearance, a smooth surface, and did not show moisture or fat release.

Large quantities of liquid egg products are pasteurized each year to produce products sold in refrigerated form with limited shelf life. Samimi et al. (1997) developed a method for pasteurization of flowable egg products using radio waves, thereby producing liquid egg products that have extended shelf lives under refrigerated conditions. Most pasteurization processes using indirect heat exchangers are designed to maximize turbulent, high shear flow in order to achieve efficient heat transfer throughout the egg product. In RF heating, particulate matter heats at the same rate as liquids, allowing the pasteurization apparatus to be designed with less concern about the flow characteristics of the egg product. In addition, shear stress on the proteins can be reduced, and the need to make highly homogeneous liquids from eggs can be eliminated. Therefore, low shear pumps may be used with this method (Samimi et al. 1997) so that the identity of the yolks and egg white is not lost. The shelf life of pasteurized liquid eggs using the RF system (in the frequency range of about 15 Mhz–150 MHz) can be extended from 4 weeks to 36 weeks (Samimi et al. 1997).

Orsat et al. (1999) determined the efficacy of RF heating to pasteurize ham by subjecting the ham samples to RF heating at 27.12 MHz to bring internal temperatures to 75 and 85°C. The ham samples were vacuum packaged and stored at 4°C for 1–28 days. The study indicated that RF heating can improve the storage ability of repacked hams by reducing bacterial load, reducing moisture loss during storage, and maintaining an overall product quality and acceptance.

Controversy surrounds the use of RF technology in treating biological membranes and microorganisms: Ponne et al. (1996) determined the suitability of RF heating for microbial inactivation. Influences of applied frequency and membrane composition were observed, and RF energy appeared to result in membrane damage that could not be attributed to thermal effects. Exposure of liposomes to RF fields with frequencies of 27 or 100 MHz resulted in lysis of vesicles. Attempts to inhibit microbial cell growth by RF treatment produced variable results—growth of *Saccharomyces cerevisiae* decreased in the presence of RF fields (27 and 100 MHz) but no inhibitory effects were demonstrated with *Erwinia carotovora* at sublethal temperature. Moreover, no additional effects (other than heat effects) were demonstrated when RF energy was applied to microbial cells. The authors concluded that RF heating has no particular advantages for use in microbial inactivation at mild temperatures.

Non-thermal Treatment of Liquid Food Products Using RF

Kozempel et al. (1999) patented a process that reduced food spoilage and extended shelf life without causing thermal damage. The process involves rapid application of electromagnetic energy (EME) such as RF and the simultaneous removal of any heat generated using a circulating cooling medium and an efficient heat exchanger.

Special Nonfood Application

For decomposition of biological materials, Knapp (1989) reviewed reduction of system errors in decomposition of samples of biological materials for trace element analysis with special reference to error due to contamination reagents, contamination by the vessel material or adsorbed impurities, losses of elements by adsorption on the vessel surface, and losses by volatilization. The author described several state-of-the-art decomposition procedures: wet chemical digestion in closed Teflon vessels at medium pressure (8 bar) with microwave heating; wet chemical digestion in closed Teflon vessels at high pressure (85 bar) with microwave heating; wet chemical digestion in closed quartz or glassy carbon vessels at 120 bar with conventional heating; low-temperature ashing with radio-frequency-induced oxygen plasma; and combustion in a dynamic system with complete recovery of all volatile elements.

Heating—Blanching

Frozen Fruit and Vegetable

It has been shown that that RF-blanched fruit and vegetables had superior sensory and nutrient content compared to conventional blanching (see "Scientific Review" section below). Conventional blanching techniques result in nutritional changes attributable to leaching and time of thermal exposure; blanching of vegetables using RF energy should provide the same thermal effect with less time, and without leaching losses to steam or water.

Canned Tomato Puree

Companies manufacturing tomato puree need pectic enzymes in tomato to be inactivated, hence blanching is a major unit operation for these industries. Conventional heating techniques have problems associated with compromised product quality due to overheating, and these companies are looking for ways to minimize the thermal dose.

Hernandez and Cano (1998) and Lopez et al. (1998) used ultra high pressure (50–500 MPa) and mild temperature (20–60°C) and manothermosonication (combined treatment of heat and ultrasound), respectively, on inactivation of pectic enzymes in tomato puree.

Scientific Review

Moyer and Stotz (1947) treated washed, prepared vegetables with RF. After the desired temperature was reached, the carton (with a cellophane liner inside) containing vegetables was placed in a −23°C cold room. After a few days of storage, physiochemical analysis was performed on blanched frozen vegetables. Results for RF-heated peas indicated that catalase was inactivated when the product reached a temperature of ~77°C; samples blanched to ~88°C using RF possessed the highest ascorbic acid content when compared with those blanched with water and steam; and carotene analysis showed little difference between samples blanched using different methods. Sensory analysis showed that blanching using RF power caused poor flavor and color compared with water and steam blanching. This may be due to inadequate cooling of the packaged product after RF blanching.

Ponne et al. (1994) studied the application of RF, microwave, microwave-steam, and IR

energy for blanching leaf vegetables (endive and spinach) and compared them with conventional hot water and steam blanching. The quality of the vegetables, both frozen and sterilized, was evaluated by instrumental and sensory analysis. Effects of blanching methods were most pronounced in frozen products. No quality differences occurred for IR and RF treatments. However, microwave energy alone or in combination with steam in the blanching process improved vitamin C retention and gave high Instron force values and better sensory properties.

Heating—Thawing and Tempering

Scientific Review

Thawing frozen foods using RF energy has been tried since the 1940s, when Cathcart et al. (1947) attempted to thaw small samples of frozen eggs, fruits, vegetables, and fish using RF heating. Results showed that problems common to frozen food thawing (discoloration and loss of flavor) were not witnessed by employing RF. In another study, Jason and Sanders (1962a, 1962b) applied RF heating to thaw herring and white fish on an industrial scale. Application of RF heat resulted in much faster thawing—12.5 minutes compared with 16 hours (air) and 3 hours (water), with much less drip loss and better odor and flavor.

Bengtsson (1963) performed lab tests to thaw frozen meat using RF heat and reported that heat treatment lasted 34 minutes, during which time 0.27 MJ/kg were absorbed at an estimated average power density of 0.125 kW/kg. Von Heeren (1964a, 1964b) thawed beef blocks weighing 30–60 kg in a 25 kW batch thawer in 1.5 hours at a power density of 0.05 kW/kg and an energy density of 0.27 MJ/kg. Both studies showed it was possible to thaw unwrapped meat or wrapped meat in plastic packaging with drip loss of < 1%.

Sun Valley Poultry (Hereford, UK) developed a system that involved conveying blocks of frozen chicken meat in plastic liners, through a defroster, while 4 RF generators produce a field of energy through which each block must pass (Anonymous 1992). An automatic control system allows energy input to be precisely programmed in four stages, and the system can be preset for different sizes of frozen meat block. Using such RF energy, defrosting times have been cut from 3 to 4 days to < 2 hours. In addition, product quality is improved and meat defrost losses are reduced by 92%.

Heating—Cooking (Baking)

Packaged Bread, Crackers, and Pizza Dough

A heating step is desired so as to render sterile bread with respect to mold growth. A study by Cathcart et al. (1947) showed a potential application of RF heating for bread wrapped in a moisture-vapor proof wrapper, with no mold development after 10 days of storage. The researchers found dielectric breakdown (arcing) between the upper electrode and the edges of the cylindrical load, which resulted in some burning at those points. During their work, this difficulty was overcome by rounding the edges of the loaves so that there were no corners adjacent to the electrodes. They also found that wrapping including waxed and glassy paper were undesirable during RF heating of packaged bread as the former softened when bread showed an internal temperature of 38°C and the latter became tacky between 52 and 60°C. Cellophane was found to be the appropriate packaging material for RF heating of

packaged bread. However, no commercial application of RF energy for heating bread has been reported so far.

It is quite possible that with the latest developments in RF heating technology, other packaging materials can also be employed during RF heating of packaged bread and may prove economical

Baking has been an important sector of the food industry. Canada has a $3.2 billion baking industry offering several baked products (Baking Association of Canada). Energy savings and large throughput have always been major requirements for the baking industry, hence bakers are always interested in oven configuration or related systems that may help them achieve such large throughput. In addition, low-fat baked products (crackers and snacks) are attracting customers. And there is need to dry these products carefully after they are cooked because the stiffness of their dough makes them especially fragile. To drive out the last 1–2% of moisture, it becomes sometimes necessary to overheat the crust, which can lead to surface checking, breaking, and crumbling (Demetrakakes 1994). Due to this dilemma and the need to achieve uniform moisture throughout the product, interest has increased for new technology such as RF that can drive moisture out through the crust, equalizing moisture throughout the product.

RF processing is a promising new technology for bakers and snack food manufacturers (Mermelstein 1998). Tom's Foods, one of the top ten snack food companies in the United States, installed an RF oven (manufactured by Proctor Strayfield) at its Columbus, Georgia, plant to remove moisture from postbaking crackers and cookies (Rice 1993). The oven operated at a frequency of 27 MHz. Using the RF oven efficiently and uniformly removed excess moisture from the crackers with repeatability in mass production and had moisture-level accuracy of ±0.2%. In addition, using RF energy to dry the baked product avoided discoloration and flavor damage due to thermal buildup. A 17-foot RF oven has similar capacity to a 70-foot conventional oven, acting more efficiently to remove excess moisture from crackers. Crackers emerging with a moisture content of 3.5–4.5% from a 180-foot conventional oven enter the RF oven at approximately 212°F. Use of the RF oven in combination with packaging line upgrades has increased factory productivity by 30%, 20% of which is accounted for by the RF oven.

Traditionally, a hot-air oven has been used to control heat and humidity for raising and baking the dough and cooking the toppings during pizza making. The time required to cook pizza is generally limited by the time required for leavened dough to rise (proofing). Shorter baking times or higher baking temperatures affect the proofing of pizza such that the dough may not rise at all or may blister and burn. The pizza toppings do not have such critical limitations and can be baked with fewer restrictions of time and temperature. In a commercial scenario, the shells are proofed in a heated, humidity-controlled chamber requiring approximately 40 minutes at a temperature of 40°C and an 80% relative humidity. Padilla (1992) invented an improved method for reducing the amount of time required to proof dough for pizza shells by constructing an RF chamber that has output power density in the range of 7.5–13.0 KW per square m at a frequency of 27.12 MHz. The inventor claimed to reduce the proof time to 20–30 minutes, thereby reducing the proof time by 25%–50%.

Grando (1993) described a kneading process for dough where the rising phase is divided into two parts. The first part is a treatment phase using traditional means but is shorter than usual. The second part is a heating phase using RF energy until the end of rising and partial cooking. This invention consists of an alimentary dough-kneading process

for baked goods, which in the rising and precooking phase uses RF heating. It is well known that various types of oven-cooked alimentary dough (bread, sweet dough, some types of biscuits) are prepared, kneaded, and leavened before cooking. The rising phase takes place in a rising chamber in which alimentary dough is maintained at controlled temperature and humidity for a given period of time (7–8 hours). Increasing the throughput during commercial application requires processes that allow reductions in rising and cooking time. The inventor demonstrated several advantages, such as uniform and compact heating, in the use of RF heating both in the rising and cooking phases. It was also noticed that the absorption of RF energy only takes place when the alimentary dough is inside rising chambers or treatment ovens; otherwise RF only dissipates energy in the generator supply, which absorbs negligible quantities of power in comparison with those used. Another advantage of this invention is that the total time from the first rising phase to the second one is by far shorter than the time necessary for the traditional rising method. Furthermore, another advantage of this invention is that the rising of goods subject to RF produces a quick increase in temperature in comparison with the normal temperature of completed rising. This allows goods to remain in the cooking oven for a shorter time, since full-cooking temperature is reached more quickly.

Extrusion Baking of Cookies Containing Heat and Shear-Sensitive Additives

Van Lengerich and Warren (1991) developed a method for extrusion baking of cookies containing heat and shear-sensitive additives. A dry flour blend, including flour, dry milk solids, and salt is added to a blending zone of a cooker-extruder with a fat or shortening. The mixture is blended and treated in a heating zone of the extruder under high temperatures without fat separation to form a heat-treated mass. The heat-treated mass is cooled in a cooling zone, followed by the addition of a sugar, heat-sensitive, and/or shear-sensitive ingredient to form a cookie composition. Water is also introduced to the cooling zone in an amount effective to produce a dough-like consistency. The heat-treated cookie composition is extruded and shaped into an unleavened and unexpanded cookie dough piece. The cookie pieces are then leavened by heating in a convection, microwave, or RF oven to produce a leavened cookie having a crumb-like structure. The heating of pieces in the RF ovens is generally performed so that proper leavening and browning is done.

Cooking Tortillas Using RF

Luz-Martinez et al. (1997) developed a method for cooking tortillas using low RF waves (RF waves of very low frequencies were generated at a power level of 500 W in order to provide an energy level of from 70 to 200 J per gram of dough pieces to be cooked). Cooking time was reduced by the application of very low RF waves simultaneously on both sides of the tortilla. This type of cooking also formed the capping layers that retained a high degree of moisture within the body of the product.

Heating—Cooking (Snack Foods)

Scientific Review

Weetabix breakfast cereals in Kettering, UK, has installed an RF heater to manufacture their cereals (Keck 1994). The RF heater used a high frequency (27.12 MHz) that results

in rapid heating or drying of food. After moulding, Weetabix are passed in rows of twelve onto the feed belt of the initial bake oven; two 50 KW RF ovens are used in sequence to remove residual moisture from the center of the central biscuit. Use of RF heating has enabled the company to increase output to fourteen million Weetabix/day.

Leavened Frozen Pastry Articles

Introini et al. (1989) developed a method of making leavened frozen pastry articles. Prior to raising and freezing, pastry articles are subjected to RF treatment in the frequency range of 10^7–10^8 Hz for 1–3 minutes. Following quick freezing, the leavened raw frozen articles (brioches and the like) can be long-term preserved within a freezer, whence they can be passed directly to the baking oven without requiring any preliminary swelling and/or additional raising step. This will obviously increase throughput and will result in energy savings.

Fat-Free Potato Chips

Petelle et al. (1995) developed a continuous process for making fat-free potato chips whereby chipping potatoes are sliced and continuously monolayered onto a conveyor, where excessive moisture and starch are removed. The potato slices are cooked in a three-zone convection oven, first by radiant heat and then by two stages of forced air convection, reducing the moisture content to 15%. Slices are then cooked in a dielectric heater in two tandem drying zones at long wavelength (65.8 feet) RF (15 mHz) from two 20 kW transmitters. The moisture content of finished slices was 7%.

Heating—Drying

Scientific Review

Drying of cereal grains is extremely important to prevent any spoilage before they are utilized. In addition, drying also requires preservation of viability of grain, that is, capability of a grain kernel to germinate. Hence, moisture content, grain temperature, and time for heat exposure, important parameters in kernel drying, play an important role in kernel germination. Due to the impact of the time-temperature on the biological quality, the temperature limitations are difficult to specify. However, a maximum temperature of 44°C is recommended to ensure the viability of seed grains. The common method of drying utilizes convective drying of cereal grains and beans that takes place in a falling rate period that is controlled by the diffusion from the interior to the material surface. However, in convective drying the surface is heated first and then heat gets diffused to the interior. This may cause problems such as cracking and case hardening. Such problems are more severe for high-protein seeds such as broad beans, lentil, or lupine as drying time for them is 4–10 times longer than for cereal grains and drying has to take place at maximum allowable temperature. This presents a scenario where dielectric-assisted convective drying can be useful. Ptasznik et al. (1990) developed a mathematical model for the RF-assisted drying of broad bean seeds with through-flow of cold air stream in order to simulate the drying and temperature curves and to optimize the operating conditions. The researchers concluded that controlling internal heat generation affected the

seed temperature and drying kinetics and posed a major problem in RF drying of broad bean seeds.

Murphy et al. (1992) investigated the drying of alfalfa by using a combination of RF power at 27 MHz and heated forced air. The application of heated forced air alone was found to dry the leaves more rapidly than the stems. Supplementing heated forced air with RF power throughout the drying process increased the overall drying rate but did not increase the evenness of drying. Similarly, applying RF power to partially dried alfalfa was not effective in leveling the moisture content of the stems and leaves.

Caldas (1992) performed a study for drying granular products (rice, maize, wheat, soy, coffee) using an RF dryer. The author found that rice is the fastest-drying product, followed by maize and wheat. Soy and coffee dry slowly, as these products contain a substantial amount of oil and tars that hinder the diffusion of moisture. Also, they are less responsive to the electric field strength. The author concluded that although introduction of RF methods increases the speed of drying operations, increasing the throughput of the plant, the question remains as to whether the throughput increase will offset the large capital and running costs associated with an RF plant. Nevertheless, RF has excellent potential for drying granulated food products.

American ginseng is a shade-requiring, herbaceous plant native to the hardwood forest of eastern North America. It is an important medicinal plant that is widely grown in Canada. The climate and soils of south-central Ontario are suited to the production of ginseng. About 85% of the root grown in North America is sold to the Asian markets. Drying is one of the most crucial steps in American ginseng production. Several techniques such as the warm/hot-air method, microwave–hot-air-drying method, belt dryers, and conveyor band dryers have been proposed or applied for drying ginseng. Most of the methods (with microwave–hot-air drying, which is not widely available, as an exception) require long drying time; many components of American ginseng are highly heat sensitive, and high drying temperature may result in browning of the processed products. Radio-frequency drying has been proposed as an alternate method for drying American ginseng (AgTIS 2000). It is possible that RF drying may result in superior retention of nutrients, flavors, and medical components, along with food safety.

Drying of Fungal Material and Resultant Textured Product

Huang and Yates (1980) developed a method of combined RF drying and hot-air finish drying of textured mass of fungal mycelial. Fibrous fungal material (*Fusarium graminearum* Schwabe) is a rich source of nutritional protein, and it contains excessive ribonucleic acid (RNA) with initial moisture content of 60–85% (wet basis). This needs to be reduced to less than 10% so it may be transported and stored without refrigeration. In addition, the dried product upon rehydration should have a meat-like resilience with adequate mechanical integrity to withstand normal cooking. In 1973, the du Pont Company entered into a joint venture with Rank-Hovis McDougal (RHM) Ltd. of England to continue its development into a new protein food for human consumption. Hot-air drying alone was found inadequate as the biomass case hardened and shrank to a dense structure that would not rehydrate to a meat-like resilience. However, RF prepuffing followed by hot-air finish drying was found to satisfy the drying requirements. It was concluded that a combination of RF prepuffing and hot-air finishing is a viable method of drying textured mycoprotein.

Heating—Cooking

Scientific Review

Industrial Apparatus to Heat Meat Products

Iacovacci and Cavestro (1998) developed industrial equipment using RF and reported that such RF process resulted in uniform heating throughout the entire mass of the food product. The apparatus has a generator (with a voltage that oscillates at a predetermined RF) that is connected to a tunnel-shaped application device. If the food products have a substantial dielectric behavior, capacitive application devices are normally used that generate an oscillating electric field involving the food product. The dielectric loss within the product causes the heating. On the contrary, if the food product substantially has a conductive behavior, inductive application devices are normally used that generate an oscillating magnetic field involving the food product. The electric currents so induced to the conductive product cause the heating. Meat products (hams made of meat, fats, and salts) behave in a way that is neither fully dielectric nor fully conductive. Therefore, the inventors devised an industrial apparatus to heat meat products; this apparatus included a generator of voltage oscillating at a predetermined RF and an application device to generate an electromagnetic field oscillating at the predetermined frequency. This combination results in uniform heating of the entire mass of meat product.

Miscellaneous Thermal Treatment Opportunities

Agriculture: Seed Treatment

Even studies conducted 80 years ago report RF treatment of seeds to improve germination. Nelson (1987) reported that some small-seeded legumes such as alfalfa, red clover, and arrowleaf clover, which often have naturally impermeable seed coats, responded consistently to dielectric heating treatments with marked increases in germination. Benefits from RF treatment have been retained in alfalfa seed for up to 21 years in storage after treatment with no evidence of any detrimental effects. Several vegetable and ornamental species did not show any improvement as a result of RF treatment, but some, such as okra, garden peas, and garden beans, responded favorably. Germination of spinach was consistently accelerated. Energy and equipment costs for capacity to treat seeds apparently precluded practical application.

Agriculture: Insect Control during Grain Storage

There has been interest in the possibility for controlling insects using RF energy for at least 70 years. Although the economies of applying such energy for insect control purposes are discouraging, interest remains for nonchemical controls due to environmental and health hazards associated with insecticide applications that are still essential for practical food production and protection of many agricultural products (Nelson et al. 1998). Nelson (1996) concluded that only some new discovery of a lethal mechanism (non-thermal effect) for insects that would permit the control of stored-grain insects with very much lower RF or microwave energy requirements would appear to make consideration of RF or microwave treatments possible. Much lower power requirements than have so far been demonstrated effective would be needed to lower energy costs and increase the throughput

capacity of the equipment. The selective heating of insects demonstrated so far in the range of 1 MHz to 12 GHz frequency is not of sufficient significance to warrant serious consideration for practical use even with continuing concern about potentially hazardous chemical controls.

RF-Assisted Nut Heating

Rowntree Mackintosh has been one of the first manufacturers to pioneer the application of RF within the food industry. The company was faced with a problem in the manufacture of its hazelnut and orange nougat called Turron, a Quality Street assortment. The production involved ingredients being mixed and heated until they reach a molten state. Then, the liquid flows into a carpet along a conveyor belt. This is then cut into inch-wide tracks and then horizontally into sweets and cooled ready for coatings and wrapping. At either side of the carpet there is some material that is not wide enough to form sweets and has to be recycled back into the process with the rest of the ingredients in a molten state. This material becomes hard and has to be brought back to molten state by using a traditional steaming oven, which takes 36 hours to complete. In addition, the quality of the material is far from satisfactory. Due to these issues, the company installed an RF processor that reduced the reheating time from 36 hours to 7 minutes and increased efficiency from 65 to 95%. Such efficiency has allowed the company to smoothly change over from one product to another (Eley 1989).

Mechanical and Microstructural Characteristics of Meat Dough

Van-Roon et al. (1994) pasteurized meat dough using RF and reported that RF-heated products were firmer and they fractured at higher stress values. In addition, for the microstructure of RF-heated products versus other samples heated in a water bath, the RF-heated products displayed a more open structure of the protein matrix with larger, irregularly shaped fat particles that were surrounded by relatively thin and compact protein bridges. The RF-heated products always displayed a thin layer of moisture on their surface and occasionally a little fat separation.

Conclusions

Table 7.1 outlines the different commercial applications of RF heating based on different unit operations.

Based on the applications review and market opportunity for RF heating, needs of the following industries need to be addressed:

1. Frozen foods and related companies (blanching and thawing)
2. Baked foods companies (drying)
3. Dry-food companies (alfalfa, soybeans, American ginseng)
4. Agriculture-based companies (stored-grain insect control and product processing)
5. Egg companies (pasteurization along with functionality improvement)

Table 7.1 Summary of applications of RF heating to food products (modified from Zhao et al. 2000)

Applications	Frequency (MHz)	Processing Temp. (°C)	Processing Time	Advantages	Food Items	References
Heating	14–17	~52–66	20–59 s	Kill mold, refresh stale bread	Bread	Cathcart et al. 1947
Blanching of vegetables	150	100	3 min	Minimum nutritional loss	Fruits, vegetables	Moyer and Stotz 1947
Thawing of frozen foods	14–17 36–40	> 1.67	2–15 min 34 min	Maintain color and flavor of thawed foods, faster thawing, less drip loss	Eggs, fruits, vegetables, fish, beef blocks, sausage, pies, bacon	Cathcart et al. 1947; Jason and Sanders 1962 a, 1962b; Sanders 1966
Baking and postbaking	27.12	~100	—	Rapid drying, improved color and flavor	Crackers, cookies, bread	Anonymous 1987, 1989; Mermelstein 1998; Rice 1993
Pasteurization of foods	9 60	~80	~10 min	Improved sensory quality, reduction of juice losses	Cured ham, sausage emulsions, meat loaf	Bengtsson and Green 1970; Houben et al. 1988, 1990, 1991; Pircon 1954

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8 *Sous Vide* and Cook-Chill Processing of Foods: Concept Development and Microbiological Safety

Vijay K. Juneja and Oscar P. Snyder

Introduction

Food safety and security are significant issues in the food industry today. Even though the United States has one of the safest food supplies in the world, there are still millions of cases of foodborne illnesses each year. The Centers for Disease Control and Prevention calculates that each year in the United States there are approximately 76 million cases of foodborne illness, with 325,000 hospitalizations and 5,000 deaths (Mead et al. 1999). Annual economic losses attributed to foodborne diseases associated with medical costs, productivity losses, and business losses due to legal problems may be as high as 5 to 6 billion dollars (CAST 1996). These food safety concerns are magnified because of consumer demands for refrigerated convenient meals, processed using only a brief/mild heat treatment. This demand has led to a growth in the application of *sous vide* and cook-chill processing technologies to extend the shelf life and to keep/enhance the quality of fresh foods (Schellekens and Martens 1992).

Demand for Minimally Processed Food

Sous vide and cook-chill pasteurized, refrigerated ready-to-eat foods were introduced in about 1970 as a more convenient food option than frozen food for the food market, deli, foodservice kitchen, and home food preparer. The refrigeration equipment industry was maturing and could be relied upon to keep food at an average temperature of 40–45°F from the manufacturer to the point of consumption. The major advantage of chilled versus frozen ready-to-eat food was that, if reheating was necessary, reheating food from a thawed condition produced a more uniformly reheated product and could be done much faster than reheating food from the frozen state. While frozen food had a 6- to 18-month shelf life, refrigerated food had only a 30 to 45-day shelf life. Today, due to improved processing technologies, these pasteurized, chilled ready-to-eat foods can have a shelf life of up to 90 to 120 days in the distribution and food market system. The actual shelf life depends on the levels and control of spoilage organisms. For example, milk, which is pasteurized at 161°F for 15 seconds, has a 14-day shelf life at 40°F, but canned crab, which is pasteurized at 185°F for 10 minutes, has a 12-month shelf life at 40°F.

Traditionally, pasteurization is thermal, but it could also be achieved using non-thermal intervention, such as high pressure, pulsed electric field, ionizing radiation, and other technologies. The value of these processes, in addition to convenience, is extended refrigerated shelf life, because the spoilage organisms have been reduced to very low numbers, and pathogens are controlled. Whereas raw food maintains good quality for only a few days, these pasteurized foods are acceptable for up to 90 days, or longer if preservatives such as

Table 8.1 *Sous vide* and cook-chill example products

Sous vide (Package-Cook)	Cook-Chill (Cook-Package)
Beef steak, chops, tenderloin, stew	Meat, poultry, fish stews
Lamb chops, shank, rack	Sauces, gravies, soups
Veal chops	Mashed potatoes, yams, refried beans
Chicken breast, squab	Pasta and sauce
Turkey breast, beef roast, ham	Salads made with precooked, cooled ingredients
Meat loaf, meatballs	
Fish fillets	
Lobster, crab, clams, scallops	
Potatoes, rice, pasta	
Apples, pears, peaches, cherries	

acid and salt are used. The time at a given pasteurization temperature depends on the tenderization needed. Table 8.1 lists examples of *sous vide* and cook-chill products (Roca and Brugues 2005).

Figures 8.1 and 8.2 show the two basic processes (NACMCF 1990). *Sous vide* is an Assemble-Package-Pasteurize-Cool-Store process. Cook-chill is an Assemble-Pasteurize-Package-Cool or Pasteurize-Cool-Package-Store process.

The *Sous Vide* Process

The *sous vide* (i.e., "under vacuum") process of cooking in a pouch or other suitable container was conceived by chefs who wanted to prepare food with a minimal cook at a low temperature in order to increase juiciness and maximize weight yield.

The great advantage of the *sous vide* process is that it allows a delicate food to be cooked more gently at a precise ±1°F, lower temperature, and pasteurized by holding for a sufficient time, as in contrast to using conventional kitchen cooking equipment, where temperatures can cycle ±10–15°F. Table 8.2 lists chemical changes in various foods as they cook over a range of 100–212°F. A small difference of 5°F can make a major difference in the quality of product. To achieve precise control of the cooking process, laboratory-grade water tanks from 1 ft^2 to 20 feet by 5 feet by 5 feet, depending on production volume, are used.

One of the first products was by French chefs preparing foie gras (goose liver). When prepared traditionally in an oven, there was excessive fat melting resulting in a 30–50% loss. When cooked to 148°F in a plastic pouch under vacuum in a 150°F water bath, then chilled, the loss was only 5%.

In operation, ingredients to be cooked are first assembled. In simple terms, this means that they can then be marinated, trimmed, flavored, seared, grill marked, and/or partially cooked. They are then packed under high vacuum (99.9% removal of the air) so that the plastic will be tight against the food and give excellent heat transfer. The bath, which has a uniformity of ±1°F, is brought to the desired temperature. The vacuum-packed food is immersed, and a planned cooking time-temperature profile is followed. Integrated lethality is calculated, and when an appropriate lethality is reached, pasteurization is complete. Since many foods are ≤ 1 inch thick, the product center temperature gets to within 2–5°F of the water temperature in 15–30 minutes, and the pasteurization time is started. Once the

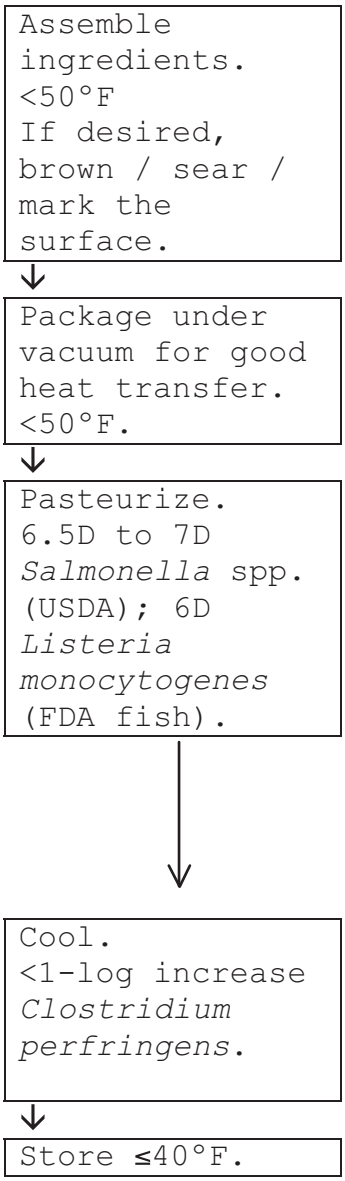

Figure 8.1. *Sous vide* assemble-package-pasteurize-cool-store.

food is pasteurized, if it is a tender cut, the food can be removed and chilled. If it is a tough cut and needs tenderization, such as lamb shank, it can be left for up to 3 days at the cook temperature, such as 135°F, before chilling.

One unique feature of *sous vide* is the ability to cook precisely. Most fish begins to "cook" at 125°F, and at 135°F, it gets tough and the muscle fibers shrink. For example, chefs like to cook salmon at about 130°F. At 135°F, meat cooks with little shrink, but by

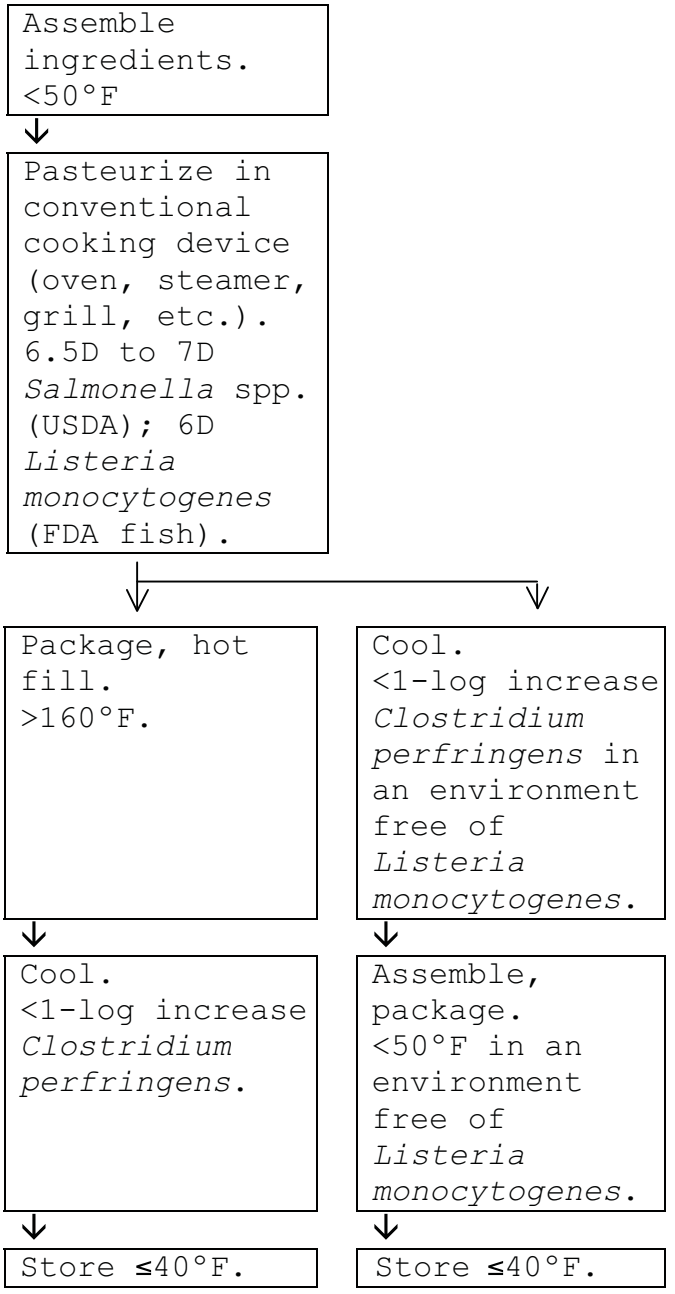

Figure 8.2. Cook-chill assemble-pasteurize-package-cool-store.

145°F, there is considerable shrink. Lamb chops cooked to 140°F are superb. Each chef can choose any degree of doneness by picking a temperature. At a lower temperature, there is little muscle fiber contraction, but tenderization can take a long time.

Another unique characteristic of *sous vide* is that a food such as a roast or beef steak

Table 8.2 Chemical changes caused by cooking

Temperature °F/°C	Fish and Shellfish	Meat and Poultry	Fruits and Vegetables
104/40	Myosin begins to denature and coagulate. Collagen sheaths shrink.		
122/50	Fish collagen begins to convert to gelatin. Myosin coagulates. Myosin-dense fish (e.g., salmon, tuna) become very succulent. Fluid leak is maximum. Enzymes are very active.	Myosin begins to denature and coagulate, leading to firmness of meat and poultry. It begins to squeeze out some water. Meat is rare.	
132/55 to 140/60	Fish begin to flake. Fish enzymes are mostly inactived.	Meat is medium rare. More protein coagulates, and meat becomes firmer. Eggs are pasteurized in shell, 135°F in 27 minutes.	Vegetables and fruits, including potatoes, sweet potatoes, carrots, beans, cauliflower, tomatoes, cherries, and apples, can be precooked to toughen them so that, when reheated, they have better texture. Pectins become more resistant to breakdown.
140/60 to 149/65	Muscle fibers begin to shrink, expressing juice, and fish becomes dry. Sheath collagen dissolves into gelatin.	Collagen begins to denature to gelatin. Meat shrinks, releasing a lot of juice. Meat becomes chewier. Proteolytic enzymes are very active. Meat is medium done at 140–150°F. Egg white thickens.	Starchy vegetables soften at this gelatinization temperature. Starch cells begin to swell.
149/65 to 158/70	Fish are getting progressively firmer and drier.	Meat becomes dry and tougher as protein fibers become more densely packed and squeeze out water. Collagen conversion to gelatin is taking place. Egg yolks thicken.	
158/70 to 167/75	Fish is stiff and dry.	Collagen connective tissue dissolves into gelatin. The gelatin provides succulence, and product becomes tender. This is a characteristic of slow-cook braises and stews. Meat is well done. Whole eggs set.	
176/80		Actin denatures and coagulates. Cells are densely compacted.	Flour starch thickens sauces and gravies.

(*continued*)

Table 8.2 Chemical changes caused by cooking (*continued*)

Temperature °F/°C	Fish and Shellfish	Meat and Poultry	Fruits and Vegetables
196/90 to 212/100			Pectins that fill the space between the cell walls begin to dissolve. Cellulose in the walls of the cells and lignin is very difficult to dissolve. Mild alkali protects the color of green vegetables. Sugars slow the solubilization of pectin. Acid added to vegetables slows the breakdown of cells.

Source: Charley 1982; McGee 2004; McWilliams 2001.

will have the same precise degree of doneness from the edge to the center of the food. The pouch is never opened until the finished product is to be served. This means the chance of cross contamination is quite remote.

To reheat the food, the pouch is put in a controlled water bath at 135–150°F for 20–30 minutes and reheated and served with a sauce, perhaps, and other items. Because the temperature is so gentle, food can be in a water bath reheater for up to 1 hour with little change.

The Cook-Chill Process

The cook-chill process has more process flexibility than the *sous vide* process, but yields are considerably lower, because a higher cooking temperature is used, such as 150–212°F. Temperatures in ovens will be 300–350°F; steamers, 212°F; grills, 300°F; and fryers, 300°F.

Once the food is cooked and flavored to the desired end point, the liquid food is added to a 1- to 1 and 1/2-gallon container such as a casing, a 1-gallon bucket, a form-fill pouch, or other suitable container. A small vacuum is drawn to reduce the chance of oxidation; the food package is cooled in a 32°F water tumble chiller, a blast chiller, or a circulating cold water bath; and then it is refrigerated. Care is taken to assure that the packaging area is kept free of detectable *L. monocytogenes*.

As an alternative, the hot, single-portion protein items may be chilled in open racks in a sanitary blast chiller; cut, flavored, sliced, and/or mixed with other ingredients in an *L. monocytogenes*-free work area at < 50°F; and then packaged in a casing or form-fill vacuum sealer.

Freezing versus Refrigerated

As originally conceived, *sous vide* and cook-chill products were refrigerated. However, that limits distribution and, very often, a manufacturer wants to distribute across the entire

United States, and even Canada. Also, manufacturers may want to produce larger batches at one time to improve inventory management. Today, many *sous vide* and cook-chilled items are frozen to extend shelf life from 60 days to 18 months. The consensus on quality is that the frozen food, when thawed, is indistinguishable from the original fresh, chilled food.

Microbiological Safety

Arguably, the most significant threat of foodborne illness via consumption of cook-chill and *sous vide* processed foods would be associated with contamination by sporeforming pathogens, such as *C. botulinum*, *C. perfringens,* and *Bacillus cereus* because of their ability to survive the heat treatment given to these products and their subsequent germination, outgrowth, and multiplication during cooling, storage, and distribution. Non-sporeforming, facultative, psychrotrophic pathogens considered prime hazards in processed products include *L. monocytogenes*, *Yersinia enterocolitica*, and *Aeromonas hydrophila*. These pathogens are capable of growth at refrigeration temperatures under anaerobic conditions (Gill and Reichel 1989; Hudson et al. 1994) and hence also pose a potential threat to consumer safety in *sous vide* and cook-chill products. Non-sporeforming, mesophilic, facultative anaerobes such as *Salmonella* spp., *Staphylococcus aureus*, or enteropathogenic strains of *Escherichia coli* may also pose a risk if foods are stored at abusive temperatures. Although all of these vegetative pathogens should be eliminated by the pasteurization step, they pose a health risk if the wide variety of raw ingredients used in these foods are of poor microbiological quality and/or if pasteurization is inadequate to destroy the high microbial load of non-sporeforming pathogens. Also, these pathogens are considered as hazards in cases of postprocess contamination prior to packaging or due to imperfectly sealed *sous vide* packages. Furthermore, these pathogens may be capable of surviving thermal processes designed for the production of these foods if they synthesize heat shock proteins and, thus, exhibit an induced thermotolerance response. The effect of heat treatment on the destruction of pathogens and the risk of the surviving pathogens on their growth, and possible toxin production, in case of sporeformers, in *sous vide* and cook-chill foods has been the aim of the research conducted using inoculated pack studies. Therefore, challenge studies conducted with formulated cook-chill and *sous vide* products to assure safety from sporeforming pathogens and the psychrotrophic, most heat resistant vegetative pathogen, *L. monocytogenes*, will be discussed.

Justification for Concern

Concerns regarding the microbiological safety and preservation of refrigerated, ready-to-eat processed foods are justified for a variety of reasons: (1) these products are generally formulated with little or no preservatives and have a low acid and high moisture (high a_w) content; (2) these products undergo minimal thermal processing, are not commercially sterile or shelf stable, and must be refrigerated; (3) vacuum packaging provides a favorable environment for anaerobic pathogens, such as *C. botulinum,* to grow and produce toxin in the processed product while the food remains edible because the spoilage microflora are inactivated; and (4) the potential exists for temperature abuse. Sufficient evidence exists to document that temperature abuse is a common occurrence at both the retail and consumer levels. Considerable fluctuations in temperature are frequently observed in commercial re-

frigerators (ICMSF 1998). According to the National Food Processors Association (1988; now called the Food Products Association), manufacturers should assume that temperature abuse will occur at some point during the distribution of a refrigerated food product. While it is relatively easy to control temperature in the institutional foodservice settings (e.g., restaurants, etc.), the potential for temperature abuse exists when the product is shipped to remote locations, during storage in the retail environment, or during handling by consumers. Thus, dependence on temperature control as the single hurdle to microbial growth is unrealistic to assure safety and extend storage life of refrigerated, ready-to-eat processed foods. Furthermore, since consumers view such packaged foods as shelf stable, there is a high risk of consumer temperature abuse, mishandling, and overextending of the product's shelf life.

Clostridium botulinum

The organism that poses the greatest threat to *sous vide* and cook-chill products is *C. botulinum* types A, B, E, and F. Proteolytic type A and B strains of *C. botulinum* produce highly heat-resistant spores and have a minimal growth temperature of 10°C. Thus, the spores of proteolytic *C. botulinum* strains will survive the mild heat treatment given to such food products. However, these are of limited significance in foods that are properly refrigerated. Nonproteolytic *C. botulinum* strain spores have reduced heat resistance but can grow and produce toxin at temperatures as low as 3.3°C. Spores of these strains that survive the thermal process would pose a botulism hazard even under proper refrigeration temperatures if a secondary barrier is not present. Therefore, without additional hurdles or barriers, heat processing must be sufficient to destroy nonproteolytic *C. botulinum* spores if the food is to be safe.

Studies have been conducted to assess the effectiveness of a combination of hurdles to render *C. botulinum* spores more sensitive to the lethal effect of heat. Since nonproteolytic *C. botulinum* spores are less heat resistant, it is practically feasible to inactivate these spores by heat. While Juneja, Eblen et al. (1995) reported that contaminated turkey should be heated to an internal temperature of 80°C for at least 91.3 minutes to give a 6-D process for type B spores, with the inclusion of 3% salt in turkey, 78.6 minutes at 80°C was sufficient to achieve a 6-D process (Juneja and Eblen 1995). Thus, incorporating low levels of salt while formulating foods and designing a reduced thermal process that ensures safety against nonproteolytic *C. botulinum* type B in *sous vide* and cook-chill processed foods will maintain the desirable organoleptic attributes of foods.

Juneja, Marmer et al. (1995) assessed and quantified the effects and interactions of temperature, pH, salt, and phosphate levels in turkey and found that the thermal inactivation of nonproteolytic *C. botulinum* spores is dependent on all four factors. Thermal resistance of spores can be lowered by combining these intrinsic factors. The following multiple regression equation predicts D-values for any combinations of temperature (70–90°C), salt (NaCl; 0.0–3.0%), sodium pyrophosphate (0.0–0.3%), and pH (5.0–6.5) that are within the range of those tested:

$$\text{Log}_e \text{ D-value} = -9.9161 + 0.6159(\text{temp}) - 2.8600\ (\text{pH}) - 0.2190\ (\text{salt}) + 2.7424\ (\text{phos}) + 0.0240(\text{temp})(\text{pH}) - 0.0041(\text{temp})(\text{salt}) - 0.0611(\text{temp})(\text{phos}) + 0.0443(\text{pH})(\text{salt}) + 0.2937(\text{pH})(\text{phos}) - 0.2705(\text{salt})(\text{phos}) - 0.0053(\text{temp})^2 + 0.1074(\text{pH})^2 + 0.0564(\text{salt})^2 - 2.7678(\text{phos})^2$$

Table 8.3. Observed and predicted D-values at 70–90°C of nonproteolytic *C. botulinum* in ground turkey

Temperature (°C)	pH	% NaCl	% Phosphate	D-value Observed (min)	D-value Predicted (min)
70	6.50	0.0	0.00	57.7	66.0
70	6.50	1.5	0.15	40.1	46.5
75	6.25	1.0	0.10	39.1	42.3
75	6.25	1.0	0.20	32.9	38.6
90	5.00	0.0	0.00	5.0	6.3
90	5.00	1.5	0.15	3.1	4.8

Source: Juneja et al. 1995b.

Additionally, Juneja, Marmer et al. (1995) developed confidence intervals to allow microbiologists to predict the variation in the heat resistance of nonproteolytic *C. botulinum* spores. Using this predictive model, food processors should be able to design thermal processes for the production of a safe ready-to-eat food with extended shelf life without substantially adversely affecting the quality of the product. Representative observed and predicted D-values at 70–90°C of nonproteolytic *C. botulinum* in ground turkey adjusted to various pH levels (5.0–6.5) supplemented with salt (0.0–1.5%, w/v) and sodium pyrophosphate (0.0–0.2%, w/v) are given in Table 8.3.

Researchers have assessed the efficacy of added preservatives on inhibiting or delaying the time to toxin production by nonproteolytic *C. botulinum* and the potential hazard due to growth and toxin production in extended shelf-life foods stored at temperatures above 3°C. For example, Meng and Genigeorgis (1994) found that sodium lactate (NaL) significantly delayed toxigenesis of *C. botulinum* nonproteolytic types B and E in commercially prepared *sous vide* products (beef and salmon homogenates) containing 0, 2.4, and 4.8% (w/w) NaL and in chicken containing 0, 1.8, and 3.6% (w/w) NaL (Table 8.4).

Maas et al. (1989) reported that proteolytic types A and B *C. botulinum* spores inoculated in ground raw turkey containing 0, 2.0, 2.5, 3.0, or 3.5% NaL and *sous vide* processed (71.1°C) was toxic after 3, 4–5, 4–6, 7, or 7–8 days, respectively, at 27°C. Thus, NaL exhibited an antibotulinal effect that was concentration dependent. Toxin production by *C. botulinum* type A and B spores was inhibited throughout the 42-day storage period at 15°C for a reformulated *sous vide* processed (75°C for 36 minutes) spaghetti and meat-sauce product containing > 1.5% (w/w) salt (Simpson et al. 1995). It is worth pointing out that none of the above studies discussed the sensory implications, if any, of the hurdles used. Therefore, research is required, as sensory acceptability may be a limiting factor in practical use.

A number of predictive models have been developed based on multifactorial design experiments and extensive data collection and analysis. These models quantify the effects and interactions of intrinsic and extrinsic factors and describe the growth responses of sporeformers (Fernandez and Peck 1997; Graham et al. 1996; Meng and Genigeorgis 1993). Meng and Genigeorgis (1993) developed the following predictive regression model for the lag phase duration of nonproteolytic *C. botulinum* type B and E spores (inoculum level: 10^2–10^4/g) in cooked turkey and chicken meats as affected by NaCl (0–5%), sodium lactate (0–3%), inoculum (I), and temperature (T) of 8–30°C and their interactions:

Table 8.4. Efficacy of sodium lactate on time to toxin detection of *C. botulinum* in *sous vide* products stored at 4–30°C

Food Product	Sodium Lactate (%, w/w)	Time (in days) to produce toxin			
		4°C	8°C	12°C	30°C
Beef	0	90	8	4	1
	2.4	> 90	90	> 40	3
	4.8	> 90	> 90	> 40	6
Chicken	0	90	16	12	2
	1.8	> 90	16	12	2
	3.6	> 90	> 90	> 40	6
Salmon	0	60	8	4	1
	2.4	90	12	6	2
	4.8	> 90	> 90	> 40	4

Source: Meng and Genigeorgis (1994)

$$\text{Log } (1/LP) = -2.29 - 0.123\ (NaCl) + 0.22(NaL) + 0.439(T) + 0.02(T)(I) \text{ with } R^2 = 0.945$$ where T equals square root of temperature

The Meng and Genigeorgis (1993) study demonstrated that the lag phase in turkey meat can be extended to > 38 days at < 8°C in the presence of 2% NaL and 1% NaCl for an inoculum of 100 spores/g. Increasing the NaCl concentration to 2% extended the lag phase to > 55 days. At a mild temperature abuse of 12°C, incorporation of 3% NaL and 2% NaCl was required to prevent toxin production for at least 36 days in turkey meat containing 100 spores/g. Such predictive models can be useful in defining microbiologically safe operating practices, such as conditions for a critical control point in a Hazard Analysis Critical Control Point (HACCP) program or predicting the growth of a microorganism in a new formulation of a product. Food processors can optimize *sous vide* product formulation by the use of these predictive models.

Clostridium perfringens

Although technically an anaerobe, *C. perfringens* is relatively aerotolerant. The pH range for growth of *C. perfringens* is pH 5.0–9.0. The temperature range for growth of *C. perfringens* is 6–50°C, with a doubling time as short as 7.1–10 minutes (Johnson 1990a). While most strains are inhibited by 5–6.5% salt, the organism has been observed to grow at up to 8% NaCl concentration in foods (Johnson 1990a). *C. perfringens* is capable of extremely rapid growth in meat systems, which makes the organism a particular concern to meat processors as well as the foodservice industry. *C. perfringens* grew faster at 45°C in autoclaved ground beef than in broth media at the same temperature (Willardsen et al. 1978; Willardsen et al. 1979). One strain of *C. perfringens* had a generation time of 7.1 minutes in autoclaved ground beef held at 41°C, although the mean generation time for an 8-strain mixture ranged from 19.5 minutes at 33°C to 8.8 minutes at 45°C (Willardsen et al. 1978). Because of its rapid growth, numbers of *C. perfringens* sufficient to cause ill-

Table 8.5. Mean[a] generation times, lag times, and D-values ± standard deviation at 99°C of spore cocktail of *C. perfringens* strains NCTC 8238, NCTC 8239, and NCTC 10288 in ground turkey that contained 0.3% sodium pyrophosphate at pH 6 and salt levels of 0, 1, 2, and 3%

	Generation Times (min)[b]		Lag Times (h)		
Product	28°C	15°C	28°C	15°C	D-value at 99°C (min)
Turkey (salt 0%)	39.4	300.0	7.3	61.6	23.2 ± 0.2
Turkey (salt 1%)	31.3	398.8	10.6	59.6	21.3 ± 0.8
Turkey (salt 2%)	24.2	238.2	11.6	106.4	19.5 ± 0.8
Turkey (salt 3%)	88.5	nd[c]	8.0	nd[c]	17.7 ± 0.3
Beef (salt 0%; pH 7)	80.1	415.9	11.55	96.06	23.3[b] ± 1.4
Beef (salt 3%; pH 7)	88.8	439.0	16.58	159.06	19.8[b,c] ± 2.1
Beef (salt 0%; pH 5.5)	122.1	4640.7	12.83	200.52	17.3[b,c] ± 0.1
Beef (salt 3%; pH 5.5)	129.2	NG	27.53	NA	14.0[c] ± 1.7

[a]Mean of two replications.
[b]Generation times calculated from regression lines for exponential growth using the Gompertz equation.
[c]not determined.
Source: Juneja and Majka 1995; Juneja and Marmer 1996.

ness, that being > 10^6 cells, can rapidly be reached under optimal conditions in meats and meat products.

Slow cooking associated with low-temperature, long-time cooking of cook-chill and *sous vide* foods can also result in growth of *C. perfringens* in foods. For instance, mean generation times in autoclaved ground beef during slow heating from 35–52°C ranged from 13 to 30 minutes with temperature increases of 6–12.5°C/hour (Willardsen et al. 1978). Another study also demonstrated growth of the organism in autoclaved ground beef during linear temperature increases (4.1°C–7.5°C/hour) from 25°C to 50°C (Roy et al. 1981).

Researchers in recent years have characterized the behavior of *C. perfringens* in *sous vide* cooked foods. The thermal resistance of *C. perfringens* spores (expressed as D-values in minutes) in turkey slurries that included 0.3% sodium pyrophosphate at pH 6.0 and salt levels of 0, 1, 2, or 3% are shown in Table 8.5. The D-values at 99°C decreased from 23.2 minutes (no salt) to 17.7 minutes (3% salt; Juneja and Marmer 1996). In a beef slurry, the D-values significantly decreased ($p < 0.05$) from 23.3 minutes (pH 7.0, 3% salt) to 14.0 minutes (pH 5.5, 3% salt) at 99°C at lower pH. While addition of increasing levels (1–3%) of salt in turkey (Juneja and Marmer 1996) or a combination of 3% salt and pH 5.5 in beef (Juneja and Majka 1995) can result in a parallel increase in sensitivity of *C. perfringens* spores at 99°C, mild heat treatments given to *sous vide* foods will not eliminate *C. perfringens* spores. In other words, spores are likely to survive the normal pasteurization/cooking temperatures applied to these foods. In fact, it is not feasible to inactivate the spores by heat. Cooking temperatures, if designed to inactivate *C. perfringens* spores, may negatively impact the product quality and desirable organoleptic attributes of foods. Mild heat treatment given to *sous vide* and cook-chill foods could also serve as an activation step for spores. Thereafter, germination and outgrowth of spores and *C. perfringens* vegetative

growth is likely to occur in such foods if the rate and extent of cooling is not sufficient or if the processed foods are temperature abused.

C. perfringens spores germinated and grew at 28°C from 2.25 to > 5 $\log_{10}$ CFU/g after 16 hours in *sous vide* processed (71.1°C) turkey samples regardless of the presence or absence of salt (Juneja and Marmer 1996). While *C. perfringens* spores germinated and grew at 15°C to > 5 $\log_{10}$ CFU/g in turkey with no salt by day 4, the presence of 3% salt in samples at 15°C completely inhibited germination and subsequent multiplication of vegetative cells even after 7 days of storage (Juneja and Marmer 1996). Growth from *C. perfringens* spores occurred within 6 days in *sous vide* processed (71.1°C) pH 7.0 ground beef samples but was delayed until day 8 in the presence of 3% salt at pH 5.5 at 15°C (Juneja and Majka 1995). *C. perfringens* growth from a spore inoculum at 4°C was not observed in *sous vide* cooked turkey or beef samples (Juneja and Majka 1995; Juneja and Marmer 1996). The generation and lag times in *sous vide* processed beef and turkey at 28° and 15°C are given in Table 8.5.

The efficacy of NaL in inhibiting the growth from spores of *C. perfringens* in a *sous vide* processed food has been assessed. Inclusion of 3% NaL in *sous vide* beef goulash inhibited *C. perfringens* growth at 15°C, delayed growth for a week at 20°C, and had little inhibitory effect at 25°C (Aran 2001). While addition of 4.8% NaL restricted *C. perfringens* growth from spores for 480 hours at 25°C in *sous vide* processed (71.1°C) marinated chicken breast, it delayed growth for 648 hours at 19°C. *C. perfringens* growth was not observed at 4°C regardless of NaL concentration (Juneja 2006). These studies suggest that NaL can have significant bacteriostatic activity against *C. perfringens* and may provide *sous vide* processed foods with a degree of protection against this microorganism, particularly if employed in conjunction with adequate refrigeration.

Since *C. perfringens* may grow and multiply rapidly after germination, cooked meat and poultry products must be cooled rapidly to restrict their germination, outgrowth, and subsequent vegetative growth. To ensure safety, cooked products must be transported, distributed, stored, and handled under proper refrigeration. Steele and Wright (2001) evaluated growth of *C. perfringens* spores in turkey roasts cooked to an internal temperature of 72°C, followed by cooling in a walk-in cooler from 48.9°C to 12.8°C in 6, 8, or 10 hours. Results of that study indicated that an 8.9-hour cooling period was adequate to prevent growth of *C. perfringens* with a 95% tolerance interval. Juneja et al. (1994) reported that no appreciable growth (< 1.0 $\log_{10}$ CFU/g) occurred if cooling took 15 hours or less when cooked ground beef inoculated with heat activated *C. perfringens* spores was cooled from 54.4° to 7.2°C at an exponential rate, that being more rapid cooling at the beginning and then slower. However, *C. perfringens* grew by 4–5 $\log_{10}$ CFU/g if the cooling time was greater than 18 hours. This implies that *C. perfringens* is capable of rapid growth in meat systems, making this organism a particular concern to meat processors, as well as to the foodservice industry.

Recent studies have shown the efficacy of certain antimicrobial agents against the growth of *C. perfringens* during cooling of meat products. For instance, Sabah et al. (2003) found that 0.5–4.8% sodium citrate inhibited growth of *C. perfringens* in cooked vacuum-packaged restructured beef cooled from 54.4°C to 7.2°C within 18 hours. The same researcher also demonstrated growth inhibition of the organism by oregano in combination with organic acids during cooling of *sous vide* cooked ground beef products (Sabah et al. 2004). Organic acid salts such as 1% sodium lactate, 1% sodium acetate, or 1% buffered sodium citrate (with or without sodium diacetate) inhibited germination and outgrowth of

C. perfringens spores during the chilling of marinated ground turkey breast (Juneja and Thippareddi 2004).

Bacillus cereus

B. cereus is widely distributed in nature and has been isolated from a variety of foods, including many ready-to-serve meals (Harmon and Kautter 1991). *B. cereus* control is a challenge in cook-chill and *sous vide* processing because the organism is a facultative anaerobe and a sporeformer. The organism is recognized as a psychrotrophic pathogen, though the temperature for growth of *B. cereus* ranges from 15 to 50°C. Foegeding and Berry (1997) screened a collection of twenty-seven *B. cereus* isolates for the ability to grow at cold temperatures. Of twenty-seven isolates, nineteen could grow in brain heart infusion broth at 7°C if previously adapted to 7°C over a 5-week period. The authors suggested that the cold adaptation response exhibited by *B. cereus* isolates should be considered while assessing shelf life or safety of foods relative to this organism. However, it is worth noting that not all foodborne isolates are psychrotrophic. The doubling time in a nutritive medium at optimum temperature is 18–27 minutes. The growth limiting pH under ideal conditions ranges from the minimum of pH 4.9 to the maximum pH 9.3. The minimum water activity for growth is 0.95 (Johnson 1990b). Growth depends upon the interactive effects of all the environmental parameters.

The heat resistance of *B. cereus* spores is a concern to the food industry and has been studied extensively. In general, the heat resistance is similar to that of other mesophilic sporeformers; however, some strains, referred to as heat-resistant strains, are about fifteen- to twentyfold more heat resistant that the heat sensitive strains (Johnson 1990b). *B. cereus* strains involved in food poisoning have D-values at 90°C ranging from 1.5 to 36 minutes. It is most likely that the organism will not be completely destroyed by the heat treatment given to most refrigerated, ready-to-eat foods. Therefore, the organism must be controlled in these foods by preventing its growth and/or restricting the shelf life of the product. As with *C. perfringens*, the risk of food poisoning due to *B. cereus* is relatively low because of the relatively high infective dose, which ranges from 10^5 to 10^7 organisms (total) for the diarrheal type and from 10^5 to 10^8 organisms per gram of food for the emetic syndrome.

A psychrotrophic *B. cereus* strain survived pasteurization and grew at 7°C in *sous vide* cooked green beans (Knochel et al. 1997). In another study (Aran 2001), no *B. cereus* growth was observed at 10°C, but after 7 days at 15°C, population densities increased by 1 $\log_{10}$ CFU/g in the *sous vide* beef goulash samples. In this latter study, calcium lactate at concentrations of 1.5% in beef goulash completely inhibited *B. cereus* growth at 20°C, but the level of NaL required to inhibit growth was 3%. Turner et al. (1996) assessed the safety of *sous vide* chicken breast with respect to *B. cereus* and evaluated the effect of processing parameters on natural microflora. The product was inoculated and processed to 77° or 94°C. *B. cereus* populations were reduced by 0.5–1.0 $\log_{10}$ CFU/g and by 3 $\log_{10}$ CFU/g in products heated to 77°C and 94°C, respectively. Thereafter, spores germinated within 1 day at 10°C, yet detectable changes in populations were not evident through 28 days of storage. Sodium lactate (2%) did not influence *B. cereus* populations or spore germination. The natural microflora were reduced by processing and levels did not increase throughout the 28 days of storage at 4°C and 10°C. Turner et al. (1996) concluded that the final temperature is important in controlling this organism even though *B. cereus* populations were reduced by the mildest heat.

Listeria monocytogenes

L. monocytogenes continues to be a foodborne pathogen of great concern to the food industry because it is ubiquitous in the environment and in a wide variety of foods. The safety concerns in *sous vide* and cook-chill processed foods relate to this microorganism's ability to grow rapidly at refrigeration temperatures and the fact that it is more heat resistant than other vegetative pathogens. Moreover, the slow heating rate/long come-up times employed in the production of foods expose the microbial cells to conditions similar to heat shock, with the possibility of rendering these cells more thermal resistant, thus facilitating a longer survival during low final cooking temperatures. Stephens et al. (1994) and Kim et al. (1994) reported that heating by slowly raising the temperature of pork exposes *L. monocytogenes* cells to conditions similar to heat stress, thereby enhancing the pathogen's heat resistance. Since recovery of heat-stressed pathogenic bacteria is increased under anaerobic conditions (George et al. 1998; Knabel et al. 1990), possible growth of heat-injured pathogens in *sous vide* products is certainly a concern. Hansen and Knochel (1996) compared the effect of slow and rapid heating regimes on the heat resistance of *L. monocytogenes* in *sous vide* cooked beef. The authors found no significant difference between slow (0.3–0.6°C/minute) and rapid (> 10°C/minute) heating and the heat resistance of *L. monocytogenes* in low pH (< 5.8) *sous vide* cooked beef prepared at a mild processing temperature. However, the latter authors did observe an increase in the heat resistance of *L. monocytogenes* in *sous vide* beef at pH 6.2. While processing at slowly rising temperatures may slightly increase the survival of *L. monocytogenes* in cooked beef, there was no evidence of an increase in subsequent growth potential of the surviving cells. In a later study, Hansen and Knochel (2001) reported that the late stationary phase cultures of *L. monocytogenes* in *sous vide* cooked beef with 95–99.9% heat-injured cells in the surviving population did not grow or repair sublethal injuries in *sous vide* cooked beef at 3°C, while repair and growth took place at 10° and 20°C. In logarithmic phase cultures, heat injury occurred very rapidly and 99.9% injury was observed in all trials in spite of much lower pasteurization values and fewer $\log_{10}$ reductions compared with late stationary phase cultures. Regardless of growth phase, all cultures where a high degree of heat injury (99%) was observed did not subsequently grow in a beef product at 3 or 10°C within 30 days. A longer lag period was observed in beef processed at slowly rising temperatures and in normal pH beef at 10°C. Quintavala and Campanini (1991) determined the heat resistance of *L. monocytogenes* strain 5S heated at 60, 63, and 66°C in a meat emulsion at a rate of 5°C/minute compared to instantaneous heating. The D-values of cells heated slowly were twofold higher than the cells heated instantaneously at all heating temperatures. Failure to inactivate *L. monocytogenes* during cooking may lead to an unsafe product even if subsequent transportation, distribution, storage, or handling are carried out properly. While it is universally agreed that proper pasteurization/cooking temperatures will destroy the organism, it is important to ensure that the mild heat treatment designed for *sous vide* foods provides an adequate degree of protection against *L. monocytogenes*.

Stillmunkes et al. (1993) evaluated the comparative effects of various additives and storage temperatures on survival of *L. monocytogenes* in *sous vide* processed nitrite-free beef roasts (62.8°C) supplemented with varying concentrations of NaL, glycerol monolaurin, or sodium gluconate. The samples were stored for up to 5 weeks at temperatures simulating the wholesale (2°C), retail (7°C), and consumer (10°C) storage conditions and temperature abuse (25°C) conditions. In this study, NaL (up to 3.5%) resulted in effective

inhibition of the pathogen. Glycerol monolaurin demonstrated a concentration-related effect (0–3.5%), but the effectiveness was not sustained beyond about 3 weeks of storage. Growth of the pathogen was suppressed by lactate but not by monolaurin in temperature abused samples. Sodium gluconate did not provide any significant control of the pathogen in cooked, uncured beef roasts. In conclusion, the use of sodium lactate can increase the microbiological safety of *sous vide* foods.

Sous vide processed fish is subjected to low time/temperature for cooking to retain intrinsic organoleptic attributes. Ben Emarek and Huss (1993) investigated the heat resistance of two strains of *L. monocytogenes* in *sous vide* cooked fillets of cod and salmon. Pasteurized salmon fillets (10.56–17.2%, w/w, fat) had one to four times higher D-values for both strains of *L. monocytogenes* than the lower fat (0.6–0.8%, w/w, fat) cod fillets. These findings document the protective effect of fatty materials in the heating medium and the importance of food type on the heat resistance of *L. monocytogenes*. Gaze et al. (1989) recommended that salmon and uncured fish fillets processed via *sous vide* should be cooked to a final temperature of 70°C and held for 2 minutes to achieve 12-D reduction of *L. monocytogenes.*

Regulations

In most countries, food industry and retail food establishments are required to comply with the published guidelines/recommendations for microbiologically safe production, distribution, and sale of ready-to-eat, refrigerated foods. Also, a code of practice, which is advisory rather than prescriptive, has been developed in some countries. In comparison with legislation, the main advantage of the codes is that they can be more easily updated to take into account the changing industry practices in response to consumer demands.

In North America, the documents are based on the principles and practices of the HACCP system and suggest that processors follow good manufacturing practices sanitation guidelines, build multiple hurdles or barriers into a particular product for an additional degree of safety and store products at refrigerated temperatures, conduct inoculated pack or challenge studies to validate the efficacy of the multiple barriers, and use time-temperature indicators (TTIs) to track the time and temperature history of the products from production to consumption (Agriculture Canada 1990; FIOC 1990; Health and Welfare Canada 1992; NACMCF 1990; Rhodehamel 1992).

In Europe, recommendations, guidelines, and codes of practice (ACMSF 1992, 1995; Betts 1996; ECFF 1996; Gould 1996; Martens 1997) have been developed to ensure the safe production of *sous vide* foods with respect to preventing growth and toxin production by nonproteolytic *C. botulinum*. Proteolytic *C. botulinum* growth and toxin production is prevented by ensuring that storage of *sous vide* foods is at temperatures < 10°C. In the UK, the Advisory Committee on the Microbiological Safety of Food (ACMSF) concluded that safety with respect to nonproteolytic *C. botulinum* could be ensured by one of the following (ACMSF 1992, 1995): (1) storage at < 3.3°C, (2) storage at ≤ 5°C and a shelf life of ≤ 10 days, (3) storage at 5–10°C and a shelf life of ≤ 5 days, (4) storage at chill temperature combined with heat treatment of 90°C for 10 minutes or equivalent lethality (e.g., 70°C for 1,675 minutes, 75°C for 464 minutes, 80°C for 129 minutes, 85°C for 36 minutes, 90°C for 10 minutes; ACMSF 1992), (5) storage at chill temperature combined with ≤ pH 5.0 throughout the food, (6) storage at chill temperature combined with a salt concentration of ≥ 3.5% throughout the food, (7) storage at chill temperature combined with

$\leq a_w$ 0.97 throughout the food, or (8) storage at chill temperature combined with combinations of heat treatment and other preservative factors that can be shown consistently to prevent growth and toxin production by *C. botulinum*. It is emphasized that some of the above recommendations cannot be applied and used for all *sous vide* foods because most of these foods have a pH $\geq$ 5.0, a high water activity, and a salt-on-water concentration $\leq$ 3.5%. To guard against the hazards for these foods, the principal factors controlling microbiological safety and quality are likely to be the heat treatment, storage temperature, and shelf life.

The time and temperature combinations recommended by the European Chilled Food Federation (ECFF 1996) were 80°C for 270.3 minutes, 85°C for 51.8 minutes, 90°C for 10.0 minutes, 95°C for 3.2 minutes, and 100°C for 1.0 min. These recommendations were based on a z-value of 7°C for temperatures < 90°C, whereas ACMSF calculations were based on a z-value of 9°C. Nevertheless, it is recommended that a heat treatment or combination process be used that reduces the number of viable spores of nonproteolytic *C. botulinum* by a safety factor of 6 $\log_{10}$ cycles (a six-decimal process) in *sous vide* foods (ACMSF 1992; ECFF 1996). A heat treatment of 90°C for 10 minutes or equivalent lethality followed by subsequent chilled storage was given as reference criteria.

Concluding Statements and Future Research Directions

The assurance of microbiological safety is a key factor in the success of cook-chill and *sous vide* processed food products. Accordingly, survival of pathogens and the occurrence of temperature abuse throughout distribution, in retail markets, and in home refrigerators is a challenge for innovative interventions. The safety of *sous vide* and cook-chill processed foods cannot be considered to rely on only one "chilled storage" factor. Since the survival during thermal processing is dependent on the pathogen per se and its initial microbial load, the microbiological quality of raw materials plays a significant role in ensuring the safety of such food products. The most critical step in the production of processed ready-to-eat products is the heating process for the inactivation of pathogens. In addition, every effort should be made to extend the lag and generation time of the pathogens in foods. The combination of heat with hurdle technology has enormous potential to improve the safety margin of *sous vide* foods. Research has assessed and quantified the effects and interactions of combinations of hurdles in foods. Combining several inhibitory parameters at subinhibitory levels, with an aim to render the pathogens more sensitive to the lethal effect of heat and to lessen the possibility of pathogen growth during storage, has proven effective. Studies that require further investigation on multiple food formulations should be aimed at identifying optimal processing time-temperature combinations and improved microbial safety during storage. This would provide secondary barriers to pathogen growth in cases of temperature abuse or failure of other primary preservative techniques. Further research employing complex multifactorial experiments and analyses to define and quantify the effects and interactions of additional intrinsic and extrinsic factors and development of "enhanced" predictive models is also needed.

Recently, challenge studies were conducted with formulated *sous vide* products to assure inactivation/control of vegetative and sporeforming foodborne microbial pathogens. In view of the continued interest that exists in lowering the heat treatment, it would be logical to define a specific lethality at low temperatures. It would be useful to determine the possible effects of injury to vegetative cells and spores that may result from mild heat treat-

ments and factors in foods that influence the recovery of cells/spores heated at these low temperatures. Also, future research should focus on conducting dynamic pasteurization (low-temperature-long-time cooking) studies to assess the integrated lethality of cooking and develop integrated predictive models for thermal inactivation, injury, repair, and behavior of target pathogens in cooked foods during storage. Further, development of quantitative risk assessment models based on product composition and formulation, and processing and storage, in conjunction with implementation of HACCP plans and employee training in HACCP principles, should provide an adequate degree of protection against foodborne sporeformers and non-sporing psychrotrophic pathogens. Also, this approach should result in a higher degree of confidence in product safety than is possible using traditional end sampling approaches to microbiological control. Finally, good manufacturing practices are advocated to enhance the safety of *sous vide* and cook-chill food products.

Note

Mention of trade names or commercial products in this chapter is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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Part Two
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9 Active Packaging: A Non-Thermal Process

Jung H. Han and John D. Floros

Introduction

Role of Packaging in the Food Chain

Packaging is a medium between product manufacturers and consumers aiding the maintenance of original quality of the packaged products and providing information and characteristics of the packaged products to the consumers (Meroni 2000). Packaging has a significant role in the food supply chain because it is an integral part of food processes and food supply (Ahvenainen 2003). The most important functions of packaging are to retard the deterioration of the value of food products, extend the shelf life, and maintain the quality and safety of the packaged foods (López-Rubio et al. 2004). To increase the effectiveness of these functions of packaging, some active agents are incorporated into the packaging system.

Package-Related Problems of Foods

Many materials are used to construct various shapes of packages. Major packaging materials are paper/paperboard, plastic polymers, glass, and metals. Glass and metals are impermeable materials and historically used for the preservation of foods. Various plastics have been substituted for the glass and metals because they are cheap and lightweight. The main disadvantage of these polymers for food packaging is that they are permeable to the transfer of gases and low molecular weight chemicals, the kinetics of which are related to the permeability, and consequently they allow the adverse effects of the environment to affect the packaged product (López-Rubio et al. 2004). Some package ingredients such as plasticizers, plastic monomers, and other plastic additives migrate into foods. Chemical migration has been controlled by regulation, including the permission of the use of the chemicals after migration studies. Important food ingredients such as flavors or other nutrients can be absorbed by hydrophobic plastic materials, resulting in the loss of nonpolar food ingredients. Besides these mass transfer phenomena reducing the quality of packaged foods, the quality also deteriorates through physiological processes (e.g., respiration of fresh produce), chemical processes (e.g., lipid oxidation), physical processes (e.g., bread staling, dehydration), microbial processes (e.g., spoilage), and infestation (e.g., insect attacks) (de Kruijf et al. 2002). Depending on the requirements of packaged food, food quality degradation can be significantly reduced by the packaging system (de Kruijf et al. 2002). An effective package must prevent the transmission of oxygen, light and water vapor, and microbial growth to retard quality deterioration of packaged foods (Singh and Singh 2005).

TABLE 9.1 Definitions of active packaging

References	Definitions
Han 2005a	Active food packaging serves beyond its conventional protective barrier property by introducing new extra functions.
López-Rubio et al. 2004	Active packaging refers to those technologies intended to interact with the internal gas environment and/or directly with the product, with a beneficial outcome.
Brody et al. 2001	Active packaging is intended to sense internal or external environmental change and to respond by changing its own properties or attributes and hence the internal package environment.
Brody 2000	Active packaging possesses attributes beyond the basic physical barrier property.
Actipak; de Kruijf et al. 2002	Active packaging changes the condition of the packaged food to extend shelf life or to improve safety or sensory properties, while maintaining the quality of the packaged food.
Floros et al. 1997; Ozdemir and Floros 2004	Active packaging is an intelligent or smart system that involves interactions between package or package components and food or internal gas atmosphere and complies with consumer demands for high-quality, fresh-like, and safe products.
Hotchkiss 1994; Rooney 1995a	Active packaging performs some desired role other than providing an inert barrier to external conditions.

Technology Backgrounds and Principles

Definitions and Applications of Active Packaging

Table 9.1 lists many definitions of active packaging. However, common ideas can be recognized from the various definitions, which indicate that active packaging has an extra function that provides beneficial conditions to the quality of packaged foods by changing the properties of foods or internal atmosphere. Active packaging changes the condition of the packaged food to give extended shelf life, improved safety, and desirably maintained quality (Ahvenainen 2003; de Kuijf et al. 2002). Active packaging is a specially designed packaging system utilizing active packaging materials and/or actively functional package objects in the forms of sachet, sheet, label, and extra (Rooney 2005a).

The most common and also the most promising active packaging applications are antimicrobial packaging systems, oxygen-scavenging systems, moisture-control systems for fresh produce and dried foods, and ethylene-absorbing systems for fruits and vegetables. These applications have been shown to have huge potential markets with benefits to the food industry and consumers.

Intelligent Packaging

The term “intelligent” implies that the package has an indicator that monitors the conditions (e.g., quality) of food products, while the “active” in active packaging indicates the procedures that consistently accommodate active interaction of the package with internal atmosphere or the package food (Meroni 2000). While active packaging changes the condition of the packaged food to give extended shelf life, improved safety, and desirably maintained quality, intelligent packaging monitors the condition of packaged foods to

provide information about the quality of the foods during transportation and storage (Ahvenainen 2003; de Kuijf et al. 2002). Intelligent packaging may sense the condition of packaged foods specifically or respond to the changes of the food condition (Rodrigues and Han 2003).

Properties and Functional Modes of Active Packaging

An active packaging system can be classified into three basic modes: absorbing mode, releasing mode, and other modes using chemical reactions (Ahvenainen 2003; Han 2003). The absorbing system scavenges adverse components against the quality of the packaged foods. Oxygen and ethylene are good examples of the adverse components of package atmosphere. The removal of oxygen and ethylene prevents the oxidation of foods and the overripening of fresh produce. The releasing system is for the addition or emission of active components into foods or package atmosphere. Ethanol-emitting sachets and chlorine dioxide–emitting films are good examples of such a releasing system. The released ethanol or chlorine dioxide can kill contaminating microorganisms. The third mode of the systems includes an immobilization system of an active agent into polymeric packaging materials, a self-heating/self-cooling system, a freshness indicating system, and so on that are not related to the absorption or release of active agents. Therefore, active packaging is not a one-step wrapping process but is a continuous non-thermal process performing an active function through distribution, storage, and consumption after the packaging process.

Drivers and Technologies of Active Packaging Applications

Active packaging is useful in extending the shelf life and maintaining the quality of many kinds of food products, and it provides viable benefits to the food industry, packaging industry, and consumers (Ozdemir and Floros 2004). Consumers may have preference for the active packaging system because the packaged foods have better quality and safety as well as decreased amounts of food additives. For example, oxygen-scavenging packaging systems can eliminate oxygen in the package atmosphere and reduce the oxidation of the packaged food without using a chemical antioxidant in the food. Therefore, consumers would ingest decreased amounts of chemical additives.

The decision to consider active packaging for a food or beverage incorporates many factors such as economic advantages, process engineering limitations, convenience in use, environmental impacts, and secondary effects resulting from some other changes in the processing or packaging (Rooney 2005a). Active packaging involves the application of specifically active functions of the packaging system for a specific food; therefore, not all active packaging systems work the same way or effectively with different foods or different packaging materials (Cutter 2002; Rooney 1995a).

In North America active packaging is very much in its infancy compared to other countries, especially Asian countries. The market for active packaging is currently largely supplier driven in North America (Brody 2005).

Active Food Packaging and Food Safety

Active packaging ensures food safety and quality through inhibition of pathogen and spoilage microorganisms, prevention of chemical contaminant migration, and display of

any package leaks present (Ozdemir and Floros 2004). Among many potential applications of active packaging, there are four applications that are closely related to the improvement in food safety: antimicrobial packaging, freshness and time-temperature integrators, tamper-resistance packaging, and electronic product code systems such as radio-frequency identification (RFID). RFID can also enhance the safety of packaged foods due to the improved traceability (Han et al. 2005). Though the freshness indicator, time-temperature integrator (TTI), and RFID are applications of intelligent packaging and not just of active packaging, these active and intelligent packaging systems can enhance the level of food safety because they can inhibit the bacterial growth in the packaged foods (antimicrobial packaging), indicate nonfresh products so that they can be removed from food supply streams (freshness indicator and TTI), and prevent the injection of foreign objects, malicious tampering, or terrorism (tamper-resistance packaging). While the conventional packaging system maintains the quality of packaged foods, active packaging can improve the quality and the safety of the foods.

Applications of Active Packaging

Active packaging involves an interaction between packaging materials, internal atmosphere, and foods that was designed intentionally to extend the shelf life of the foods (Cutter 2002). Because there are many factors of quality deterioration that could be prevented, active packaging has various applications. Table 9.2 summarizes the commercial packaging materials that possess active functions for food packaging systems.

Antimicrobial Packaging

Microbial growth in packaged foods significantly reduces the safety and quality of foods (Han 2005b). Most foods are perishable and very good media for the growth of pathogenic microorganisms. The composition, water activity, and oxidation-reduction potential of food are very important parameters that affect microbial growth and proliferation (Cousin and Rodriguez 1987; Cutter 2002; Han 2003, 2005b). There are also some extrinsic factors influencing microbial growth: storage temperature, storage period, and gaseous atmosphere (Cutter 2002). Alteration of the above parameters can affect the growth of microorganisms. However, for the purpose of the best quality maintenance of foods, most of the above parameters of packaged foods may not be changeable.

Antimicrobial packaging extends the lag period of microbial growth and reduces the growth rate and maximum growth number (Han 2000). Antimicrobial packaging systems consist of antimicrobial agents (biocide additives in Europe), packaging materials, internal atmosphere, and foods. Antimicrobial packaging attracts more attention from the food and packaging industry because of the increasing demand for minimally processed preservative-free products. The low levels of total preservatives in the packaging system, compared to the amount of directly added preservatives into foods, are also desirable (Han 2000; Vermeiren et al. 2002). Antimicrobial agents may be released from packaging materials through diffusion or vaporization onto the food surface or into the headspace atmosphere. To eliminate adverse effects of the migration of antimicrobial agents, the agents could be immobilized by spacing chemical cross-linkers into polymeric packaging materials.

Various antimicrobial agents could be incorporated into packaging materials or as inserts in the package. They can be classified into three groups: (1) chemical agents; (2) nat-

Table 9.2 Commercial functional films and sachets with application in the active packaging of foods (modified from Han 2005b; López-Rubio et al. 2004)

Company	Brand Name	Active Principle	Internet Information or References
Oxygen-Scavenging Films			
Colormatrix	Amosorb	Polyunsaturated polymers	http://europe.colormatrix.com
Chevron-Phillips	OSP OS 2000	Ethylene methacrylate	http://www.sealedair.com/products
		Cyclohexene methacrylate	http://www.cpchem.com/osp/TechLibrary/Factorsdoc.pdf
Cryovac	Stealth, OS 1000	Polybutadiene + metal salts	Brody et al. 2001
Darex	Dareval	Ascorbate	http://www.darexcontainer.com/dareval.html
Constar	Oxbar	Nylon MXD-6 oxidation	http://www.constar.com/Technology_Oxbar.htm
Toyo-Seikan	Oxyguard	Metal powder	http://www.toyo-seikan.co.jp/e/oxyguard.html
	Oxyblock	Metal powder	http://www.toyo-seikan.co.jp/e/oxyblock.html
Ciba	Shelf-Plus O2	Ascorbate	http://www.cibasc.com/pf/pf_tn.asp?ind=21&sind=45&tn=SHELFPLUS+O2+%2D+2400
Multisorb Technologies	FreshMax		Vermeiren et al. 1999
Carnaud Metal Box	Oxbar		Vermeiren et al. 1999
Advanced Oxygen Tech.	Smartcap		Vermeiren et al. 1999
Americal Can Com.	Miraflex 7	Al-oxide, hydrogen, catalyst	
Oxygen-Scavenging Sachets			
	Oxyban	Glucose oxidase	
	Oxyguard	Iron powder	
Mitsubishi Gas Chem.	Ageless	Iron powder, salts	
Carbon Dioxide–Emitting Films			
CO2 Technologies	Active-strip	Bicarbonate	http://www.co2technologies.com/products.htm
Ethylene, Ammonia, and Hydrogen Sulfide–Scavenging Films			
Grofit Plastic	Bio-Fresh	Minerals	http://www.grofitplastics.com/technical_info.HTM
Chantler Packaging	PeakFresh	Minerals	http://www.chantlerpackaging.com/PF_PackagingNotes/htm/
Evert-Fresh Co.	Evert-Fresh	Ceramics	Vermeiren et al. 1999
Cho Yang Heung San	Orega	Zeolite, metal oxide, carbon	Vermeiren et al. 1999
Peakfresh Products	Peaksorb	Superabsorbant (sheet)	Brody et al. 2001
DuPont Polymers	Bynel IXP101	Modified ethylene-copolymer	Rooney 1995b
?	Profresh	ADI 50	Vermeiren et al. 1999

(*continued*)

Table 9.2 Commercial functional films and sachets with application in the active packaging of foods (modified from Han 2005b; López-Rubio et al. 2004) (*continued*)

Company	Brand Name	Active Principle	Internet Information or References
Aroma-Emitting Films			
Japan Liquid Crystal Corp.		Flavor—cyclodextrin	Anon. 1987
TechniChem Inc.		Fragrance	Brody et al. 2001
Ethylene, Ammonia, and Hydrogen Sulfide–Scavenging Sachets			
Ethylene Control Inc.		$KMnO_4$	Brody et al. 2001
Rengo Packaging Sys.	Green Pack	$KMnO_4$ + silicon oxide	Brody et al. 2001
Humidity-Absorbing Films			
CSP Technologies	Activ-pak	Dessicant	http://www.csptechnologies.com/
Pty Ltd.	Thermarite	Superabsorbant (sheet)	Brody et al. 2001
Toppan Printing Co.	Toppan Sheet	Superabsorbant (sheet)	Brody et al. 2001
Peakfresh Products	Peaksorb	Superabsorbant (sheet)	Brody et al. 2001
Humidity-Absorbing Sachets			
Capitol Specialty Plastics Inc.		Desiccant	Brody et al. 2001
Grace Davison/WR Grace	Flo-tech	Desiccant	Brody et al. 2001
Hammond WA Drierite	Drierite	Desiccant	Brody et al. 2001
Multisorb Tech. Inc.	FreshMax	Desiccant + humectants	Brody et al. 2001
Antimicrobial Films			
Sinanen Zeomic Co.	Zeomic	Ag-zeolite	http://www.zeomic.co.jp/english/
AgIon Technologies	AgIon	Ag-zeolite	http://www.agion-tech.com/Default.asp
Kobico	Apacider	Ag-zeolite	http://www.kobico.co.kr/english
Daikoku Kasei Co.	Piatech	Ag oxide	Brody et al. 2001
Nimiko Co.	Silvi Film	Ag oxide	Brody et al. 2001
Okamoto Industries	Super Wrap		Brody et al. 2001
Sangi Co.	Apacider	Ag zeolite and others	Brody et al. 2001
Shinanen New Ceramics	Zeomic	Ag zeolite	Brody et al. 2001
Kanebo Co.	Bactekiller	Ag zeolite	Brody et al. 2001

Table 9.2 Commercial functional films and sachets with application in the active packaging of foods (modified from Han 2005b; López-Rubio et al. 2004) (*continued*)

Company	Brand Name	Active Principle	Internet Information or References
Mitsubishi Int. Corp.	Zeomix		Brody et al. 2001
DuPont	MicroFree	Ag, CuO, zinc silicate	Brody et al. 2001; Vermeiren et al. 2002
Milliken Co.	Novaron	Ag zirconium phosphate	Vermeiren et al. 2002
Surfacine Development	Surfacine	Ag-halide	Vermeiren et al. 2002
Ishizuka Glass Co.	Ionpure	Ag/glass	Vermeiren et al. 2002
Microban Products Co.	Microban	Triclosan	Brody et al. 2001
Sanitized AG/Clariant	Sanitized, Actigard, Saniprot	Triclosan and others	Suppakul et al. 2003; Vermeiren et al. 2002
Thomson Research Asc.	Ultra-Fresh	Triclosan and others	Vermeiren et al. 2002
Green Cross Co.	WasaOuro	Allyl isothiocyanate	Brody et al. 2001
Rhone-Poulenc	MicroGarde	Clove and others	Brody et al. 2001
Takex Co.	Take Guard	Bamboo extract	Brody et al. 2001
Freund Industrial Co.	Acticap	Ethanol	Smith et al. 1987
Micro Science Tech Co.	Biocleanact	Antibiotics	http://www.biocleanact.com; Han and Moon 2002
Bernard Technologies/ Southwest Research Institute	Microatmosphere	Chlorine dioxide	Brody et al. 2001
Antimicrobial Sachets			
Bourbon Co.		Encapsulated ethanol	Brody et al. 2001
Freund/Biddle Sawyer	Ethicap	Encapsulated ethanol	Brody et al. 2001

Table 9.3 Examples of food-grade antimicrobial agents that can be used for antimicrobial food packaging systems

Classification	Chemical Antimicrobial Agents	Natural Antimicrobial Agents
Organic acids and their derivatives	Acetic acid, benzoic acid, mixture of organic acids, potassium sorbate, sodium benzoate, sorbic anhydride, benzoic anhydride, propyl paraben, methyl paraben, ethyl paraben	Acetic acid, lactic acid, citric acid, malic acid, propionic acid, sorbic acid, succinic acid, tartaric acid, mixture of organic acids
Alcohol		Ethanol
Bacteriocins		Nisin, pediocin, subtilin, lacticin
Fatty acids and fatty acid esters	Lauric acid, palmitoleic acid, glycerol mono-laurate	
Chelating agents	EDTA	Citrate, lactoferrin
Enzymes		Lysozyme, glucose oxidase, lactoperoxidase
Metals	Silver, copper, zirconium, titanium oxide	
Antioxidants	BHA, BHT, TBHQ, iron salts	
Antibiotics		Natamycin
Fungicides	Benomyl, imazalil, sulfur dioxide	
Sanitizing gas	Ozone, chlorine dioxide, carbon monoxide, carbon dioxide	
Sanitizers	Cetyl pyridinium chloride, triclosan, trisodium phosphate, cresol	Acidified NaCl
Polysaccharide		Chitosan
Phenolics	Hydroquinone	Catechin
Plant volatiles		Allyl isothiocyanate, cinnamaldehyde, eugenol, isoeuginol, linalool, terpineol, thymol, carvacrol, pinene
Plant/spice extracts		Grape seed extract, grapefruit seed extract, hop beta acid, Brassica erucic acid oil, rosemary oil, oregano oil, basil oil, clove oil, cinnamon oil, other herb/spice extracts and their oils
Probiotics		Lactic acid bacteria

Modified from Han 2000, 2003, 2005b; Suppakul et al. 2003

ural agents; and (3) probiotics (Han 2005b). Table 9.3 shows potential antimicrobial agents and food-grade preservatives that can be utilized for constructing antimicrobial packaging systems. Because antimicrobial packaging systems are designed to control the growth of microorganisms in packaged foods, the system always consists of antimicrobial agents, target microorganisms, internal atmosphere, packaging materials, and foods. These five components are related to one another for designing the most efficient system features (Han 2005b). Antimicrobial packaging systems have characteristic antimicrobial activity and spectrum to different microorganisms. Therefore, the system should be custom designed based on the characteristics of the five components.

Many antimicrobial systems that exhibit strong antimicrobial activity against target mi-

Table 9.4 Various oxygen scavengers and their mechanisms

Mechanisms	Chemical Scavengers	Natural Scavengers
Radical acceptors	BHA, BHT, TBHQ, PG, photosensitive dyes	
Oxygen interceptors or reducing agents	Aromatic nylon, ferrous iron, sulfites, unsaturated hydrocarbons (isoprene, butadiene), boron, hydrogen with palladium catalyst	Carotenes, tocopherol, ascorbic acid, unsaturated fatty acids
Oxygen absorbers		Glycol, sugar alcohols
Others		Glucose oxidase

croorganisms in culture media do not possess the same effectiveness when they are tested with real foods. This phenomenon clearly shows there is an interaction effect of food ingredients, microorganisms, and antimicrobial agents to the antimicrobial activity (Han 2005b). Therefore, it is necessary to conduct experiments for the effectiveness of an antimicrobial packaging system using a real food instead of culture broth or agar plates.

Oxygen-Scavenging Packaging

Despite the advanced protection of foods by many packaging materials, foods are susceptible to deterioration through biochemical reactions or physical assaults. Food quality is mainly degraded oxidatively. Oxygen-scavenging packaging retards the oxidative degradation of packaged foods. The oxidative degradations happen due to the existence of oxygen, which can be produced by enzymatic or microbial production, leakage of sealing, gas exchange through the packaging body, and oxygen originally existing in the internal atmosphere (Rooney 2005b). The use of oxygen scavengers prevents oxidation of food ingredients, insect damage, and proliferation of aerobic microorganisms (Vermeiren et al. 2003). Oxygen scavengers remove internal oxygen through their oxygen-removing mechanisms, which react with radicals (radical acceptor), oxidize faster than food ingredients (oxygen interceptor), or absorb oxygen on the solid surface physically (oxygen absorber). Table 9.4 lists the oxygen-scavenging agents that have been incorporated into packaging materials or packaging systems. Generally these oxygen-scavenging chemicals are packaged in a sachet that is incorporated with the food enclosed by high-oxygen barrier materials during the packaging process (Cutter 2002). The disadvantages of sachet-type oxygen scavengers are (1) accidental ingestion of the sachet by consumers, (2) additional operations and equipment necessary to insert the sachet into each package, (3) potential leakage of sachet ingredients, causing the contamination of the foods, and (4) limited applications only to dried products, not available to liquid or semiliquid foods (López-Rubio et al. 2004). To overcome these disadvantages, research and development on the oxygen-scavenging films has been actively performed, and there are many commercial film products available.

Ethylene-Scavenging Packaging

Ethylene gas is an endogenic plant hormone that increases respiration rate, leads to maturity, softens the tissue, and therefore accelerates senescence of fruits and vegetables. Due

to the undesirable changes induced in fresh produce by ethylene, the removal of ethylene has been attempted using ethylene-absorbing materials. The most common ethylene absorber is potassium permanganate ($KMnO_4$); however, this chemical is toxic and can not be integrated into food contact materials. For controlled atmosphere storage, large amounts of $KMnO_4$ have been used in the air circulation equipment to remove atmospheric ethylene. For modified atmosphere packaging, $KMnO_4$ can be included in a sachet to prevent the direct contact of the chemical to the foods. To reduce toxicity, alternative absorbers are used. Such absorbers are clay, zeolite, ceramic powder, and mineral oxide powder. A fine powder of these materials absorbs ethylene gas effectively due to the increased surface area contacting the atmosphere. These fine powdery materials can be incorporated into polymers to develop ethylene-absorbing films. However, these films become opaque and do not absorb enough ethylene (López-Rubio et al. 2004). The addition of these powdery materials could change the permeability of the packaging materials. The changes in permeability may create some advantages to modified atmosphere packaging applications because the permeability of oxygen, carbon dioxide, and ethylene are modified. Besides the powdery absorbers, some synthetic chemicals have been incorporated into plastic film materials, such as tetrazine and methyl cyclopropane (Rooney 2005a).

Moisture Control

There are three common moisture-related problems in packaged foods: (1) moisture loss from fresh produce, meats, poultry, fish, and other processed foods; (2) moisture absorption of dried and semidried foods from humid environments; and (3) condensation of moisture inside the package. The first and second phenomena could be prevented by packaging materials that have very low water vapor permeability. For example, polyolefins such as polyethylene and polypropylene are generally good water vapor barrier materials and can prevent the water vapor exchanges between internal and external atmosphere of the packages. However, for the case of fresh produce, meats, poultry, and fish, the water vapor barrier packaging material increases the relative humidity (RH) of the internal atmosphere. The RH can be increased as a result of normal respiration, evaporation, microbial activity, or other reasons. The internal RH over 95% could deposit liquid water by moisture condensation when the storage temperature of the packaged products decreases (Brody et al. 2001). The condensation can moisten the product surface and cause the leakage of soluble nutrients, microbial growth, texture changes, and the loss of product value.

Historically several desiccants were widely used to reduce the RH inside a package. Silica gel, clay, or calcium sulfate sachets have been used for dried foods and pharmaceutical products. The amount of desiccants and the water vapor permeability of sachet membranes govern the capacity and kinetics of moisture removal, respectively (Powers and Calvo 2003). The mass ratio of various humectants or powdered salts to the desiccants is an important factor of the final equilibrium of RH inside the package. The mixtures of desiccants with humectants and salts have RH buffering activity and specific range of RH controlled for moisture-sensitive products. They can prevent moisture condensation inside the package as well as moisture loss from fresh produce, meats, poultry, and fish. Besides the common desiccants, molecular sieves can remove moisture effectively. The porous structured materials can trap moisture in their pores. The sieves will release the trapped water molecules by dehydration process and can be reused. These sieves and other desiccants can be mixed with humectants to maximize the effectiveness of moisture control.

Moisture-control systems have mostly sachet applications. This system is designed as a form of sachet and inserted into packages during the packaging process. However, by transferring these sachet desiccant materials into polymeric films or sheets, and after many consequent studies, commercial moisture-controlling films were developed. The desiccant materials can be sandwiched between a permeable inner layer and a moisture barrier outer layer. Due to some limitation of physical and mechanical property changes, there are still more applications and markets for sachet desiccants; however, the film applications will provide extra benefits of convenience in processing equipment and better consumer acceptance for the food industry, pharmaceutical industry, and specialty packaging industry.

Besides the use of desiccants, microporous films can be used for the entire packaging system to allow the excessive moisture inside the package to escape to the outer atmosphere and reduce the RH lower than the critical moisture content of condensation when the storage temperature decreases to saturated wet bulb temperature (dew point) in psychrometry.

Liquid water in the package also affects the RH of the interior atmosphere. The liquid water originates not only from condensation but also from the drip of tissue fluid from cut meats and produce. Liquid absorbents can remove the liquid water. The absorbent can be incorporated into the packages as forms of absorbing pad, tray, sheet, or sachet.

Freshness Indicators and Time-Temperature Integrators (TTIs)

A freshness indicator is designed to identify a specific target compound inside the package. The target compound could be a product of an unwanted chemical reaction or a metabolite of undesired microbial growth (Smolander 2003). A TTI is an indicator recording the time-temperature profile of the product. It responds to time and temperature changes. A TTI records the history of temperature to identify preexisting temperature abuse (Taoukis and Labuza 2003). The temperature abuse can cause physicochemical changes of food products and the growth of microorganisms. While the freshness indicator detects the growth of pathogens or their metabolites, the TTI indicates that the food product has been improperly treated at an abnormal temperature that may provide favorable conditions to pathogens (Smolander 2003). Both TTIs and freshness indicators are good applications of active packaging to ensure the quality and safety of the packaged foods.

The freshness indicator generally changes its color after reacting with the target chemical agents by the use of indicator dye compounds. There are several commercially available detecting mechanisms to design freshness indicators, which cause the color change or other visual indication. The target compounds include the proton (changes in pH from organic acids or alkali chemicals), nitrogen compounds (ammonia, biogenic amines), sulfuric compounds (H_2S), biochemical metabolites (diacetyl, aldehydes, alcohols, and other fermentation products), and energy resources (glucose or ATP) (Brody et al. 2001; Smolander 2003).

Regardless of mechanisms or principles of freshness indication, the indicating reaction should be irreversible, remaining the unsafe sign of the indicator even after the specific target compounds are diminished. Freshness indicators and TTIs neither improve nor maintain the degree of safety or security. However, these technologies can be used effectively for screening unsafe products and removing them from food chain.

Table 9.5 Materials used for edible films and coatings (modified from Han and Gennadios 2005)

Functional Compositions	Materials
Film-forming Materials	*Proteins*: collagen, gelatin, casein, whey protein, corn zein, wheat gluten, soy protein, egg white protein, fish myofibrillar protein, sorghum protein, pea protein, rice bran protein, cottonseed protein, peanut protein, keratin *Polysaccharides*: starch, modified starch, modified cellulose (CMC, MC, HPC, HPMC), alginate, carrageenan, pectin, pullulan, chitosan, gellan gum, xanthan gum *Lipids*: waxes (beeswax, paraffin, carnauba wax, candelilla wax, rice bran wax), resins (shellac, terpene), acetoglycerides
Plasticizers	Glycerin, propylene glycol, sorbitol, sucrose, polyethylene glycol, corn syrup, water
Functional Additives	Antioxidants, antimicrobials, nutrients, nutraceuticals, pharmaceuticals, flavors, colors
Other Additives	Emulsifiers (lecithin, Tweens, Spans), lipid emulsions (edible waxes, fatty acids)

CMC = carboxy methyle cellulose; MC = methylcellulose; HPC = hydroxypropyl cellulose; HPMC = hydroxypropyl methylcellulose

Edible Coatings and Films

The use of edible films and coatings as carriers of active substance was suggested as a promising application of active food packaging (Cuq et al. 1995; Han 2000, 2001). However, the use of edible films and coatings for food packaging without active substances is also one of active food packaging applications, since the edibility and biodegradability of the films/coatings are additional functions not offered by conventional packaging materials (Han 2002).

Edible films and coatings consist of edible biopolymers and food-grade additives (Table 9.5). Film-forming biopolymers are proteins, carbohydrates, gums, or lipids (Gennadios et al. 1997). Plasticizers and other additives are combined with the film-forming biopolymers to improve the physical properties or functionality.

Edible films and coatings protect food products from physical, chemical, and biological deterioration and enhance the quality (Guilbert et al. 1996; Kester and Fennema 1986). The application of edible films and coatings can readily strengthen food products mechanically, reduce particle clustering, and improve appearance and tactile features on product surfaces (Cisneros-Zevallos et al. 1997; Cuq et al. 1995). It can also prevent moisture migration, oxidation of nutrients, microbial growth on the surface, light-induced chemical changes, and so on (Kester and Fennema 1986). Edible films and coatings also act as barriers against oils, gases, or vapors and as carriers of active substances (Gennadios and Weller 1990; Guilbert and Gontard 1995; Kester and Fennema 1986; Krochta and De Mulder-Johnston 1997; Miller et al., 1998;). These protective functions are subject to enhanced quality of food products, resulting in the extension of shelf life and the improvement of safety (Gennadios and Weller 1990).

Obviously all edible film and coating materials are biodegradable (Krochta 2002). The

biodegradability is one of the greatest advantages of edible films and coatings, along with edibility (Debeaufort et al. 1998). There are also many potential nonfood applications for edible films and coatings; for example, films for agricultural uses (e.g., mulching, tunnel and bale wrap), grocery bags, paper/paperboard coatings, or cushioning foams (Guilbert and Gontard 1995; Han and Krochta 1999, 2001). The use of edible films and coatings as primary packaging may replace conventional packaging materials, partially or totally, and consequently reduce the overall usage of the packaging materials (Krochta and De Mulder-Johnston 1997; Petersen et al. 1999), or simplify the total packaging structure (Debeaufort et al. 1998; Krochta and De Mulder-Johnston 1997).

Electronic Product Code

The electronic product code (EPC) is a compact device identifying each product, carton, box, or pallet in supply chains (Michel 2005). Manufacturers, distributors, and retailers could have better and more accurate management, inspection, and exchange of information by wireless scanning of the electronic tag. Radio-frequency identification (RFID) systems are an application of EPC systems currently exploited by large-scale chain stores and distribution channels. RFID tags are noncontact transducers emitting a coded radio-frequency signal of programmed information (Han et al. 2005).

The use of RFIDs provides benefits to the food industry in at least four ways: shipping and receiving, product theft, counterfeiting, and product recall (Michel 2005). RFID facilitates accurate delivery of the exact amount of the correct food products. Since an RFID system keeps a record of the chains of custody for each product, it prevents item loss or theft. The difference in identification signal of each RFID can determine the authenticity of a shipment, resulting in prevention of counterfeiting. Most of all, an efficient product recall would be the biggest benefit to maximize the ability of RFID for enhancing food safety. By enabling more accurate identification, the RFID system allows immediate access to the exact location of recalled products in the distribution channel. Therefore, the RFID system improves the traceability of food products, resulting in a consequence of safety assurance. Traceability is the ability to trace the history, application, or location of an entity by recorded identifications (ISO 8402). More traceable food products may enhance the safety of food systems. Therefore, the RFID system can enhance the level of safety by tracking the food products more accurately, precisely, and immediately and improving the traceability of the foods.

Commercialization and Further Development

Technical Considerations

Some technical considerations should be taken into account for the commercialization of active packaging materials. When active agents are incorporated into packaging materials, the agents may change the physical and chemical properties of polymeric packaging materials such as permeability, undesired migration of low molecular weight agents, and desorption kinetics of the agents from the materials (López-Rubio et al. 2004). The fundamental barrier functions of packaging systems should be maintained after the incorporation of active agents. The addition of active agents into polymeric materials may not significantly change the mechanical strength of the materials within a practical amount of incorporation; however, the visual or appearance characteristics of the packaging materials, that is, color, transparency, and gloss, can be altered by the small amount of the agents (Han 2003; Han and Floros 1997).

Regulations

In the United States, most active agents can be considered food contact substances. However, when the food contact substance migrates into food or is added into packaging materials for the purpose of changing the characteristics of packaged food intentionally, it should be classified as a food additive (Song and Hepp 2005). Therefore, the clearly authorized classification of the active agent is required before any commercialization considerations of the active packaging material.

The active substances in the active packaging system are not conventional agents for the packaging material. No matter whether the agent releases into the packaged food or not, the active packaging materials are new materials and should be regulated as food contact substances. When the active packaging releases an agent into foods to control the undesirable phenomenon of quality degradation specifically, the migration profile of the agent should also be regulated as a food additive. There could be two legal interpretations: (1) the migrating active agent is a food additive (direct or indirect); or (2) the migrant is a contaminant. There is no specific regulation currently covering the use of active packaging for foods. In the United States, the migrating active agent could be classified as a food additive because the agent has been incorporated into packaging materials intentionally for release into foods and for changing the characteristics of the packaged foods by extending shelf life or preserving quality (Song and Hepp 2005). European regulations may classify the active agent as a contaminant if it has not been listed in the list of the compounds allowed to contact the packaged food (de Kruijf et al. 2002). In addition, the release profile should satisfy the overall migration maximum of 60 mg kg^{-1} food in Europe. When the incorporated agent does not migrate into foods in the case of an absorbing mode system, the agent absorbing target chemicals could be classified as a food contact substance in the United States. In most cases, with some exceptions, the active agent could be either a food additive or a food contact substance.

Consumer Satisfaction and Acceptance of the New Active Packaging Systems

Due to the extra active functionality of active packaging, active packaging would be designed differently from the common barrier packaging system. The active packaging, therefore, could confuse the users and cause fear for consumers. The consumers can confront the new materials and design or question potential health-related perceptions of the interaction between food and packaging (Meroni 2000). The new active packaging system may require some new procedures of processing and consumption different from the traditional uses of packages, equipment, utensils, and household appliances. This could create huge disadvantages to the manufacturing and logistics by increasing costs of installation and new system establishment. However, the active packaging can reduce the dependence of food products on the traditional preservation systems such as a cold-chain system or thermal processing for commercial sterilization.

Collaborating R & D with Related Technologies

The principles upon which packaging acts consist of many scientific disciplines. Chemical reactions have been used to eliminate atmospheric gases such as oxygen, carbon dioxide, and ethylene. Water is removed by substances with high affinity, such as silica gel, dehy-

drated lime, or polyol humectants. Volatile organic matters are largely adsorbed by porous solids such as zeolites or inorganic solids powder through vapor stage equilibrium. Self-heating/self-cooling packages normally involve exothermic or endothermic chemical reactions in a separate compartment of the package (Rooney 2005b). Antimicrobial packaging requires fundamental knowledge of microbial physiology. Biodegradable and edible packaging materials could be developed after studying their polymer material science characteristics. Most active packaging applications utilizing absorption and release modes should be carefully studied for better understanding of the mass transfer profile of the substances. For the successful development of an active packaging system, it is essential to manage the tight collaboration between various knowledge domains.

Conclusions

Active packaging is a packaging system that possesses extra processing functions that do not exist in conventional barrier packaging. Due to these extra functions, active packaging performs its special task during storage, distribution, and consumption. Therefore, active packaging can be a very special non-thermal processing application. More effective technology to enhance the performance of the packaging system has been developed and will continue to be advanced in the future. Active packaging has many advantages and applications for the food industry. The biggest market of active packaging applications is in the area of antimicrobial packaging and oxygen-scavenging packaging. For producers and processors, better protection by extended shelf life and more convenient systems by reduction in process time and skilled labor would be preferred for the successful utilization of active packaging development and applications. For consumers, fresher quality of products and more convenient use of the products will be attractive. New active packaging materials or systems may have a large market in the food industry if the cost-to-benefit ratio is favorable. Since current packaging is not always optimum for many different food products, there are still many opportunities for packaging improvement and consequently the quality and safety management of packaged foods.

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10 The Ozonation Concept: Advantages of Ozone Treatment and Commercial Developments

John S. Novak and James T.C. Yuan

Introduction

Alternative food industry sanitizers to inexpensive chlorine compounds have gained popularity in recent years due to concerns over hazardous chlorinated by-products including trihalomethanes. Ozone, a relatively unstable allotrope of oxygen, has been suggested as an effective antimicrobial sanitizer for water, food, and food processing surfaces and equipment. Although ozone decomposes to a number of free radicals, no residual components are left on products when decomposition is complete, liberating the main product of oxygen. The relatively indiscriminate attack of organic compounds by free radicals from ozone decomposition inactivates microorganisms while conjointly resulting in self-depletion. This chapter examines the advantages while exposing the drawbacks to ozone applications in the food industry. Potential applications for the future are explored as well with respect to current research developments.

An individual's first introduction to "ozone" may bring about recollections of elementary school science and a study of atmospheric conditions. It is true that in the upper atmosphere, a layer of ozone helps to protect the Earth from higher energy ultraviolet (UV) irradiation. At lower altitudes, local weather reports frequently warn of high ozone days and corresponding poor air quality. This chapter will highlight another aspect of ozone—as a chemical disinfectant for industrial uses with respect to foods.

Ozone (O_3), or triatomic oxygen, an allotropic form of oxygen, is produced naturally from oxygen as a result of lightning or UV radiation interactions (Kim et al. 1999). Synthetically, ozone can similarly be manufactured using UV light or corona discharge generators. During ozone production, oxygen molecules (O_2) are split, producing highly reactive free radicals that react with other oxygen molecules, forming ozone (see fig. 10.1). The biocidal activity comes from the molecule's inherent instability. Ozone is a bluish gas at ambient temperatures and pressures that dissolves readily in water at refrigeration temperatures and acidic pH values (Gordon 1995). As the pH or temperature is elevated, ozone decomposes into a variety of reactive free radicals. As a result, ozone is a potent oxidizing agent and effective antimicrobial.

Historical Perspectives

Historically, ozone was first discovered by Schonbein in 1840, followed by a U.S. patent being issued to Fewson in 1888 for use in deodorizing sewer gases (Graham 1997). The first commercial-scale disinfection of potable water with ozone was put into practice in France in 1906 (Graham 1997). The first water treatment plant to use ozone in the United States was installed in 1940, leading to more than 350 municipal water treatment plants

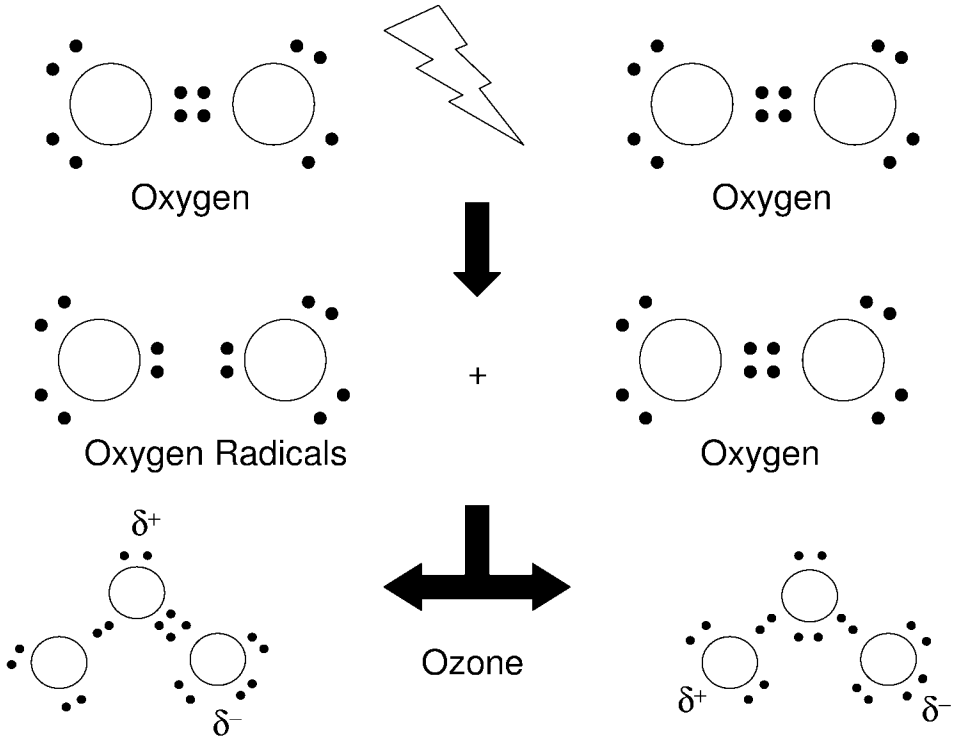

Figure 10.1. Formed from oxygen free radicals, ozone molecules are unstable allotropes of oxygen.

using ozone in the country today (Overbeck 2000). Although numerous research studies have examined the antimicrobial properties of ozone, in practice the technology has been limited exclusively to water treatment for the last hundred years.

In 2001, the U.S. Food and Drug Administration (FDA) approved the use of ozone as an antimicrobial agent in the treatment, storage, and processing of meats and produce (*Federal Register* 2001). Studies have been published recording mostly the research applications of ozone in shellfish disinfection (Violle 1929), prevention of overripening in produce (Gane 1936; Kuprianoff 1953), and refrigerated shelf-life extension of meats (Rusch and Kraemer 1989). Ozone has been proven to be a very potent oxidizing agent that readily inactivates microorganisms in aqueous solutions (Broadwater et al. 1973). Unfortunately, in association with foods, many organic components are reactants as well, resulting in self-depletion of ozone and reduced biocidal effectiveness (Khadre et al. 2001). As a result, an important consideration in the use of ozone should involve the minimum concentration of ozone necessary to decrease microbial concerns while at the same time minimizing damage to the food being treated (Rice et al. 2002). The minimum and maximum ozone exposures required may be food product specific irrespective of the microbial efficiency.

Table 10.1 A comparison of ozone characteristics with respect to commercial applications

Properties of Ozone	Positive Attributes	Negative Attributes
Physical state (gas)	Highly reactive > 100 times chlorine	Unstable $T_{1/2}$ normally < 20 min @ 20°C
Generation (UV, corona discharge)	System can be tailored to specific applications; Produced on-site	Must be produced on-site
Specificity	Indiscriminant attack, multiple targets	Indiscriminant attack, high organic load will deplete ozone
Solubility in water	High at low temperature and low pH	Low at high temperature and high pH
Toxicity	Effective oxidizing agent against a variety of microorganisms	Hazard to workers; Code of OSHA Regulations < 0.1 ppm over the course of an 8-hour work shift
End products	Oxygen	Not applicable
Cost	Long range more economical	Short-term expensive system investment

Ozone Usage: Advantages Outweigh Disadvantages

In evaluating any technological advancement for commercial applications, a comprehensive analysis of beneficial and undesirable attributes must be engaged. Table 10.1 provides a summary comparison of ozone characteristics. The predominant characteristic of ozone is that under standard atmospheric conditions it is a gas as a result, many potential applications are defined by this physical state. As a gas, ozone is capable of penetrating microscopic crevices that may be restrictive to a liquid or solid. The high reactivity of ozone (greater than 100 times chlorine) is thought to be a result of the molecule's instability (half-life of less than 20 minutes in water at 20°C) (Meddows-Taylor 1947). Despite an oxidation potential 1.5 times greater than that of chlorine, ozone at times may appear no more effective. Treatment of foods with bleach leaves a residual that continues to work as an oxidant against microorganisms. The lack of a residual following ozone use can also be considered an environmental advantage over the more commonly used chlorinated compounds that instead result in carcinogenic trihalomethanes following prolonged contact with organic wastes.

Another aspect of ozone's unstable nature is that it cannot be stored in gas cylinders as most other gases. Ozone must be generated on-site and the application system must be tailored to specific uses. This would eliminate the storage and inventory issues of chemicals and enable optimal utilization for each application, such as a surface sanitizer of industrial equipment, an air purifier, a reconditioning potable water treatment, or a food product antimicrobial wash. Although equally unstable in water, ozone is soluble in water and that solubility could be increased by a reduction in water temperature and pH. Therefore, ozone must be more actively replenished when applications require higher temperatures and pH. The solubility of ozone in water also enables the oxidant to be a more effective antimicrobial in damp, cool, refrigerated atmospheres (meat or produce storage rooms) as opposed to warmer, more arid atmospheres. The economic considerations of each tailored ozone application would be expected to likewise fluctuate.

As an oxidant, the reaction mechanism of ozone is rather nonspecific in attacking unsaturated carbon double (C=C) bonds. Bacterial cell death may be attributed to changes in cellular permeability leading to lysis from the oxidation of glycoproteins, glycolipids, or proteins containing sulfhydral groups at the cellular membrane level (Greene et al. 1993). Obviously, some amino acids are more susceptible than others. In descending order these include cysteine, methionine, tryptophan, tyrosine, histidine, cystine, and phenylalanine (Menzel 1971). Ozone has been shown to be reactive as a general protoplasmic oxidant responsible for the destruction of dehydrogenating enzyme systems resulting in interference with cellular respiration (Ingram and Haines 1949). Additionally, the major cause of poliovirus I inactivation by ozone is nucleic acid damage (Roy et al. 1981). Therefore, ozone has been reported to attack numerous cellular constituents including proteins, unsaturated lipids, peptidoglycans, enzymes, and nucleic acids (Khadre et al. 2001).

Unfortunately, the relative nonspecificity of ozone toward animal, plant, or bacterial cells decreases ozone's efficiency when used in systems with a high organic load, as in the case of foods. Antimicrobial applications with high organic load place a higher demand on the available ozone and as a result it must be continually replenished during the treatment. The effectiveness of ozone depends on the amount of residual ozone remaining to react with microorganisms after demands have been met (Kim et al. 1999). As a result, it is necessary to monitor the residual ozone during the course of experiments in order to avoid underestimation of the actual ozone dose (Kim et al. 1999). For these reasons, ozone may be best applied as a terminal stage in a combination processing system that first reduces organic load through another method such as filtration.

Despite effective oxidizing potential against microorganisms, the toxicity of ozone warrants monitoring to prevent worker exposure to more than 0.1 ppm of ozone for an 8-hour work shift, or more than 0.3 ppm of ozone two times for 15 minutes (*Code of Federal Regulations* 1997). Fortunately, ozone can be detected at much lower concentrations (a magnitude of 10 times) by the human nose (Scott and Lesher 1963). Symptoms of overexposure include irritation of eyes, headache, dry throat, and coughing. Higher level exposure could lead to vomiting, shortness of breath, lower blood pressure, and pulmonary edema leading to death. Monitoring by workers is constantly recommended as human senses may be desensitized over prolonged subtle exposure to the pungent oxidant. In addition product ozone overexposures have been reported to lead to discoloration or bleaching and undesirable odors from overoxidation of the foodstuffs (Kim et al. 1999).

One of the positive end products of ozone degradation is oxygen. In water the main oxidizing intermediate formed from the decomposition of ozone is the hydroxyl radical ($\cdot OH$) (Hoigne and Bader 1975). Other free radicals such as the superoxide ($\cdot O_2^-$) and hydroperoxide radical ($HO_2\cdot$) are formed from the reaction of the hydroxyl radical with remaining ozone molecules (Khadre et al. 2001). There has not been an extensive evaluation of damage at the molecular level to foods exposed to these intermediates and whether free radical damage may be passed onto consumers. Some evidence of mutagenic effects of ozone has been investigated in nonhuman studies, however (Brinkman et al. 1964; Fetner 1958; Hamelin and Chung 1975). Such extrapolations to human studies are not typically valid, although they do suggest the need for more extensive research on potential intermediates that may implicate long-term safety hazards associated with ozone-processed foods.

In terms of commercial acceptance for ozone, paramount importance is placed on the economics of the process. In one example, surface sanitation using ozone was examined at a food processing facility in terms of cost analysis (Rice et al. 2002). The facility, which

typically spent $6,000 per year in chemical costs, lowered its wastewater disposal from 15,000 gal/day to 6,000 gal/day for a total annual savings of $18,960, incorporating ozone for sanitation despite an initial bias toward a more "economical" chemical treatment (Rice et al. 2002). Although initial costs for process changes and implementation of ozone technology may seem higher than current practices, analyses should examine the cost savings over an extended time period in years. Many of the savings include recycling of a valuable life-sustaining natural resource, such as water, that increases environmental responsibility and consciousness.

Potential Applications

Industrial applications of ozone can be proposed based upon two primary considerations: (1) the type of product contact—direct or indirect, and (2) the application state—aqueous or gaseous. Examples of direct contact applications using aqueous ozone include the sanitation of water and turbidity clarification for drinking water, swimming pools, spas, cooling towers, irrigation systems, and sewage plants. In addition, ozonated water could be used to provide direct surface antimicrobial disinfection for produce or certain meats, and seafood products pending additional processing. Potential applications for gaseous direct contact with ozone include the destruction of airborne toxins, deodorization of sewer gases, and improvement in air quality. Gaseous ozone has also been used as an antimicrobial to prevent fungal growth during the storage of blackberries (Barth et al. 1995). Ozone has also been used as an alternative to other less environmentally friendly oxidizing or bleaching agents in the pulp and paper industry.

Indirect ozone applications include the aqueous use of ozonated water to wash processing equipment to prevent cross contamination. Ozonated water has been used as acceptable recycled process water in gelatin, casein, and albumin production. Other applications include microbial load reduction in food processing operations that rely heavily on large quantities of recycled water, such as poultry processing or frankfurter skinning operations. Indirect gaseous ozone applications include the maintenance of air quality in produce and meat storage coolers for product shelf-life extension as well as in combination with packaging materials.

Although the protozoan parasite *Cryptosporidium parvum* is capable of surviving water chlorination, a recent report showed that *C. parvum* was 90% inactivated following 5 minutes of treatment with 1 ppm aqueous ozone (Korich et al. 1990). Other researchers have since shown that the *C. parvum* oocysts could be rendered susceptible to chlorinated water if they were first exposed to aqueous ozone (Gyurek et al. 1996). Similarly, water filtration combined with ozonation could lessen the ozone demand in water with high organic debris and improve water quality for recycled water use.

In aqueous solutions, ozone has biocidal activity against bacterial vegetative cells of *Escherichia coli*, *Bacillus cereus*, and *B. megaterium*, but low ozone concentrations (0.12–0.19 ppm) at a contact time of 5 minutes were ineffective when organic matter was present to interfere with the action of ozone on the bacterial cells (Broadwater et al. 1973). Bacterial spores of *B. cereus* and *B. megaterium* were more resistant, requiring a minimum lethal concentration of ozone at 2.29 ppm (Broadwater et al. 1973). Ozonated water was also shown in another study to effectively kill spoilage bacteria, *Pseudomonas aeruginosa*, yeast, *Zygosaccharomyces*, bacilli, fecal contaminants, *Enterococcus faecalis*, and foodborne pathogens of concern, *Listeria monocytogenes*, *Yersinia enterocolitica*, and

Staphylococcus aureus (Restaino et al. 1995). Typically 5–7 $\log_{10}$ reductions in viable cells were seen if the ozone levels were not reduced by the ozone demand of organic nutrients also present in the aqueous solutions. Regardless of the potential inactivation efficiency of ozone against a specific microorganism (complete inactivation of 7 $\log_{10}$ CFU/ml in H_2O), the microorganism was protected against the oxidizing properties of ozone when attached to a food product. Ozone treatments of bacteria on food products unimpressively reduced viability measurements by approximately 1 $\log_{10}$ with respect to untreated conditions. Greater reductions (up to 7 $\log_{10}$ CFU/ml) in microbial viability were seen when the microorganisms were attached to stainless steel surfaces, suggesting a more beneficial role for ozone as a terminal disinfectant of food processing equipment surfaces (Greene et al. 1993; Moore et al. 2000).

Recent Research Findings: A Hurdle Approach

Frequently combination treatments including heat, irradiation, acidic pH, and competitive microflora enhance microbial inactivation in foods (Scott 1989). Others have reported that food preservation practices involving heat, cold, irradiation, high pressure, osmotic stress, and pH modifications have the potential to increase the resistance and virulence of microorganisms (Abee and Wouters 1999; Aldsworth et al. 1998; Bower and Daeschel 1999). The survival potential of *E. coli* O157:H7, *Clostridium perfringens*, and *L. monocytogenes* strains was examined on beef following ozone treatment (3 ppm O_3 for 5 minutes) in combination with mild heat (55°C for 30 minutes), pH variations in the 6–12 range, and NaCl stress from 0 to 4% (Novak and Yuan 2003), and for *C. perfringens* cells, heat treatment at even higher temperatures (60°C) (Novak and Yuan 2003). *C. perfringens* cells exposed to 3 ppm O_3 for 5 minutes following mild heat exposure (55°C for 30 minutes) were, however, more susceptible to ozone treatment (Novak and Yuan 2003). *L. monocytogenes* and *E. coli* O157:H7 stress responses were different from those for *C. perfringens* and were more subdued. Aqueous ozone treatment of meat was found to increase the likelihood that the bacteria surviving initial processing conditions could become more susceptible to subsequent heat, pH variations, or higher salt concentrations (Novak and Yuan 2003).

The spores of *C. perfringens*, which have been reported to survive boiling water for up to 60 minutes (Rhodehamel and Harmon 1998), were more susceptible to 55°C or 75°C following 5 ppm of aqueous ozone for 5 minutes (Novak and Yuan 2004a). The results supported application of ozone treatment of raw meats prior to fabrication to reduce the surface microbial load and increase susceptibility to subsequent heat treatments (Novak and Yuan 2004a). In a continuation of that work, shelf-life extension of meat processed with 5 ppm O_3 for 5 minutes and containing *C. perfringens* spores combined with modified atmosphere packaging (MAP) as a "hurdle" technology was proven to be effective in inhibiting spore germination and outgrowth over 10 days storage at CO_2 concentrations above 30% and 4°C (Novak and Yuan 2004b). These findings strengthen arguments for ozone integration into current food processing schemes.

Ozone Commercial Developments: The System Approach

Although ozone cannot be bottled, shipped, or stored, if combined with the proper system design, this powerful, toxic oxidizing agent can be transformed into a safe disinfectant. An application-specific integration system is necessary for each industrial use and must incor-

porate several essential components that determine overall efficiency. One of the factors is electric power necessary to generate ozone, which could be an economic deterrent to some industrial uses. Another essential component is the selection of an ozone generator, equipped with either ultraviolet irradiation or corona discharge ensuring an adequate O_3 supply for the intended industrial application. In terms of a feed gas, usually air or oxygen may be used, although high-purity oxygen will increase generator efficiency and reduce additional generator maintenance. As ozone gas is a more effective sanitizer in moist air, environmental humidity is an important application consideration. For aqueous ozone solubility, mass transfer of ozone gas into liquid needs to be optimized with respect to the delivery system conditions of system temperature, distribution, and organic load.

An obvious consideration in ozone system design is the selection or replacement of all plumbing, tubing, and hardware having ozone exposure with ozone resistant/compatible materials such as stainless steel, specialized plastics, and elastomers. Oxidizable materials such as rubber, iron, copper, or brass are unacceptable and unsafe. In order to ensure safety, the ozone system should be well contained and frequently monitored during operation with various ozone detectors for any leaks. Appropriate ventilation and destruction of escaping ozone or off-gas is necessary prior to release into the open atmosphere.

Certain commercial applications require a tailored ozone system to accommodate specific requirements resulting in optimum efficiencies. For example, if water is to be recycled, then filtration may be necessary to remove suspended organic matter that may react with the available ozone in the system, reducing its efficiency toward sanitation. Ozone monitoring with regards to product exposure is essential, as the generated ozone is constantly being degraded. In terms of the product load being treated, the actual available reactive ozone in any one system at any given time can vary widely. Therefore, the final system applications will only be as effective as the variables built into the system design through careful and thoughtful consideration of all possible product scenarios.

Conclusion

There is great potential for using the reactive, antimicrobial properties of a natural environmental compound such as ozone when synthesized in a controlled system for food-based applications. Key to the optimization of ozone's applications will be the enhanced utilization of favorable properties while reductively controlling the unfavorable properties. Although ozone technology has existed for over a hundred years, recent acceptance fueled by environmental and health concerns now poises this technology for future longevity and increased usage. Appropriate analysis of industrial application variables ensures the highest antimicrobial efficiencies, whether aqueous or gaseous in application, while reducing product damage and safety concerns. Ultimately system design and monitoring will enable this technology to succeed for future applications whether based on water purification recycling, air quality improvement, product extended storage, and/or equipment surface sanitations.

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11 Electronic Pasteurization

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Introduction

According to the Centers for Disease Control and Prevention (CDC), foodborne illnesses account for millions of infections and fatalities around the world. Foodborne illnesses are, however, preventable. They can be prevented by improved food production methods, improved food processing technologies, and improved food preparation and consumption habits within homes. A number of food processing technologies have been developed and employed in recent years. Electronic pasteurization, a food processing technology involving the use of ionizing radiation to destroy microbial pathogens, is a feasible solution to prevent foodborne illness and enhance global competitiveness. Electron beam (E-beam)–based electronic pasteurization is a technology with worldwide applicability. Electronic pasteurization, however, is intended to be used only in conjunction with a comprehensive Hazard Analysis Critical Control Point (HACCP) plan and should never be considered a substitute for good manufacturing practices and sanitary procedures. Given the need for pathogen-free foods and the ability to prevent foodborne illnesses, there should be a concerted attempt at streamlining the federal and local regulations that can facilitate the adoption of this technology worldwide.

A number of food processing technologies have been developed in recent years. There is probably no other food processing technology that has been as extensively researched as electronic pasteurization (Smith and Pillai 2004). However, none of the technologies have had the same level of promise, criticisms, and man-made hurdles as electronic pasteurization. Electronic pasteurization involves the use of ionizing radiation (electrons) to destroy pests and pathogens from food and agricultural products. Food irradiation can be accomplished using either radioactive isotopes or using accelerated electrons (electron beam/electronic pasteurization). This chapter discusses the underlying technologies and the potential research areas as they relate to electronic pasteurization.

The Societal Implications of Foodborne Pathogens

Recent estimates in the United States show that foodborne illnesses cause over $7 billion in economic loss each year (Mead et al. 1999). Over seventy-four million pounds of contaminated meat and meat products were recalled between 2000 and 2003 (USDA/FSIS 2004). Pathogen contamination is supposedly responsible for over five thousand deaths (see fig. 11.1b) and approximately seventy-six million cases of illnesses (see fig. 11.1a) in the United States on an annual basis (Mead et al. 1999). A majority of pathogens that are known to cause human foodborne illnesses reside in food animals such as cattle, poultry, and swine. These animals harbor highly infectious pathogens such as *Salmonella* spp., *Campylobacter* spp., and toxigenic *E. coli* strains. These pathogens are responsible for causing sporadic illnesses and chronic complications in millions of people around the world (Tauxe 1997). Meat and poultry products are normally contaminated due to inade-

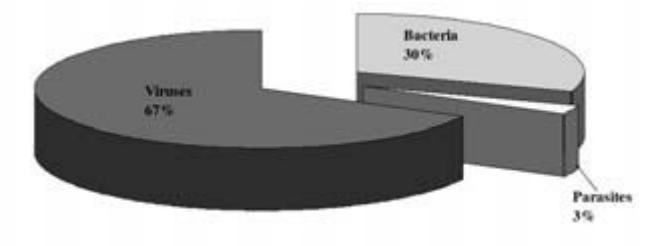

Figure 11.1a. Etiology of foodborne human illnesses in the United States on an annual basis (modified from Mead et al. 1999).

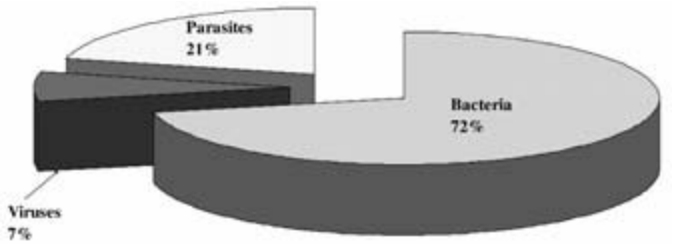

Figure 11.1b. Etiology of foodborne human deaths in the United States on an annual basis (modified from Mead et al. 1999).

quate disinfection, cross contamination, environmental contamination, and improper cooking and handling practices within homes. Environmental contamination of products such as cooked sausage, cooked corned beef, and luncheon meats by *Listeria monocytogenes* and *Salmonella* spp. is a major problem facing the ready-to-eat (RTE) food industry (Levine et al. 2001). Additionally, contaminated irrigation water can lead to the contamination of fruits and vegetables that are consumed either raw or with minimal processing (e.g., green onions, cantaloupes, cilantro, and lettuce). In addition to bacterial pathogens, foodborne viral agents such as Norovirus and hepatitis A virus (HAV) are responsible for a significant number of human infections. Over five hundred people were infected from a single restaurant in Pennsylvania by HAV after they consumed salsa that was prepared using green onions that were contaminated by the virus through irrigation water (CDC 2003). Human enteric caliciviruses (HECVs) are also recognized as key agents responsible for outbreaks of acute nonbacterial gastroenteritis (Koopmans and Duizer 2004). Norovirus, a genus of HECVs, is among the leading cause of foodborne and waterborne gastroenteritis in the United Kingdom, United States, and the Netherlands (deWit et al. 2003; Fankhauser et al. 2002; Hale et al. 2000).

The Underlying Technology

Ionizing radiation as a pathogen inactivation technology was patented as early as 1905, and the technology was evaluated as early as 1921 to destroy trichinae in pork (Josephson 1983). Ionizing radiation is defined as radiation that has enough energy to remove electrons from atoms, thereby leading to the formation of ions. The unit for the dose of ioniz-

ing radiation (i.e., the amount of energy deposited per unit mass of material) is gray (Gy). One-thousand (1,000) Gy = 1 kGy (1 kGy = 1 joule/gm). Ionizing radiation can occur naturally from radioactive isotopes or from artificially created isotopes using nuclear reactors and linear accelerators. There are different types of ionizing radiation such as X-rays, gamma rays, and beta rays, depending on the source. However, all ionizing radiations function the same way, namely, "stripping" electrons off the atoms, thereby causing ionizations of the atoms in the food materials. The irradiation sources that have been internationally approved for food processing include gamma rays produced from radioisotopes cobalt-60 (1.17 and 1.33 MeV) and cesium-137 (0.662 MeV); machine-generated (via linear accelerators) electron beams (max. energy 10 MeV); and X-rays (max energy 5 MeV) (Codex 1984) (recent legislation in the United States has permitted the use of X-rays up to 7 MeV).

There are unique aspects to the construction and engineering specifications of individual facilities, depending on the type of ionizing radiation produced. For example, gamma facilities such as those employing cobalt-60 have specific characteristics to protect workers and the surrounding environment from the radioactive isotopes and for storing the isotope material under water when not in use. Electron (E-beam) (e-rays) and X-ray (photon) facilities, on the other hand, do not have these requirements. Irrespective of the source and the facility providing the irradiation, all types of ionizing radiation destroy biological entities by essentially the same process: the radiation causing "breaks" in the DNA or RNA double helix. It is believed that ionizing radiations disrupt normal cellular activity by damaging the nucleic acids by "direct" and/or "indirect" effects. Specifically, single- or double-stranded breaks occur on the DNA or RNA. The observed lethal effect to RNA containing viruses subjected to these types of ionization is evidence that RNA is also a target for electronic pasteurization. Ionizing radiation does not discriminate between pathogens and nonpathogens, and so both the indigenous normal flora and pathogens on a food product can be inactivated.

Linear accelerators employ commercial electricity to generate accelerated electrons. These electrons are propelled to just below the speed of light and are used to bombard the food item. The accelerated electrons during E-beam irradiation (electronic pasteurization) damage the nucleic acids by direct "hits." Additionally, damage to the nucleic acids can also occur when the radiation ionizes an adjacent molecule, which in turn reacts with the genetic material. Water is very often the adjacent molecule that ends up producing a lethal product (Grecz et al. 1983). Ionizing radiation causes water molecules to lose an electron, producing H_2O^+ and e^-. These products react with other water molecules to produce a number of compounds, including hydrogen and hydroxyl radicals, oxygen, and hydrogen peroxide. These products in turn react with other water molecules, with nucleic acids, and with other biologically sensitive molecules. The most reactive by-products arising from the hydrolysis of water are the hydroxyl radicals (OH^-) and hydrogen peroxide (H_2O_2). These molecules are known to react with the nucleic acids and the chemical bonds that bind one nucleic acid to another in a single strand, as well as with the bonds that link the adjacent base pair in an opposite strand. Since the precise location of where the ionization of water occurs and where the direct DNA hits occur are random, the damage sites on the DNA molecule are random. The indirect effects can also cause single- and double-stranded breaks of the nucleic acid molecules. Though biological systems do have a capacity to repair both single- and double-stranded breaks of the DNA backbone, the damage occurring from ionizing radiation is so extensive that the bacterial repair of radiation damage is nearly impossible.

Many kinds of ionizing radiation occur in nature. The types differ in terms of the dis-

tance they can penetrate, and the amount of energy they deposit when crossing an individual cell. However, it is generally the *total amount of energy deposited*, not the type of depositing radiation, that determines the magnitude of the damage to prokaryotic cells. Thus, only the two types of radiation that are most easily managed, electrons up to 10 MeV and photons (X-rays or gamma rays) up to 5 MeV, are normally considered for radiation processing applications. These two types of radiation are not only relatively easy to produce, they also hold the advantage of not producing radioactive materials. It generally requires the addition of a neutron to the nucleus of an atom to convert that atom to a radioactive form; electrons and photons at these energies cannot do it. The accelerators that we use to produce the electrons (E-beam) and photons (X-rays) cannot produce neutrons.

Electrons and photons are used for food processing because these particles can carry large amounts of energy, up to 10,000,000 electron volts (by comparison, a visible light photon carries about 2 electron volts) and can easily transfer enough energy to a molecule to break chemical bonds. Actually, when a photon interacts with a molecule the photon will lose most, if not all, of its energy, but only about 3 eV actually goes into breaking chemical bonds. The rest of the photon energy goes to the electron knocked out of the molecule. This high-energy electron, in turn, *ionizes* (thus the term *ionizing*) or excites almost every molecule that it passes, producing many more damaged molecules. Most of the chemical changes are produced by these energetic electrons.

The primary difference between photon and electronic pasteurization is efficiency. One can use electrons in an X-ray tube to produce photons, but this is a relatively inefficient process (only 20% of the energy of the electrons is converted to photon energy), and the resulting photons have a relatively low probability of transferring energy to electrons, so some of them will go through any given target and deposit their energy in the surrounding shielding material. On the other hand, electrons have relatively well-defined, short range in most materials so they can deposit nearly all of their energy in a target of the right thickness. So, if a charged particle accelerator is used to generate the electrons, nearly all of the energy can be deposited in the target, but if electrons are used to make X-rays, much of the energy will be wasted as heat in the X-ray tube target, and some of it will be wasted in the shielding needed around the irradiation system. Generally speaking, if the thickness of the object to be irradiated is greater than twice the penetrating depth of the electrons, the only option is to use X-rays or gamma rays from a radioactive source such as cobalt-60 or cesium-137.

Cobalt-60 and cesium-137 are relatively inexpensive by-products of atomic fission. However, there are significant environmental and occupational issues to be considered when employing these radioactive isotopes for food processing. A major drawback compared to E-beam pasteurization is that the radiation source cannot be switched "off" and "on." The isotopes are normally stored underwater to quench the radiation. So regulatory issues related to permits and approvals for radioactive isotope handling, storage, and disposal have to be addressed. The food materials to be irradiated are placed around the "source," which is often raised into position from the storage mode. The processing temperature is controlled by adjusting the temperature of the entire room, or the products are placed within temperature-controlled containers. The half-life of cobalt-60 is 5.27 years, compared to cesium-137, which is approximately 30 years. The dose rate from cobalt-60 is approximately 100 Gy/min. Electron beam–based pasteurization has a higher dose rate (10^3–10^6 Gy/sec), which translates to a higher energy deposition. In contrast to radioisotope sources, the E-beam sources (i.e., linear accelerators) can be turned off and there are

Table 11.1 D_{10} values for selected pathogens on meat and poultry products (adapted from Molins 2001)

Target Organism	Product Temp (°C)	Product	D_{10} value (kGy)
Staphylococcus aureus	5	Turkey breast meat	0.45
Campylobacter jejuni	30	Ground turkey meat	0.16
	5		0.19
	−30		0.29
Salmonella Heidelberg	0	Poultry (air packed)	0.24
	0	Poultry (vacuum packed)	0.39
	3	Ground beef	0.55–0.78
Salmonella spp.	5	Turkey breast meat	0.71
Listeria monocytogenes	5	Beef	0.45
E.coli O157:H7	5	Ground beef patties	0.27–0.38

no residual worker or environmental safety issues. The food sample to be irradiated is placed on a conveyor belt and the product exposed for a defined period of time to achieve varying doses. The mono-directional characteristic of the radiation permits a greater flexibility in product package design. The penetration capacity of the E-beam source does depend on its energy. Electrons from a 10 MeV source are more penetrating than electrons from a 4 MeV source. However, E-beam has a limited (approximately 3 inches) penetration capacity compared to X-rays and gamma rays from radioisotope sources. To overcome penetration depth issues, most E-beam pasteurization facilities have linear accelerators positioned on the top and bottom of a conveyor belt to maximize penetration. The technological advantages of E-beam are seconded by some of its financial highlights. Linear accelerators that are currently used in the food industry in the United States cost approximately $1.5 million. The current commercial cost in the United States for E-beam irradiated ground beef is approximately $.05–$.08 per pound.

Future Research Directions

Sensitivity of Foodborne Viruses to Electronic Pasteurization

Foodborne viruses are responsible for a large number of human illness cases. Enteric viruses such as rotavirus, enteric adenoviruses, Noroviruses, HAV, hepatitis virus type E (HEV), and astrovirus can be transmitted through foods (Goyal 2004). Studies are needed to identify the electronic pasteurization dose and food matrix properties that can eliminate viral pathogens, especially in RTE foods and fruits and vegetables that are minimally processed and yet highly vulnerable to fecal contamination.

Sensitization of Pathogens and Sensitivity to Electronic Pasteurization

Studies have shown that a number of factors can affect the radiation sensitivity of microbial pathogens (table 11.1). Certain chemical components, when added extraneously, can significantly reduce the D_{10} value of a particular pathogen. The precise mechanisms that are involved in this radio-sensitization of microbial pathogens need to be further elucidated. A better understanding of the factors controlling the sensitization of microbial pathogens can allow for the incorporation of specific "sensitizing" molecules directly to

the food, the matrix, or into the packaging materials to attain or prevent a certain desired level of nucleic acid damage. Buchanan et al. (1999) reported that different strains of the same pathogen can exhibit significant differences in radiation sensitivity, presumably a reflection of their physiological status. Microbial cells in the starvation mode can also exhibit increased resistance to ionizing irradiation. Studies have also shown that carbon monoxide in modified atmosphere packaging (MAP) and hydrogen peroxide treatments can also be protective to varying degrees. A number of other stress factors such as osmotic stress, heat stress, and alkali stress can also enhance radiation resistance. The precise mechanism of protection or repair needs to be elucidated so that appropriate strategies can be adopted when irradiating such foods.

Organoleptic Attributes

There is a need for better standardization of sensory changes or organoleptic attributes of irradiated products as it relates to irradiation sources, irradiation conditions, dosimetry, and product profiles. Without standardization, it would be difficult to compare and analyze electronic pasteurization results. Technologies such as multidimensional gas chromatography for analytical determination of specific odoriferous compounds in irradiated foods need to be explored.

Low-Dose Electronic Pasteurization and Dosimetry

Improvements in dosimeters, radiochromic film, and sensitivity of electron spin resonance (ESR) instruments are needed. Instruments that can measure radiation doses in the range of 10 Gy to 1,000 Gy are needed as the products and the objectives of electronic pasteurization expand. A dosimeter certification service will be needed to assure consistent results at different processing and research facilities. These analytical improvements will require the development of standardized techniques for dosimeter placement and methods for reading dosimeters.

Product Packaging

Research on the next generation of packaging materials that can retard possible negative changes in sensory attributes or to enhance desired sensory attributes during electronic pasteurization is needed. Studies suggest that MAP in combination with irradiation can improve the chemical, physical, and microbiological safety of a variety of foods (Ahn et al. 2003; Fan and Sokorai 2002; Fan et al. 2003; Song et al. 2003). Research on synergistic action by antimicrobial coatings/antioxidant additions and irradiation can provide avenues that could potentially extend the product lines for which electronic pasteurization becomes a viable option. The development of "intelligent" packaging material or indicators that can visually denote an irradiated product, or dose range, or detect adverse changes in a product can also find commercial application.

Multicomponent Foods

Multicomponent foods will find increasing demand due to changing human lifestyles. Developing electronic pasteurization protocols for these type of foods will become a key

requirement as food pasteurization moves forward. The issues of dosimetry and pathogen reduction and sensory issues will be significant in multicomponent foods because of the anticipated differences in the food matrix, the potential varying pathogen loads, the types of pathogens that could be encountered, and the critical need to retain the sensory attributes of the packaged meals.

Conclusions

The technology to prevent foodborne illnesses is available. Millions of lives can be saved if the microbiological safety of the foods is improved. There needs to be a concerted attempt at giving the consumers the "choice" to purchase irradiated food products. Without this choice consumers cannot be expected to show that there is a demand for irradiated food products. With wider acceptance of this technology, international trade in irradiated foods will also increase. Given the proven toxicological and nutritional safety of irradiated food products and ingredients it can be argued that regional or national barriers to irradiated foods constitute "illegal trade barriers."

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12 High-Pressure Processing of Foods

Gaurav Tewari

Introduction

High-pressure processing (HPP) is gaining in popularity with the North American food industry because of its capacity to inactivate pathogenic microorganisms with minimal heat treatment, resulting in the almost complete retention of nutritional and sensory characteristics of fresh food without sacrificing shelf life. Other advantages of HPP over traditional thermal processing include reduced process times; minimal heat damage problems; retention of freshness, flavor, texture, and color; no vitamin C loss; no undesirable changes in food during *pressure-shift freezing* due to reduced crystal size and multiple ice-phase forms; and minimal undesirable functionality alterations. Changes that may be made improve functional properties of food constituents resulting in value-added products. Minimization of damage during pressure-shift freezing and thawing using HPP, non-thermally-induced enzyme inactivation, and desirable changes in starch-gelatinization properties are some other examples of potential benefits of HPP. However, spore inactivation is a major challenge for HPP. Methods used to achieve full inactivation of spores using HPP are yet to be developed. In thermal processing, D (time required in minutes to reduce the microbial population tenfold), Z (temperature in °C yielding a tenfold change in D), and F_o (the integrated lethal value from all heat received by a treated food with a reference temperature of 121.1°C and assuming a Z-value of 10°C) values are standard processing parameters; however, there is a need to develop and standardize HPP process parameters with respect to *microbial inactivation,* because none exist. This is essential before *commercialization* of this technology can take place. In this chapter, basic principles of HPP are explained and the major research done on various HPP applications around the world is critically evaluated. Recommendations are made for major research areas in HPP for commercialization of this technology.

Non-thermal food processing techniques are regarded with special interest by the food industry. Among non-thermal techniques (pulse-electric field pasteurization, high-intensity pulsed lights, high-intensity pulsed-magnetic field, ozone treatment), HPP is also gaining in popularity with food processors not only because of its *food preservation* capability but also because of its potential to achieve interesting functional effects (Leadley and Williams 1997). High-pressure processing has had application for years in other industries that process or use ceramics, carbon graphite, diamond, steel/alloy, and plastics. Hite et al. (1914) were the first researchers who reported the effects of HPP on food microorganisms by subjecting milk to pressures of 650 MPa and obtaining a reduction in the viable numbers of microbes. For the last 15 years, the use of HPP has been explored extensively in food industry and related research institutions due to the increased demand by consumers for improved nutritional and sensory characteristics of food without loss of "fresh" taste. In recent years, HPP has been extensively used in Japan and a variety of food products like jams and fruit juices have been processed (Cheftel 1995). There have been ten to fifteen types of pressurized foods on the Japanese market, but several have disappeared, and those

that remain are so specific that they would have little interest to European or American markets. Nevertheless, interest in HPP derives from its ability to deliver foods with fresh-like tastes without added preservatives. Examples of commercial pressurized products in Europe or the United States are (1) orange juice by UltiFruit®, Pernod Richard Company, France; (2) acidified avocado puree (guacamole) by Avomex Company in the United States (Texas/Mexico); and (3) sliced ham (both cured-cooked and raw-cooked) by Espuna Company, Spain. Volumes produced are still very small. Pressurized fruit preparation from yogurt should be coming soon on the market. The European "Novel Foods" Directive (May 1997) has introduced regulatory problems and slowed the introduction of new pressurized products. The capability and limitations of HPP have recently been reviewed and studied extensively by food scientists and food engineers (Autio 1998; Cheftel 1995; Farr 1990; Knorr 1995; Leadley and Williams 1997; Thakur and Nelson 1998). The aim of this chapter is to provide a current review of different aspects and potential applications of HPP and to critically examine HPP-related studies.

Mechanism of HPP

Any phenomenon in equilibrium (chemical reaction, phase transition, change in molecular configuration), accompanied by a decrease in volume, can be enhanced by pressure (Le Chatelier's principle: Leadley and Williams 1997). Thus, HPP affects any phenomenon in food systems where a volume change is involved and favors phenomena that result in a volume decrease. The HPP affects noncovalent bonds (hydrogen, ionic, and hydrophobic bonds) substantially, as some noncovalent bonds are very sensitive to pressure, which means that low molecular weight food components (responsible for nutritional and sensory characteristics) are not affected, whereas high molecular weight components (whose tertiary structure is important for functionality determination) are sensitive. Some specific covalent bonds are also modified by pressure. The other principles that govern HPP are the *isostatic principle,* which implies that the transmittance of pressure is uniform and instantaneous (independent of size and geometry of food)—however, transmittance is not instantaneous when gases are present—and the *microscopic ordering principle*, which implies that at constant temperature, an increase in pressure increases the degree of ordering of the molecules of a substance (Heremans 1992). Another interesting rule concerns the small energy needed to compress a solid or liquid to 500 MPa as compared to heating to 100°C, because compressibility is small. HPP offers several advantages: reduced process times; minimal heat penetration/heat damage problems; freshness, flavor, texture, and color are well retained; there is no vitamin C loss; multiple changes in ice-phase forms resulting in pressure-shift freezing; and functionality alterations are minimized compared with traditional thermal processing (Cheftel 1995; Farr 1990; Knorr 1995; Leadley and Williams 1997; Mertens 1992; Williams 1994).

Applications of HPP

Sensory and Nutritional Characteristics—Retention, Shelf-Life Extension, and Value-Added Products

As mentioned above, HPP does not depreciate the nutritional and sensory characteristics of food, and yet it maintains shelf life (table 12.1). Eshtiaghi and Knorr (1993) compared the effect of HPP with water blanching on the microbial safety, quality (softness), and

Table 12.1 Application of HPP in retention of sensory and nutritional characteristics of fruits and vegetables

Reference	Product	Pressure (MPa)	Holding Time (min)	Temperature (°C)	Microorganisms Tested	Other Parameters Studied
Eshtiaghi and Knorr (1993)	Potato cubes	400	15	5–50		Microbial safety, softness, functionality
Rovere et al. (1997)	Chopped tomatoes	400, 600, or 800	3, 5, or 7			Color, sugar content, pH
Maggi et al. (1994)	Apricot nectar, distilled water	600–900	1–20	20	*Byssochlamys fulva*, *B. nivea*, *Neosartorya fischeri*, *Talaromyces flavus*	
Kimura et al. (1994)	Jams					Quality (volatile flavor components, anthocyanins, browning index, furfural, sucrose, vitamin C, microbiological stabilization)
Moio et al. (1995)	White and red grape must	304–811	1–5	25		Microbiological stabilization
Dong et al. (1996)	*Angelica keiskei* juice	0.01	7	25		Sensory and shelf life
Kloczko and Radomski (1996)	Fresh apples, pears, bananas, parsley, potatoes, celery, carrot juice, apple juice, vitaminized carrot, mixed apple and vitaminized carrot juice	300, 370	6, 15	25		Preservation, aroma, flavor, microbial quality
Pehrsson (1996)	Citrus juice	500–700	1–1.5	0–5		Freshness
Donsi et al. (1996)	Orange juice	350	1	30		Microbial activity and chemical composition

(*continued*)

Table 12.1 Application of HPP in retention of sensory and nutritional characteristics of fruits and vegetables (*continued*)

Reference	Product	Pressure (MPa)	Holding Time (min)	Temperature (°C)	Microorganisms Tested	Other Parameters Studied
Butz et al. (1997)	Vegetable juices (beets, carrots, cauliflower, spinach, tomatoes, kohlrabi, grapefruit, strawberries	400, 600	10	35		Anti-mutagenic activity
Gow and Hsin (1996)	"Guava puree"	400, 600	15	25		Quality and shelf life
Severini et al. (1997)	Extra virgin olive and seed oils (grape seed, sun-flower, soyabean, peanut, and maize)	700	10	25		Lipid oxidation

functionality (polyphenoloxidase [PPO] activity, leaching of potassium, and loss of ascorbic acid) of potato cubes. Total inactivation of microbes and PPO activity occurred at 20°C (using dilute citric acid solution at 0.5 or 1.0% as immersion medium). Water-blanched and high-pressure-treated potato cubes had similar softness but potassium leaching was reduced by 20%; in addition, ascorbic acid was better retained (90% at 5°C to 35% at 50°C) in high-pressure-treated vacuum-packaged samples. However, they did not investigate use of different pressure ranges, which may have resulted in different functional characteristics. Rovere et al. (1997) studied the effects of HPP on chopped tomatoes. They pretreated tomatoes at 25, 50, or 85°C, followed by cooling and vacuum packaging. After this, the tomatoes were subjected to pressures. They reported that color, sugar content, and pH were affected by pressure, and that viscosity decreased with increasing blanching temperature and increased with increasing pressures. They also found that pressure has a significant inactivating effect on polygalacturonase-type pectic enzymes, but had only little effect on pectin esterases (PEs). They concluded that HPP may control various quality parameters of chopped tomatoes.

Kimura et al. (1994) compared the quality (volatile flavor components, anthocyanins, browning index, furfural, sucrose, and vitamin C content) of pressure-treated and heat-treated jams during storage at 5 and 25°C for 1–3 months. Immediately after processing, the pressure-treated jams had better fresh quality than heat-treated jams, and the quality was maintained in both at low-temperature storage, but not at room temperature for pressure-treated jams. The presence of dissolved oxygen and enzymes was believed to have resulted in deterioration of pressure-treated jam held at ambient temperature. However, pressure-treated jam could be stored at refrigeration temperatures with minimal loss in sensory and nutritional characteristics for up to 3 months. In their study for microbiological stabilization of white and red grape must during HPP, Moio et al. (1995) concluded that the rate of microbial inactivation was directly proportional to pressure used. Stabilization of white wine must occurred at 507 MPa for 3 minutes, whereas complete microbial inactivation was not achieved in red must (even at 811 MPa for 5 minutes), which could have been due to its high concentration of suspended solids. Gow and Hsin (1996) compared the quality and shelf life of pressure-treated "guava puree" with thermally pasteurized (88–90°C for 24 seconds) guava puree. A substantial inactivation of microbes (< 10 cfu/mL) was observed at 600 MPa, and the pressure-treated samples showed no color change, no degradation of pectin, no cloud formation, and had the same ascorbic acid content as fresh samples. However, enzyme inactivation was more pronounced in thermally treated samples. The pressure-treated guava puree (600 MPa) maintained good quality (similar to freshly extracted guava puree) for 40 days when stored at 4°C. It is to be noted that guava puree is particularly sensitive to enzymatic browning reactions, which are inhibited during HPP to some extent.

Dong et al. (1996) studied the effect of HPP on the shelf life and sensory characteristics of *Angelica keiskei* juice by subjecting it to a pressure of 0.01 MPa for 7 minutes and storing it at 4°C. *Pseudomonas* spp., *Escherichia coli*, and coliform bacteria were totally inhibited by HPP. During storage, microbiological quality and sensory characteristics were monitored and it was found that HPP did not significantly influence freshness, sweetness, and bitterness, but after 8 days (at 4°C), pressure-treated juices had better freshness ratings than controls (nonpressurized juice). Kloczko and Radomski (1996) studied preservation of fresh fruits, vegetables, and fruit and vegetable juices by subjecting them to pressures and subsequently storing them at 6°C. They reported that HPP had no beneficial effects on

keeping quality of fruits and vegetables, whereas immediately after pressurization, and after 55 days of refrigerated storage, pressure-treated juices had better aroma, flavor, and microbiological quality than untreated controls, and vitamin C content remained the same or declined slightly. Pehrsson (1996) described experiments on HPP of microbially stable citrus juice, where they processed juices for 60–90 seconds at refrigeration or freezing temperature. The product was stored and distributed under refrigeration. The pressure-treated juice was stable for 6 months at 4°C, without losing any freshness (as compared to juices thermally treated at 98°C for 10 seconds). Donsi et al. (1996) studied the high-pressure stabilization of orange juice by evaluating microbial activity and the chemical composition of orange juice treated at different pressure levels for various operating times. They obtained a 2-month shelf life for pressure-treated orange juice (at 350 MPa for 1 minute at 30°C) stored under refrigeration. Butz et al. (1997) studied the effects of HPP on antimutagenic activities of fruit and vegetable juices. The antimutagenic activity was compared with raw and heated samples (100 or 50°C for 10 minutes). They reported that antimutagenicity of strawberry and grapefruit juices was not affected by heat and pressure. Also, vegetable juices exhibited moderate to strong antimutagenicity, whereas the antimutagenic activity of carrot, leek, spinach, kohlrabi, and cauliflower juices was sensitive to heat treatment but remained unaffected by pressure treatment.

Moormans et al. (1996) investigated the use of high-pressure throttling (HPT) as an alternative to thermal processing of milk before acidification to yogurt. They reported that when HPT was used to increase milk temperature to 80°C (above minimum pasteurization temperature), followed by rapid cooling to 40°C, this resulted in a 4-log reduction of microbial numbers and increased viscosity, yielding a thick and creamy yogurt (requiring no further addition of polysaccharides).

Severini et al. (1997) studied the effects of high pressure on the lipid oxidation of extra-virgin olive and seed oils. Peroxidase value, p-anisidine value, rancimat test, and volatile hydrocarbons were the analytical parameters measured to study the oxidative stability of the pressure-treated oils. They reported pressure treatment changed p-anisidine values but not others (peroxidase values and volatile hydrocarbons). Other parameters affecting high-pressure treatment were origin, composition, initial quality, and age of the oils, and it was found that olive oils were more resistant to oxidation, which suggests need for replacement of seed oil with extra-virgin olive oil during HPP to extend shelf life of foods. They conducted their study using only one pressure, temperature, and time period. Further studies should be conducted using different combinations of pressure, temperature, and time intervals, which may result in other value-added products and opportunities.

Microbial Inactivation

Application of HPP as a method for microbial inactivation has stimulated considerable interest in the food industry. The effectiveness of HPP on microbial inactivation has to be studied in great detail to ensure the safety of food treated in this manner. Currently, research in this area has concentrated mainly on the effect of HPP on spores and vegetative cells of different pathogenic bacterial species. Detectable effects of HPP on microbial cells include an increase in the permeability of cell membranes and possible inhibition of enzymes vital for survival and reproduction of the bacterial cells (Farr 1990). To design appropriate processing conditions for HPP of food materials, it is essential to know the precise tolerance levels of different microbial species to HPP and the mechanisms by which

that tolerance level can be minimized. A knowledge of critical factors that affect the baroresistance of different bacterial species will help in the development of more effective and accurate high-pressure processors. Inappropriate use of a variety of parameters like pressure range, processing temperature, initial temperature of sample, holding time, and packaging type may adversely affect the outcome of HPP. Thus a thorough understanding of the effect of a variation in critical factors on the intracellular changes undergone by pressure-treated microbial species is essential for documentation of a safe HPP. The physicochemical environment can adversely change the resistance of a bacterial species to pressure. In most cases, the effect of HPP on Gram positive bacteria is less pronounced than on Gram negative species. Factors such as the water activity and pH also influence the extent to which foods need to be treated to eliminate pathogenic microorganisms.

Hoover et al. (1989) reported that most bacteria are baroduric; that is, they are capable of enduring high pressures but grow well at atmospheric pressures. Hauben et al. (1997) studied HPP resistance development in *E. coli* MG1655 mutants. Three barotolerant mutants (LMM1010, LMM1020, LMM1030) were isolated and pressure treated. Mutants showed 40–85% survival at 200 MPa for 15 minutes (ambient temperature) and 0.5–1.5% survival at 800 MPa for 15 minutes (ambient temperature). In contrast, survival of the parent strain (MG1655) decreased from 15% at 220 MPa to 2×10^{-8}% at 700 MPa. It should be noted that pressure sensitivity of the mutants increased from 10 to 50°C, as opposed to the parent strain, which showed minimum sensitivity at 40°C. This research indicated that the development of high levels of barotolerance should be properly understood in order to predict the safety of HPP. Similar studies are needed to document the barotolerance of other potentially pathogenic bacterial species.

Maggi et al. (1994) studied the effects of HPP on the heat resistance of fungi in apricot nectar and distilled water. They reported complete inactivation of *T. flavus* ascospores at 900 MPa for 20 minutes at 20°C and a 2-log cycle reduction in *N. fischeri*, but no effect was seen on *B. fulva* and *B. nivea* populations. In contrast, preheating apricot nectar (50°C) followed by pressure treatment of 800 MPa for 1–4 minutes resulted in complete inactivation of all four species. Also, lower pressure resistance was observed in distilled water samples.

Factors Affecting Microbial Inactivation

Several theories on the effect of HPP on bacterial species have been proposed over the years to explain the mechanism behind microbial inactivation and to better optimize HPP of foods. The processing conditions (initial sample temperature, circulating water temperature, pressurizing medium, holding time) under which high pressures are applied significantly influence the level of inactivation as well as the overall effect on the nutritional and sensory characteristics of food. Ye et al. (1996) studied the pressure tolerance of *Saccharomyces cerevisiae*, *Escherichia coli*, and *Staphylococcus epidermidis* in various media (agar, broth, apple jam, and juice) by subjecting the inocula to a pressure of 300 MPa at 5–25°C for 1–20 minutes. They reported that media pH played a very important role in the destruction of microbes; *S. epidermidis* was inhibited > 90% at 300 MPa in 11.2 minutes at pH 7.2 and in 4.8 minutes at pH 4.0. Variations in the pH and water activity of foods can result in different levels of lethality to a particular bacterium for the same high-pressure processing parameters. Studies conducted by Timson and Short (1965) on *B. subtilis* showed that the pressure resistance of the bacterium (when subjected

to 483 MPa for 30 minutes) was decreased as the pH in milk medium was lowered or raised from a pH value of 8. This value is not a constant for all microorganisms and the survival of *B. subtilis* at a specific pH can vary with the pressure and temperature of treatment. The type of culture medium used for growing the microbial species can also have a significant impact on the pressure and heat resistance of any microorganism. In general, the richer the growth medium, the better the baroresistance of the microorganisms. This is thought to be because of the increased availability of essential nutrients and amino acids to the stressed cell. It must be kept in mind that the parameters governing pressure tolerance are not constant for every bacterial species; they vary from one bacterium to another and may also be different for a single species grown under different conditions or in different growth media. It is very likely that the application of pressure affects a multitude of functions in a cell, thus interacting to retard or even kill the cell (Hoover et al. 1989). Therefore, studies related to the inactivation of bacterial species during HPP should specifically describe and document the processing conditions under which the inactivation took place. Also, specific information should be given about the variation in sample temperature during HPP.

Hayakawa et al. (1994) compared the pressure resistance of spores of six *Bacillus* strains. The spores were cultivated on nutrient agar and suspended in cold sterile distilled water with the filtrate being heated for 30 minutes at 80°C to destroy any vegetative cells. Spores of these six strains were then treated under pressures ranging from 196 to 981 MPa at 5–10°C for holding times of 20–120 minutes. It was found that *B. stearothermophilus* IAM 12043, *B. subtilis* IAM 12118, and *B. licheniformis* IAM 13417 had the most resistance to pressure, but *B. coagulans* was actually activated when treated with high pressures. There was no actual correlation between pressure and heat resistance, although they chose rightly a number of sporeforming bacteria varying widely in heat resistance. This could be due to applications of very low pressure in most cases. However, variables including initial and final spore levels and dilution levels were not specified, which may help in understanding the results better. It should also be noted that, in most cases, the reference heat treatment is much more inactivating than the pressure treatment to be compared; therefore, direct comparisons between heat resistance and pressure resistance are not possible.

Patterson et al. (1995) studied the sensitivity of vegetative pathogens (*Yersinia enterocolitica* 11174, *Salmonella typhimurium* NCTC 74, *Salmonella enteritidis*, *Staphylococcus aureus* NCTC 10652, *Listeria monocytogenes*, *E. coli* O157:H7) in buffer (pH 7.0), UHT milk, and poultry meat to high pressures up to 700 MPa at 20°C. A 10^5 reduction in numbers was obtained in all cases when pressures in the range of 275–700 MPa for 15 minutes were applied at 20°C. Different strains of *L. monocytogenes* and *E. coli* O157:H7 showed significant variations in pressure resistance, which were further used to examine the effect of substrates on pressure sensitivity and indicated that substrate affected the baroresistance of the microorganisms significantly.

The pH of a food material plays a very important role in determining the extent to which HPP affects the microorganisms under study. Several studies have documented and analyzed changes in heat resistance of organisms grown under different pH conditions, but there have not been very many studies on the pressure resistance of spores at different pH values. Roberts and Hoover (1996) investigated the effects of changes in pH values combined with a variety of other factors on the pressure-resistance of *B. coagulans* ATCC 7050. They reported an increase in the effectiveness of pressurization as the pH of the buffer was lowered. A decrease of an additional 1.5 log was observed as the pH was de-

creased from 7.0 to 4.0. On the basis of these results, it is highly probable that, other factors remaining constant, a neutral pH value is most conducive to high-pressure resistance in the cells. Earlier, Timson and Short (1965) observed that at high pressure the spores were most resistant at neutral pH, and at low pressure, most sensitive to neutral pH. That is perfectly in line with other findings that a pressure between 50 and 200 MPa enhances germination followed by kill and direct killing at high pressures above 900 MPa. In contrast, Sale et al. (1970) reported that the inactivation of bacterial spores by pressurization was maximum when the buffer was at a pH near neutral and was lowest at extreme values of pH. The difference in pH was thought to affect membrane ATPase and intracellular functions of the spore, thereby destabilizing the microorganisms (Macdonald 1992). This effect of pH on the pressure resistance of any microorganism is accentuated by other factors like the addition of salts, temperature conditions, and general process parameters. A better understanding of the exact process by which the variations in the pH affects the stability of the spore is still awaited and should give a better overall picture of the effect of high pressures. Therefore, studies to examine the effect of pH variation on the inactivation of different types of spores by HPP need to be done.

The water activity (a_w) of cells also affects the in pressure resistance. It is reported that the lower the a_w, the higher the pressure resistance of cells. Palou et al. (1996, 1998) studied the combined effect of HPP and a_w on *Zygosaccharomyces bailii* inhibition. They reported complete inhibition of yeast at $a_w > 0.98$, and an increase in the surviving fraction with a decrease in a_w. They concluded that addition of sucrose (to decrease a_w) acts as a baroprotective layer, preventing inhibition of yeast even at high pressures. Such a mechanism can be utilized to prevent inhibition of favorable microbes during HPP. More work is needed in this area.

Combined Processes

Pressure and Temperature

Alpas et al. (1998) studied the interaction of pressure, time, and temperature on the viability of *Listeria innocua* strain CWD47 in peptone solution by subjecting samples to pressures in the range of 138–345 MPa, temperature in the range of 25–50°C, and exposure times of 5–15 minutes. They showed that the combination of 345 MPa, 50°C, and 9.1 minutes could reduce the microbial population by 7 logs, with a Z-value (temperature change in °C causing a tenfold reduction in D-value; here it stands for pressure in MPa needed to reduce D-value by tenfold) of 173 MPa. Such studies resulting in description of Z-values for a specific microbial species are aimed toward a standardization of HPP parameters, which will facilitate commercialization of HPP.

Maggi et al. (1995) studied the use of HPP for inactivation of *Clostridium pasteurianum* spores isolated from peeled tomato and inoculated into tomato serum. They reported that high-pressure treatments of 900 MPa for 5 minutes at 60°C completely destroyed the spores (which was not obtained at temperatures < 60°C). Pressures of 700 or 800 MPa for 5 minutes at 60°C resulted in D-values of 2.4 and 3.4 minutes, respectively. They also studied spore inactivation using pressure pretreatment followed by heat treatment and found that a pretreatment of 300 MPa or 500 MPa for 1 minute at 60°C reduced heat resistance of spores by one-third and half, respectively. Their documented D-values for *C. pasteurianum* may be used for developing a database for pressure-temperature-time requirements

for complete destruction of different types of spores. Roberts and Hoover (1996) studied the inactivation of *Clostridium sporogenes* PA3679 and *Bacillus subtilis* 168 using a combination of pressure, temperature, acidity, and nisin. They exposed the samples (buffer at pH 4–7, inoculated with spores) to 405 MPa at different temperatures (25–90°C) for 15 or 30 minutes. After pressure treatment, spores were pour plated into agar with or without nisin. They reported an increase in spore inactivation with a decrease in pH. Also, sterilization was achieved using higher temperatures and pressures; for example, pressurization to 405 MPa at 45°C for 30 minutes resulted in complete inhibition of *C. sporogenes* at pH 4, while 90°C yielded complete inhibition over a pH range of 4–6. *B. subtilis* was completely inhibited by pressurization to 405 MPa at 70°C for 15 minutes at pH 4. Addition of nisin resulted in further reduction of microbial numbers. Unfortunately, this study was limited to only one pressure and D-value was not documented. The use of other combinations of pressures and temperatures may have given more promising results.

Maggi et al. (1996) studied the combined effect of pressure and temperature on the inactivation of *C. sporogenes* PA 3679 (ATCC 7953) spores in liquid media at pH 7.0 (beef or carrot broth medium, and phosphate buffer). They reported that 1,500 MPa at 20°C for 5 minutes resulted in no spore inactivation, whereas 1,500 MPa at 60°C fully inactivated the spores, and a pressure of just 800 MPa at 80–90°C resulted in sterilization of beef or carrot broth. Rovere et al. (1996) studied the effect of high pressure (up to 1,500 MPa) and temperature (20–88°C) on the destruction of *C. sporogenes* strain PA 3679, ATCC 7955, and reported that total bacterial spore inactivation could be obtained using a combined pressure (1,000 MPa) and temperature (50–60°C) treatment. Butz et al. (1996) also reported a combined pressure-temperature treatment for the inactivation of *Byssochlamys nivea* DSM 1824 ascopores. Researchers (Awao and Taki 1990; Clouston and Wills 1969; Gould and Sale 1970; Mallidis and Drizou 1991; Nishi et al. 1994; Seyderhelm and Knorr 1992; Taki et al. 1991) have also attempted to inactivate bacterial spores (*B. stearothermophilus, B. licheniformis, B. cereus, B. coagulans, C. botulinum*) using combined high pressure (> 0.7 MPa) and moderate temperatures (> 50°C) and found satisfactory results. However, for complete spore inactivation, pressures > 100 MPa in combination with temperature 60–80°C are required.

Sonoike et al. (1992) examined the death rates of *Lactobacillus casei* Y1T9018 and *E. coli* JCM1649 under various temperatures (0–60°C) and pressures (0.1–400 MPa). They reported that death rates of both strains decreased with rising temperatures under a high pressure, and contours of constant death rates of both strains on the pressure-temperature plane were elliptical and similar to that of a free-energy difference for pressure-temperature-reversible denaturation of proteins.

Hashizume et al. (1995) studied the inactivation of yeast using HPP at low temperatures by subjecting *Saccharomyces cerevisiae* IFO 0234 to a pressure range of 120–300 MPa at −20 to 50°C. After performing regression analysis of forty-three inactivation rates, they reported that the same degree of inactivation was achieved at higher pressures and higher temperatures as compared with low pressures and low (subzero) temperatures (e.g., the inactivation effect at 190 MPa and −20°C was similar at 320 MPa and room temperature). They concluded that high-pressure treatment applied at subzero temperatures requires lower pressures to achieve the same degree of microbial inactivation than when conducted at high temperatures. Since only a few studies have been performed using HPP at subzero temperatures, further studies are needed in this area to demonstrate the robustness of HPP at low temperatures, which may lead to interesting results.

Pressure and Other Processes

Crawford et al. (1996) studied the combined use of pressure and irradiation to destroy *C. sporogenes* spores in chicken breast. They reported a 5-log reduction at ambient temperature (25°C) with a pressure of 689 MPa applied for 60 minutes; heating samples at 80°C for 20 minutes before pressurization resulted in the lowest number of survivors. They also reported that a 3.0 kGy irradiation treatment before and after pressurization at 80°C for 1, 10, and 20 minutes did not show any significant differences in spore numbers between samples that were pressurized and then irradiated or vice versa. However, the irradiation D-value of *C. sporogenes* decreased from 4.1 kGy to 2 kGy at high pressures (> 600 MPa at 80°C for 20 minutes); their research showed that high pressure reduced the irradiation dose required to produce chicken with an extended shelf life. They concluded that pretreatment with irradiation (prior to HPP) is a useful technique for inactivating *C. sporogenes* spores, thereby reducing the radiation dose required to eliminate the spores by irradiation alone.

Fornari et al. (1995) studied the inactivation of *Bacillus* spp. using a combination of pressure, time, and temperature. They studied four *Bacillus* spp. (*B. cereus,* SSICA/DA1 [from wheat flour], *B. licheniformis* SSICA/DA2 [from spices], *B. coagulans* SSICA 1881 [from tuna in tomato sauce], and *B. stearothermophilus* SSICA/T460 [from spoiled canned peas]) by subjecting prepared samples to a pressure range of 200–900 MPa for 1–10 minutes at 20, 50, 60, or 70°C. They also examined the effect of pressure cycling on spore inactivation (pressure treatment of 200–500 MPa followed by 900 MPa). They found that *B. cereus* spp. were more sensitive to pressure treatment (inactivation of $4 \times 10^{+5}$ endospores/mL was achieved at ambient temperature by treatment at 200 MPa for 1 minute followed by 900 MPa for 1 minute). However, for other species, a combination of higher pressure and moderate temperature was needed for significant reduction (*B. licheniformis* was inactivated at 800 MPa for 5 minutes at 60°C; *B. coagulans* was reduced to 10^{-4} endospores/mL at 900 MPa for 5 minutes at 70°C; *B. stearothermophilus* was inactivated at 70 MPa for 5 minutes at 70°C).

Aleman et al. (1996) studied the effects of pulsed and static HPP on fruit preservation by inactivating *S. cerevisiae* 2407-1a in unsweetened pineapple juice. They applied sinusoidal and step-pressure pulses and compared the inactivation effects with static pressure treatments. They reported that no inactivation was observed after the application of 40–4,000 fast sinusoidal pulses (10 cycles/second) at 4–400 seconds over a pressure range of 235–270 MPa, whereas static pressure treatments of 270 MPa at 40 and 400 seconds gave 0.7 and 5.1 decimal reductions, respectively. Also, slower 0–270 MPa step pulses at 0.1 (10 pulses), 1 (100 pulses), and 2 (200 pulses) cycles/second with total time of 100 seconds resulted in 3.3, 3.5, and 3.3 decimal reductions, respectively. They also reported that the ratio of on-pressure time to off-pressure time affected inactivation (e.g., on-pressure time of 0.6 seconds and off-pressure time of 0.2 seconds resulted in a 4 decimal reduction in 100 seconds). They concluded that slower step pulses resulted in increased effectiveness of HPP as more reduction in microbial numbers was observed in less time in step-pressure processing. However, studies need to be done using step-pressure processing for inactivating other microorganisms and documenting their destruction kinetics. More studies are needed along the same lines for inactivation of other baroresistant microbes (e.g., Gram positive bacteria, spores) using cycled pressure treatment. However, the high resistance of bacterial spores to HPP is still a major outstanding issue and is the subject of

a variety of reports (Crawford et al. 1996; Maggi et al. 1996; Roberts and Hoover 1996; Shimada 1992).

Shimada (1992) reported that the combined treatment of high pressure and alternating current yields lethal damage in *E. coli* and *B. subtilis* spores. Earlier, Shimada and Shimahara (1985, 1987) found that the exposure of *E. coli* cells to an alternating current (ac) of 50 Hz caused the release of intracellular materials located in the nucleus region within the cells, causing a decrease in the resistance to basic dyes. This was believed to have resulted from loss and/or denaturation of cellular components responsible for the normal function of the cell membrane, which suggested that the lethal damage to microorganisms may be enhanced when the organisms are exposed to ac before or after the pressure treatment. Shimada (1992) subjected the *E. coli* cells to 300 MPa for 10 minutes immediately after ac exposure and *B. subtilis* suspension to 400 MPa for 30 minutes before ac exposure. Exposure was carried out at 0.6 A/cm^2 for *E. coli* cells at 35°C for 2 hours and at 1 A/cm^2 for spores at 50°C for 5 hours. They found that the surviving fractions of *E. coli* cells and *B. subtilis* spores treated with ac and pressure were significantly reduced. It was also found that the susceptibility of *E. coli* cells and *B. subtilis* spores to some chemicals increased after the combination treatment, suggesting that the combined use of pressure and ac also lowers the tolerance level of microorganisms to other challenges.

Response to pressure cycling (Honma and Haga 1991; Knorr 1994), ultrasound with pressure (Knorr 1994), and additives plus pressure (Knorr 1994; Papineau et al. 1991; Popper and Knorr 1990) have been studied, and significant interactive effects on microbial inactivation have been found. In addition, sensory and functional characteristics of foods were enhanced. Knorr (1994) reported that neither ultrasonic nor high-pressure treatment alone was capable of inactivating *Rhodoturola rubra*; however, pretreatment of samples with ultrasonic waves (100 W/cm^2, 25°C, 25 minutes) followed by HPP (400 MPa, 25°C, 15°C) resulted in complete inactivation of *R. rubra*. Popper and Knorr (1990) demonstrated the effectiveness of combinations of enzymes like lysozyme, lactoperoxidase, and glucose oxidase on the inactivation of microbes at atmospheric pressure. It is highly likely that the combination of enzyme pretreatment with HPP may result in significant inactivation of microbes even at low pressures.

The work done in combined pressure-temperature, pressure-ac exposure, pressure cycling, and pressure-ultrasound areas is at a preliminary level and the exact mechanisms of spore inactivation by such combined processes are not well known. There is no doubt that combined high pressure and moderate temperature (60–80°C) or high pressure and ac exposure will have a beneficial impact on the sensory characteristics of heat-sensitive products, yet no such processes can be commercialized until microbial safety can be guaranteed. In most of the studies researchers did not evaluate the effect of pressure-induced "adiabatic" heating on sample temperatures nor did they examine spore inactivation during pressure "come-up" time, both of which may affect inactivation kinetics significantly. Also, the operational efficiency of a high-pressure food processor is very important during combined treatments; therefore, proper equipment maintenance and effective training of personnel are prerequisites for such studies. A detailed evaluation of the database accumulated from studies that used a combination of pressure and other treatments (temperature, ac exposure, cycling) is needed prior to commercialization of HPP. Also, corresponding D-values for specific microorganisms need to be documented and validated to standardize HPP before it can be commercialized.

Protein Denaturation and Enzyme Inactivation

The four levels of protein structure are characterized as primary (amino acids in a polypeptide chain joined by covalent bonding), secondary (coiling of peptide chains joined with hydrogen bonding), tertiary (arrangement of chains into globular shape by noncovalent bonding), and quaternary (various compact structures or subunits joined by noncovalent bonding) (Heremans 1995; Leadley and Williams 1997). The secondary, tertiary, and quaternary structures can be significantly affected by HPP (because high pressure affects noncovalent bonds); therefore, HPP can result in novel functional properties because tertiary structure is important in determining protein functionality. Pressures of 700 MPa have been shown to coagulate egg albumen completely (Bridgmann 1914). The amount of pressure required depends upon the type and concentration of protein, pH, and ionic strength of treated solutions (Leadley and Williams 1997). Some of the HPP applications used in the food industry found to affect protein functionality include induction of coagulation and gelation in egg white, egg yolk, Alaskan pollack paste, rabbit meat paste, and soy protein (Hayashi 1989; Hayashi et al. 1989; Okamoto et al. 1990); production of pressure-induced gels in whey protein, egg white, blood plasma, and hemoglobin protein concentrates (Van Camp and Huyghebaert 1995); induction of gelation in different surimi types (Shoji et al. 1990); increased elasticity, breaking strength, and syneresis resistance of pressure-treated skimmed milk due to the production of acid-induced gels (Johnston et al. 1992); and increased rates of acid hydrolysis of proteins (Hayashi et al. 1990).

Ludikhuyze et al. (1997) determined kinetic parameters of pressure-temperature inactivation of *B. subtilis* alpha-amylase under dynamic conditions of pressure cycling. They reported that multiple pressure treatments had a more pronounced effect on the inactivation of *B. subtilis* alpha-amylase as compared to a single-cycle process. They attributed this result to more extensive and frequent temperature variation during multiple-pressure cycling.

Ohshima et al. (1993) studied the effect of high pressures on fish muscle proteins. Myofibrils were first prepared by removing the water soluble glycolytic enzymes and proteins like collagen. Contractile, regulatory, and elastic structural proteins are the major components of muscle cells and their constituent myofibrils. When the myofibrils were pressurized at 150 MPa for 30 minutes, the microscope revealed that destruction of the arrangement of myofibrils and cohesion of filaments occurred. Treatment at 38°C for 2 hours did not reverse the change. It was suggested that this denaturation of myofibrils was caused by depolymerization of myofibrillar proteins or aggregation of myosin (Hayashi et al. 1990). When carp muscle was subjected to pressures of 200, 350, and 500 MPa and stored at 5°C, a delay in the natural decrease of inosine monophosphate (IMP) levels was observed at pressures of 350 and 500 MPa (Ohshima et al. 1993). This was important because IMP is responsible for the fresh taste of fish. Thus, it was suggested that the enzymes involved in ATP breakdown undergo denaturation and deactivation. Recently, Hauben et al. (1996) studied the effect of high pressure on the disruption of bacterial outer-membrane permeability under pressures of 220–320 MPa. In their experiments, *E. coli* was found to be sensitive to lysozyme and nisin, which are normally excluded by the outer cell membrane. Also, a periplasmic enzyme, ß-galactosidase, leaked into the extracellular medium under high pressure. This observed membrane damage was rectified after pressurization, thereby leading the authors to believe that the damage was either spontaneously reversible or repaired by enzymes. Another area that needs to be explored is the effect of high pressure on the constituent lipids and fatty acids of cells. Predictably, it has been reported that

lipids in fish muscle are also affected by subjecting them to high pressures due to lipolysis (Ohshima et al. 1993).

Macdonald (1992) reviewed the effects of HPP on natural and artificial membranes and reported that pressure increases the order of model and natural lipid bilayers, which diminishes the void volumes between hydrocarbon chains and converts the liquid crystalline state and orthodox bilayer structure to an ordered gel state and partial interdigitated bilayer (phase changes), respectively. Due to sufficient ordering of bilayer by pressure, the integral and peripheral proteins get detached from the plasma membrane. Also, high pressure (> 150 MPa) inactivates the ATPase activity and increases the enzyme's solvation. Due to membrane permeabilization, HPP may facilitate the penetration of antimicrobials into vegetative cells.

Timson and Short (1965) suggested that a possible mechanism for protein denaturation under high pressures in the 345–2,175 MPa range is ionization and subsequent precipitation of protein complexes. On the application of high pressure the solvation of ions and the ionization of weak electrolytes is increased. At these pressures the weakly charged molecules that are compressed together into the charged ionic groups increase the total charge. This gives the oppositely charged groups enough energy to combine, which may not happen under normal conditions. Thus ionic bonds are formed, decreasing the solubility of protein molecules due to a decrease in the number of free hydrophilic groups. As a result the proteins precipitate from the solution. Also, electrostriction has been shown to occur under pressure, corresponding to charge separation and dissociation of ionic interactions. However, this may be reversible when pressure is released. Cheftel (1995) indicated that gel networks formed due to pressurization are weaker, if they are mainly comprised of hydrophobic interactions, than those in gels formed at atmospheric pressure. In contrast, gels formed mainly with hydrogen bonds are further reinforced due to pressurization (Cheftel 1995). To fully understand the mechanisms of protein denaturation by high pressure, the effect of HPP on different protein types needs to be studied in depth. The extent of reversibility of denaturation for given pressure conditions also needs to be analyzed.

HPP affects enzymes in a variety of ways depending on other parameters of processing and also the type of enzyme (Cheftel 1995). However, it should be noted that pressure-resistant enzymes can also affect HPP outcomes (Rovere et al. 1994). Enhanced enzyme activity after HPP may be due to enzyme release from cellular compartments and closer contact with substrates. Rovere et al. (1994) first treated PPO, which is responsible for browning of fruits, at levels necessary to achieve complete inactivation of microbes. At these levels PPO was not inactivated. Other researchers (Asaka and Hayashi 1991; Gomes and Ledward 1996) also studied the effect of HPP on PPO activity and reported incomplete inhibition of PPO. Gomes and Ledward (1996) investigated the effects of pressure treatment (100–800 MPa for 1–20 minutes) on commercially available mushroom tyrosinase and tyrosinases extracted from mushrooms, potatoes, and apples. They reported that the activity of a commercial PPO extract from mushrooms decreased at a constant rate with applied pressure and time in phosphate buffer at pH 6.5, with complete but reversible inactivation being obtained at 800 MPa for 5 minutes. The response of PPO extracted from potatoes and mushrooms was different than for commercial PPO. For potatoes, PPO activity was consistently lost with increasing pressure, but after 10 minutes at 800 MPa, 40% of PPO activity remained. For mushrooms, PPO activity was increased after treatment at 400 MPa for 10 minutes, and even at 800 MPa for 10 minutes, 60% of PPO remained. They concluded that PPO types affect the efficacy of HPP in their inactivation and recommended that HPP

should be coupled with other treatments to completely inhibit PPO activity. Jaenick (1991) found that enzyme inactivation under HPP was brought about by intramolecular structural changes. It was also reported that HPP increases reaction rates that involve enzymes. Thus the catalytic behavior of enzymes is affected by the application of pressure and this is probably due to volume changes resulting from changes in protein conformation. It has been found that pressures ranging from 102 to 304 MPa cause reversible denaturation and pressures over 304 MPa result in irreversible denaturation. Jolibert et al. (1995) studied the effects of HPP on PPO in fruits (plums, apricots, strawberries, and apples). They found that HPP increased apple browning but reduced PPO activity in plums, and they suggested that a total inactivation of fruit PPO activity may be achieved at 600 MPa. They reported irreversible inactivation of PPO, which was also dependent on the substrate, fruit species, and pH (maximum inactivation occurred at low pH). They recommended addition of preservatives like sucrose, ethanol, or ascorbic acid to facilitate inhibition of fruit PPO by HPP.

A reduction in pectin esterase (PE) was observed by Ogawa (1992), Seyderhelm and Knorr (1992), and Berg (1996), but results obtained were not promising as PE showed baroresistance even at high pressures (900 MPa). This resistance increased with an increase in soluble solids (Ogawa 1992) and high sucrose concentration (Seyderhelm and Knorr 1992). PE is an enzyme that is found in citrus juices and can cause a loss of the cloudiness in fresh juices. Goodner et al. (1998) studied the inactivation of PE in orange and grapefruit juices by high pressure. They reported that isostatic pressure of 600 MPa caused substantial inactivation of the heat-labile form of PE, whereas the heat-stable form was not affected. Treatment times significantly affected total PE inactivation in orange juices (but not in grapefruit juices). They also reported that heat-labile grapefruit PE was more sensitive to pressure than orange PE. Basak and Ramaswamy (1996) performed a kinetic study on the pectin methyl esterase (PME) during HPP of orange juice and evaluated the effects of pH and soluble solids concentration on PME inactivation. They subjected nonpasteurized and pasteurized orange juice to pressures of 100–400 MPa for 0–720 minutes and reported that PME inactivation was dependent on the pressure level, holding time, pH, and total soluble solids. They found primary pressure inactivation to be dependent only on pressure level and secondary inactivation to be dependent on holding time at each pressure level. Rovere et al. (1996) also studied the inactivation of PME, peroxidase (POD), and PPO activities by combining pressures up to 100 MPa with temperatures of 20–88°C. They concluded that all of the enzymes could be inactivated using 100 MPa at 50–60°C. Seyderhelm et al. (1996) studied the effects of HPP on PME, lipase, PPO, lipoxygenase, peroxidase, lactoperoxidase, phosphatase, and catalase by subjecting samples to a pressure range of 0.1–900 MPa at 25–60°C for 2–45 minutes (pH 3–7). They ranked these enzymes based on their pressure-induced inactivation and reported that a combination of pressure and temperature increased the degree of enzyme inactivation. They also mentioned that actual foods provide a baroprotective layer preventing the inactivation of these enzymes during HPP and concluded that inactivation of enzymes depends upon immersion medium, pH, temperature, and processing time.

Tamagawa et al. (1996) studied the effect of HPP on sterilization, viscosity, and browning of grated yam. They reported complete inactivation of most fungi and Gram negative bacteria and a decrease in viscosity at pressures > 500 MPa (for 10 minutes at 20°C). At pressures > 500 MPa, a suppression of browning in yam was observed, suggesting inactivation of PPO by this treatment. Use of HPP for inhibition of proteolysis in milk whey (Okamoto and Hayashi 1990); protease activities in meat (Ohmori et al. 1991); and activity of en-

zymes like polygalacturonase as well as pectin methylesterase, PPO, and POD (Dörnenburg et al. 1996) have also been studied further. Enhanced proteolysis of ß-lactoglobin (e.g., trypsin) under pressure has been suggested for use in preventing the allergenicity of dairy proteins. Castellari et al. (1997) studied the effect of HPP on PPO activity of grape must and reported that limited PPO inhibition was obtained between 300 and 600 MPa. They suggested that to totally inactivate PPO activity, very high pressures (> 900 MPa) along with mild thermal treatment (40–50°C) should be used. Cano et al. (1997) studied the pressure and temperature effects on inactivation of POD, PPO, and PME enzymes in strawberry and orange products. They subjected the samples to a pressure in the range of 50–400 MPa combined with heat treatment (20–60°C). They found that significant inactivation of strawberry PPO activity (60%) occurred up to 250 MPa and POD activity was reduced by 25% up to 230 MPa. In orange juice, POD and PME activities were reduced by 50% at 35°C. Ibarz et al. (1996) also investigated inhibition of PPO activity in apple slices during HPP. They immersed apple slices in water or ascorbic acid, citric acid, or 4-hexylresorcinol (4-HR) solutions as antibrowning agents at 50 ppm, prior to subjecting them to high pressures in the range of 138–690 MPa for 5 minutes. They did not find any PPO inactivation at 138 MPa (except in slices immersed in 4-HR) and suggested that to inhibit PPO activity, pressures > 276 MPa should be used or prior treatment with 4-HR should be done.

Ashie and Simpson (1996) studied HPP to control enzyme-related fresh seafood texture by subjecting enzyme extracts (chymotrypsin, collagenase, cathepsin C, trypsin) from bluefish and sheephead fishes to pressures of 7–21 MPa. They found that fish enzymes were more sensitive to HPP as compared to their mammalian counterparts. They also concluded that HPP may serve as a tool to control the deterioration of seafood texture. Enzymes, like microorganisms, are affected by HPP depending upon the amount of pressure applied, holding time, process temperature, and environmental/substrate conditions (Leadley and Williams 1997). Nevertheless, enzymes may be inactivated reversibly or permanently depending on the amount and duration of pressure as well as the nature of associated processing conditions. However, more studies need to be done in the enzyme inactivation area using pretreatment with different combinations of processing conditions to optimize HPP and yield foods with excellent quality.

Food Packaging

The type of food packaging used also plays a very important role in HPP. Two basic requirements that the packaging material needs to possess are the ability to withstand the magnitude of pressure under operating conditions and good heat sealability. Currently, several different types of packaging are in use for HPP, like plastic stomacher bags, sterile tubes, polyester tubes, polyethylene pouches, nylon cast polypropylene pouches, and various other flexible pouch systems. The physical and mechanical properties of the material greatly influence the effectiveness of HPP on the food material. Nachmanson (1995) discussed various issues related to packaging materials used in HPP. He stated that the packages must have the ability to prevent any deterioration in the product quality during HPP, and excellent logistics should be applied to distribute the pressure-treated products. Foods to be treated by HPP may be either bulk or individually (consumer) packaged before or after (direct) processing. Nachmanson (1995) also stated that the presence of headspace must be kept as small as possible because air and other gases are compressed to ~zero volume under high pressure, leaving deformation strains on the packages. Therefore, each

package should be tested for permissible headspace because headspace cannot be avoided in practical situations. Film barrier properties and structural characteristics of polymer-based packaging material were unaffected when treated at 400 MPa for 30 minutes at 25°C (Nachmanson 1995).

Masuda et al. (1992) also studied the effects of HPP on packaging materials for food (mainly gas barrier properties). They examined water vapor and oxygen permeability, tensile strength, and heat seal performance of pressurized gas barrier composite films, as affected by HPP. They reported that pressure treatment of 600 MPa at 40°C for 10 minutes or 5 pulses of intermittent treatment at 300 MPa, 20°C, for 2 minutes did not affect the barrier properties. No change in the superior aromatic-proof properties of EVOH and PVOH films was observed (400 MPa, 20°C for 10 minutes). However, they found that voids appeared in composite films, including hydrophilic films (OH, EVOH, PVOH), which they suggested could be avoided by providing thick hydrophobic film lamination, altering the ethylene content of EVOH, or reducing HPP time. They concluded that the properties of EVOH and PVOH are little affected by HPP and can be easily adapted for HPP to preserve freshness of food. Also, delamination of some multilayer composite films has been reported.

Starch

A few studies (Ezaki and Hayashi 1992; Hayashi and Hayashida 1989; Muhr et al. 1982; Muhr and Blanshard 1982; Thevelein et al. 1981) have been done to examine the effect of HPP on starch. A phenomenon similar to heat gelatinization has been observed with starches. Ezaki and Hayashi (1992) studied the effect of pressure on twenty starches and compared effects with that of temperature for specific applications. They investigated the pressure effects of starches according to their A, B, or C-type classification (based on X-ray diffrectograms). A-type starches (corn, wheat, rice) were most susceptible to pressure, that is, enzyme digestibility and swelling degree increased at about 200–400 MPa (> 70% digestibility increase was obtained at 500 MPa). B-type starches (potato, marron, lily) were less susceptible to pressure than A-type starches, that is, enzyme digestibility and swelling degree increased at ≥ 500 MPa. C-type starches (sweet potato, tapioca, mung bean) showed intermediate susceptibility between A and B-types. Ezaki and Hayashi concluded that the structure of pressure-treated starch was different from heat-gelatinized structure. Pressurization swelled starch granules, allowing them to keep their granule-like structure and improving enzyme digestibility and gelatinized structure (without retrogradation).

Thevelein et al. (1981) examined the gelatinization temperature of starch under HPP. They reported that the application of high pressure results in an upward shift of gelatinization temperature (highly swollen stage of starch at a particular temperature or temperature at which an equal modulus of elasticity is obtained for gels formed at different pressures), which was 3–5°C/7 MPa. These studies were done on a laboratory scale and give limited insight to HPP of starches. Further work needed in this area should compare the effectiveness of HPP under different operating conditions, as it is highly probable that starch with unique functional properties can be obtained if treated with high pressures.

Pressure-Shift Freezing and Thawing

The rates of freezing and thawing influence the quality of food because they cause changes in the texture and cooking properties believed to result from destruction of membrane

structure and changes in the concentration of solutes (Bevilacqua et al. 1979). Even cryogenic freezing may result in fracture of food products (as volume is decreased during cooling, followed by an increase in volume during freezing) (Kalichevsky et al. 1995). High pressure depresses the freezing temperature of water, which means that HPP (200 MPa) can result in unfrozen water existing even at −21°C. However, upon release of pressure, ice crystals are formed uniformly as the pressure applied is isostatic. Thus, three potential applications of HPP can be envisioned, namely pressure-shift freezing; super cooling by storage at subfreezing temperatures without freezing; and pressure-shift thawing (Deuchi and Hayashi 1990, 1991, 1992; Leadley and Williams 1997). Only a few studies (Haas et al. 1972; Knorr et al., 1998) have been done in this area.

Haas et al. (1972) investigated the quality of pressure-frozen foods with that of freeze-dried products. They concluded that pressure freezing followed by air drying resulted in less textural damage, less shriveling, more rapid dehydration and more uniform rehydration. Deuchi and Hayashi (1992) examined the application of HPP at subzero temperatures to preservation, rapid freezing, and rapid thawing of foods. They stored noncooked foods, microorganisms, and freeze-sensitive microorganisms at temperatures from −5 to 20°C with pressure applied from 50 to 200 MPa for a few days or weeks. They found strawberries retained fresh flavor, color, and texture; raw pork with no drippings; most microorganisms (coliforms, *Enterobacteriaceae,* Gram negative and Gram positive psychrophiles, enterococci, and LAB) were reduced in number; and freeze inactivation of enzymes was partially prevented. They reported that storage of foods under moderate pressure at subzero temperatures preserved the natural characteristics of foods without microbial spoilage and damage. They also examined the rapid thawing of frozen foods by HPP. During their experiments, they pressurized ice (at −10, −15, −20, and −30°C) from 50 to 200 MPa, held samples at 5°C for 30 minutes, and found that all ice was completely thawed. They recommended that rapid thawing of foods is a possible way to avoid qualitative changes, provided processing conditions (temperature, pressure, time) are carefully selected. They also studied the rapid freezing of food using HPP (pressurization up to 200 MPa followed by cooling to −20°C and rapid release of pressure) and found the procedure useful because of uniform formation of small ice crystals throughout the food material, preserving the sensory characteristics of food.

Knorr et al. (1998) studied the effect of HPP on phase transitions of food and performed a study of high-pressure freezing and thawing of potato. They designed a high-pressure vessel that can withstand subzero temperatures and suggested that due to the transient nature of heat transfer, fast removal of latent heat is a major engineering challenge during pressure freezing and thawing. They further stated that "food research and development has until recently neglected taking advantage of the phase diagram of water; understanding phase changes during pressure-assisted freezing and thawing of foods can aid food process and product development" (Knorr et al 1998).

Kanda and Aoki (1992) used this method to freeze tofu and found that ice crystals were uniform, granular, and very small in size. They thawed tofu at room temperature and found that the original shape, structure, taste, and texture of tofu were restored. Fuchigami et al. (1998) studied high-pressure freezing effects on the textural quality and histological structures of Chinese cabbage by subjecting the cabbage to high-pressure freezing (100–700 MPa at −20°C). Freezing of samples at 100 MPa (ice I) and 700 MPa (ice VI) resulted in increased rupture strain; however, texture was comparatively intact at 200 MPa (liquid), 340 MPa (ice III), and 400 MPa (ice V). Also, pectin release and histological damage in

samples frozen at 200 and 340 MPa were less than in those frozen at 100 and 700 MPa. They reported an increase in both textural and histological attributes of pressure-frozen samples as compared to results from traditional freezing. More studies are needed in high-pressure freezing and thawing, which may result in an excellent new processing technique for heat-sensitive food materials.

Meat and Fish Industry

Researchers have studied the application of HPP in the meat industry using several combinations of pressure, time, and temperature (table 12.2). Carlez et al. (1992) studied the effect of high pressure and bacteriostatic agents on the destruction of *Citrobacter freundii* in minced beef muscles and also gave a pressure-destruction-kinetic equation for further comparison with thermal processing. By analogy with D-values (for thermal destruction), they calculated a decimal reduction time $D_{230\ MPa/20°C}$ of 14.7 minutes, that is, the period of time at 230 MPa (pressure used in their study) and 20°C required to cause a decimal reduction in surviving microorganisms. They also studied the probable synergy between pressure and bacteriostatic substances (sorbic acid, benzoic acid, and CO_2 as a gas or in a supercritical state) and found little effect on the bacterial destruction. They suggested that essential oils or their constituents could be used as adjuncts for further study. Shigehisa et al. (1991) reported complete destruction of *Salmonella typhimurium* at 300 MPa after 10 minutes at 25°C. Carlez et al. (1993) reported that *Citrobacter freundii*, *Pseudomonas fluorescens*, and *Listeria innocua* were completely inactivated at pressures > 280, 200, and 400 MPa, respectively, at 20°C. They also noticed a paler color in samples of minced beef treated at pressures > 150 MPa and grayish color in samples at pressures > 350 MPa. In addition, they also calculated decimal reduction times (D)-values for all microorganisms tested. When studies generate D-values, then data can further be compared with other work, and eventually yield a database of D-values (at a constant pressure and temperature) for different microorganisms that will be essential for standardization and commercialization of HPP.

Miyao et al. (1993) investigated the effects of HPP on microorganisms in surimi paste. All of the microbes were destroyed at 300–400 MPa; fungi showed highest sensitivity to HPP, followed by Gram negative and Gram positive bacteria. They also identified pressure-resistant bacteria, for example, *Moraxella* spp. (viable at 200 MPa); *Acinetobacter calcoaceticus* (viable at 300 MPa); *Streptococcus faecalis* (viable at 400 MPa); and *Corynebacterium* spp. (viable at 600 MPa). They also reported a long lag phase in the growth curve of pressure-treated bacteria as compared to nontreated bacteria (e.g., *Streptococcus faecalis* subjected to 400 MPa showed a lag phase extended by 20 hours). In their study with pressurized minced mackerel meat, Fuji et al. (1994) reported that in pressure-treated samples, *Bacillus, Moraxella, Pseudomonas,* and *Flavobacterium* spp. were totally inactivated, whereas *Staphylococcus* and *Micrococcus* spp. dominated during storage after pressurization. The changes in freshness indicators (pH, amine content, histamine, TBA, lactic acid content) were related to bacterial growth; however, from sensory and freshness points of view, a 4-day increase in shelf life of pressure-treated samples was obtained.

Carlez et al. (1994) stored pressurized minced meat samples for 16–23 days at 3°C. Gram negative bacteria were more sensitive to HPP than Gram positive types. Total inhibition of microorganisms occurred at 400–450 MPa. However, *Pseudomonas* spp. were

Table 12.2 Application of HPP in the meat industry

Reference	Meat Type	Pressure (MPa)	Holding Time (min)	Processing Temperature (°C)	Microorganisms Tested	Other Parameters Studied
Carlez et al. (1992)	Minced beef muscle	230 MPa		20	*Citrobacter freundii*	Synergy between HPP and bacterio-static substances
Carlez et al. (1993)	Minced beef muscle	50–400	20	4, 25, 35, and 50	*Citrobacter freundii, Pseudomonas fluorescens, Listeria innocua*	
Carlez et al. (1994)	Minced beef muscle	200–450	20	20	Gram negative and Gram positive	Repair and/or recovery mechanism following pressure stress of microorganisms
Carlez et al. (1995)	Minced beef muscle	200–500				Color and myoglobin content of minced beef samples
Shigehisa et al. (1991)	Pork slur-ries	300	10	25	*Salmonella typhimurium*	
Miyao et al. (1993)	Surimi paste	100–600			*Moraxella* spp., *Acinetobacter calcoaceticus*, *Streptococcus faecalis*, *Corynebacterium* spp.	
Fuji et al. (1994)	Minced mackerel meat	203	60	5	*Bacillus* spp., *Moraxella* spp., *Pseudomonas* spp., *Flavobacterium* spp., *Micrococcus* spp.	Freshness indices
Carpi et al. (1995)	Creamed salmon	700	3	2–8 (refrigeration temperature)	*Salmonella typhimurium*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Saccharomyces cerevisiae*, *Pencillium expansum*, *Rhizopus oryzae*, *Clostridium sporogenes*, *Lactobacillus casei*, *Enterobacteriaceae*	Chemical and sensory changes

Table 12.2 Application of HPP in the meat industry (*continued*)

Reference	Meat Type	Pressure (MPa)	Holding Time (min)	Processing Temperature (°C)	Microorganisms Tested	Other Parameters Studied
Ananth et al. (1995)	Fresh pork	414–826	< 30 min	2 or 25		Sensory quality
Ananth et al. (1998)	Fresh pork loins	414–827	30	2 or 25	*Listeria monocytogenes* Scott A, *Salmonella typhimurium* ATCC 13311	Color, texture, moisture, water-holding capacity, and sensory qualities
El Moueffak et al. (1996)	Duck foie gras	300 and 400	10	50	Coliforms, *Staphylococcus aureus*, vegetative mesophilic, psychrotrophic contaminants	
O'Brien and Marshall (1996)	Freshly ground raw chicken meat	408–818	10	25	*Carnobacterium divergens*, *Serratia liquefaciens*	
Nishiwaki et al. (1996)	Rabbit meat	< 200				Mg enhanced ATPase activity of rabbit myofibril
Brunn and Skibsted (1996)	Model cured meat system	< 300		15		Oxidation of nitrosylmyoglobin (an important pigment in cured meat products)
Cheah and Ledward (1996)	Minced pork	< 800	20	20		Lipid oxidation
Cheah and Ledward (1997)	Fresh beef	800–1,000	20	25		Inhibition of metmyoglobin formation
Paul et al. (1997)	Lamb meat	200	30	30	Coliforms, *Staphylococcus* spp.	
Takahashi and Haga (1997)	Hams	300	5, 15, or 25	20	*Lactobacillus* SK-1001, *Staphylococcus* spp., Coliforms	

detected after 3–9 days at 3°C, which means that they were not fully inactivated but stressed during HPP. Therefore, HPP should be coupled with some other treatment (e.g., moderate temperature of 50°C) to eliminate viable *Pseudomonas* spp. In a subsequent study, Carlez et al. (1995) investigated the effects of HPP on color and myoglobin content of minced beef samples packaged under vacuum, air, or oxygen. They noticed a pink color of meat treated at 200–350 MPa (increase in L, lightness, color values), which turned gray-brown at 400–500 MPa (a decrease in L values). Also, a decrease in myoglobin content at 200–500 MPa, a decrease in oxymyoglobin, and an increase in metmyoglobin at 400–500 MPa were noticed. They also evaluated the effect of HPP in combination with ascorbic acid, cysteine, nicotinamide, nicotinic acid, sodium nitrite, sodium chloride, and an oxygen scavenger (Ageless FX-100, Mitsubishi France) in vacuum-packaged meat to prevent oxidation and yield less discoloration of meat. Only sodium nitrite, sodium chloride, and the oxygen scavenger provided some protection against oxidation. They suggested that meat discoloration during HPP is due to a whitening effect at 200–300 MPa, caused by globin denaturation, haem displacement or release, or oxidation of ferrous myoglobin to ferric myoglobin at 400 MPa. However, there was no significant increase in haem iron extractable during HPP at different levels. They also mentioned that interaction between pressurized meat samples and chemicals from packaging was not taken into account, which may have had a significant effect on meat discoloration. They used only two types of packaging materials, and it could be possible that other types may prevent meat discoloration. A separate study having packaging material as another variable is needed to examine this possibility. In this study, only one type of oxygen scavenger was tested, and there is significant variation in O_2 absorption rate among individual O_2 scavengers of the same type (Tewari et al. 1999). Therefore, use of different custom-designed oxygen scavengers with better O_2-absorbing kinetics may be useful in preventing meat discoloration. Specially designed oxygen scavengers to prevent natural meat color are being commercialized by Tewari De-OX Systems' patents pending "zero-oxtech" packaging program (www.tewarisystems.com). Further study should be conducted in this area.

Carpi et al. (1995) inoculated smoked creamed salmon samples with a variety of microorganisms: *Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus, Saccharomyces cerevisiae, Pencillium expansum, Rhizopus oryzae, Clostridium sporogenes, Lactobacillus casei, and Enterobacteriaceae*. They reported that an extended shelf life from 60 to 180 days at 3 or 8°C (without significant chemical, microbiological, or sensory changes) was obtained for high-pressure-treated samples. They also suggested that high-pressure-treated samples must be stored at temperatures < 3°C to prevent outgrowth of surviving *Clostridium botulinum* spores. They did not mention the temperature used during HPP, which can have a significant effect on the lethality of any pressurized process. Ananth et al. (1998) studied the shelf-life extension, microbiological safety (*L. monocytogenes* Scott A and *Salmonella typhimurium* ATCC 13311), and quality of fresh pork loins using HPP. They reported the highest $D_{414\ MPa/25°C}$ value of 2.17 minutes for *L. monocytogenes* and highest $D_{414\ MPa/2°C}$ value of 1.48 minutes for *S. typhimurium*. They did not find any significant difference in color (L^*, a^*, b^*), texture, and water-holding capacity between pressure-treated (414 MPa, 13 minutes, 25°C) samples and controls. However, pressure-treated samples at low temperature and processing time (414 MPa, 9 minutes, 2°C) resulted in improved texture as compared to the controls. They reported that the level of psychrotrophs was 5.7 log cfu/g for pressure-treated samples at 25°C as compared with 7.0

log cfu/g for controls. They recommended HPP for extending the shelf life of fresh pork loins; however, they did not report any significant difference in sensory characteristics between pressure-treated pork and controls. They performed a systematic study by calculating D-values and determining processing conditions for pork using HPP. More such studies are needed for other meat products.

El Moueffak et al. (1996) compared the effects of high pressure (300 and 400 MPa) at 50°C on microbial inactivation of duck foie gras with thermal pasteurization (80°C at the coldest spot). They reported a reduction of vegetative mesophilic and psychrotrophic contaminants, destruction of coliforms, and *Staphylococcus aureus* (similar to pasteurization) in samples treated at 400 MPa for 10 minutes at 50°C. It is unfortunate that different processing times were not used and that D-values were not calculated, which would have been useful in developing a database for microbial inactivation. O'Brien and Marshall (1996) studied the microbiological quality of freshly ground raw chicken meat (initial microbial population of 10^6 cfu/g) sealed in polyfilm pouches using HPP. They reported that application of 408, 616, and 818 MPa at ambient temperature for 10 minutes resulted in spoilage times of 27, 70, and > 98 days, respectively. They also concluded that facultative anaerobic psychrotrophs like *Carnobacterium divergens* and *Serratia liquefaciens* were highly resistant to applied pressures. Again, experiments were not performed at different holding times, preventing calculation of D-values to provide comparison with other similar studies. Nishiwaki et al. (1996) studied the effects of HPP on Mg-enhanced ATPase activity of rabbit myofibrils, to examine the effect of high pressure on actin/myosin interactions in rabbit meat. They reported similar Mg-enhanced ATPase activity and its sensitivity to ionic strength in myofibrils from pressure-treated muscles (up to 200 MPa) and from muscles conditioned for 7 days. A slight increase in ATPase activity was reported when isolated myofibrils were pressure treated. These changes in ATPase activity may reduce time required for conditioning of muscles. Also, release of soluble material from pressure-treated myofibrils was increased significantly at pressures > 150 MPa, which may further lead to tenderization. Brunn and Skibsted (1996) studied the effects of high pressure on the oxidation of nitrosylmyoglobin (an important pigment in cured meat products). They pressurized model cured meat systems (based on horse nitrosylmyoglobin) up to 300 MPa and reported decreased oxidation of nitrosylmyoglobin with increasing pressure. At 15°C in an air saturated solution (ionic strength 0.16 and pH 6.8), the first order rate constant for nitrosylmyoglobin oxidation was smaller by a factor of 5 at 300 MPa when compared with that at atmospheric pressure and ambient temperature. They explained that the pressure effect on oxidation was due to protein denaturation; reduced oxidation rates favor the use of HPP with cured meats.

Cheah and Ledward (1996) studied the effects of HPP on lipid oxidation in minced pork by treating some samples at 800 MPa for 20 minutes at 20°C and cooking other samples at 80°C (controls were untreated). The samples were stored for 8 days at 4°C. They reported that pressure-treated samples showed faster oxidation than controls; however, a significant increase in the rate of oxidation in minced pork was observed only at pressures > 300 MPa. Also, a significant denaturation of myofibrillar and sarcoplasmic proteins and conversion of reduced myoglobin/oxymyoglobin to the oxidized ferric form were also observed at pressures > 400 MPa. In a subsequent study, Cheah and Ledward (1997) also studied the effect of HPP on inhibition of metmyoglobin formation in fresh beef *longissimus dorsi* and *psoas major* muscles. The samples were treated at 80–100 MPa for 20 minutes at 2 or 20 days postmortem, and it was found that during early postmortem, color

stability was improved (a greater effect was observed on *longissimus dorsi* than *psoas major* muscles), whereas little effect on color stability was found during the latter postmortem period. This is an important study that may lead to the solution of the transient discoloration problem in centrally prepared retail beef cuts packaged under a controlled atmosphere of 100% CO_2/N_2, due to the presence of residual oxygen. However, a study using modified atmosphere packaging (high anoxic packaging) coupled with pressure treatment to reduce discoloration of beef has not been documented.

Paul et al. (1997) studied the effect of HPP, gamma irradiation, and combined treatments on the microbiological quality of lamb meat during chilled storage. Gamma irradiation (1.0 kGy) or pressure (200 MPa for 30 minutes at 30°C) or a combination of both was used to examine the shelf life of minced lamb meat after subsequent storage at 0–3°C. All coliforms were inactivated by either of these two treatments, whereas *Staphylococcus* spp. showed a reduction of only 1 log cycle when treated with irradation or pressure alone. In contrast, a combination treatment resulted in complete inactivation of *Staphylococcus* spp. (initial number in the untreated sample was 10^4 cfu/g) in samples immediately after the treatment. Also, a low number of injured cells, which were not present in the samples treated with irradiation or pressure alone, were recovered only after 3 weeks of storage following the combined treatment. This suggests an interactive effect against *Staphylococcus* spp. However, it should be noted that *Staphylococcus* spp. do not grow at temperatures < 9°C, therefore the nonpermissive storage temperature used following treatment could have provided opportunity for organisms to recover from injury sustained. Additionally, their study was restricted to one level of irradiation and pressure treatment. It is likely that with some other levels of treatments total inhibition of *Staphylococcus* spp. may be obtained, and calculation of D-values for these organisms would have been possible. Cheftel and Culioli (1997) reviewed the effects of HPP (100–800 MPa) on different components and quality attributes of meat. A detailed explanation and review of research done on various aspects (meat enzymes, meat structures, isolated myofibrillar proteins, meat texture, pressure-induced gelation, meat myoglobin, meat lipids, meat microorganisms, as well as subzero processing of meat) of pressurized meat is given. However, they did not discuss the effect of combined HPP and gamma irradiation on the microbiological quality of meat. There was also no attempt to generate D-values for HPP treatment of meat.

Takahashi and Haga (1997) studied the HPP of uncooked pickle-cured and fermented hams by monitoring changes in microbial growth, color, and composition of hams. They injected porcine *longissimus thoracis* muscle with *Lactobacillus* SK-1001 (10^6 cells/g) that was cured (3% salt) and fermented for 1 week at 5°C and then samples were subjected to a pressure treatment. They reported reduction in the survival of staphylococci and coliforms in pressure-treated fermented hams after pressure treatment as compared to untreated ones; however, there was no significant change in the number of lactic acid bacteria within 25 minutes after pressurization and 1 week later. Also, a significant increase in pH and a color change was noticed in the pressure-treated fermented hams (L* and H-0 values increased and a*, b*, and c* values decreased).

Acceleration of changes that take place during ageing, better meat tenderization (Macfarlene 1985; Suzuki et al. 1992), coagulation of pork slurries (Shigehisa et al. 1991), increase in the tensile strength of beef patties (Macfarlene et al. 1984), and changes in color and myoglobin content of minced beef (Cheftel 1996) are other effects induced by HPP treatment that may have further application in the meat-processing industry.

Dairy and Egg Industry

High-pressure processing may also have application in the dairy and egg industries due to changes induced in the functional properties of whey protein, as well as in other milk components and native constituents (table 12.3). Dumay et al. (1994) investigated the effects of HPP on the unfolding and aggregation of an industrial beta-lactoglobulin (ß-LG) protein isolate prepared from sweet or mixed whey. For processing, ß-LG solutions having pH of 7.5 with 0–5.0% sucrose (a baroprotectant) were prepared. Significant unfolding of the proteins occurred as ΔH (residual enthalpy of denaturation) was decreased by 44 or 54% when the 2.5 or 5.0% protein solutions were pressure processed at 0% sucrose, respectively. However, ß-LG remained soluble. The solubility of ß-LG in 2M ammonium sulphate decreased due to pressure-induced protein aggregation. A partial reversibility of pressure-induced unfolding and aggregation were obtained on storage for 7 days at 4°C. It was also found that the presence of 2.5 or 5.0% sucrose reduced ß-LG unfolding and slightly increased the recovery of protein solubility in 2M ammonium sulphate. Galazka et al. (1995) studied the effect of HPP on the emulsifying behavior of whey protein concentrate (WPC). The pressure was applied to the protein before homogenization or to the emulsion prepared with native WPC. Functional properties of WPC were examined along with the relationship between stability of WPC emulsions and degree of adsorption of the protein emulsifier. Galazka et al. found that oil-in-water emulsions (0.4 wt.% protein, 20 vol.% n-tetradecane, pH 7) prepared with pressure-treated WPC solutions gave a broader droplet size distribution than emulsions made with native untreated protein. An inverse relationship was obtained between emulsifying efficiency and applied pressure plus treatment time. Also, HPP had little effect on the stability of WPC emulsions made with native protein.

Lopez et al. (1996) studied the effects of HPP on whey protein denaturation and cheese-making properties of raw milk. They reported that high pressure slightly improved the microbiological quality of milk without modifying lactoperoxidase activity (a native milk enzyme). ß-LG was denatured by pressures > 100 MPa, whereas α-lactalbumin and bovine serum albumin were pressure resistant (400 MPa for 60 minutes). An increase in cheese yield was found (at 300 and 400 MPa) in conjunction with additional ß-LG and moisture retention. They concluded that HPP can improve the coagulation properties of milk and can increase moisture retention of fresh cheese. Buchheim and Frede (1996) investigated the effect of HPP on the crystallization of emulsified fats, using model emulsions (ultra-high-temperature, UHT-treated whipped cream and coffee cream, having 30 and 10% fats, respectively). They found that pressure treatment resulted in higher content of solid fats, and this result was also affected by pretreatment tempering at 37 or 60°C and the average fat globule size. They also demonstrated combination of pressure and temperature used during HPP of dairy products, which may be used for the optimization of HPP control unit (pressure-temperature-time) parameters. Their study was geared toward standardization of HPP for improvement in the functional characteristics of dairy fats and they were able to describe a relationship between pressure-temperature-time, which may lead to a better design of high-pressure food processors exclusively used for accelerating crystallization processes for emulsified fats and permit development of other value-added dairy products. Pittia et al. (1996) investigated changes in structure and surface properties of ß-LG by pressurizing 0.25% (w/v) ß-LG to 300 MPa for 10 or 30 minutes; 600 MPa for 10 or 15 minutes; and 900 MPa for 5 or 10 minutes. Pressure-treated ß-LG showed reduced

Table 12.3 Application of HPP in the dairy and egg industries

Reference	Product	Pressure (MPa)	Holding Time (min)	Processing Temperature (°C)	Microorganisms Tested	Other Parameters Studied
Dumay et al. (1994)	Whey	450	15	25		Unfolding and aggregation of an industrial ß-LG protein isolate
Galazka et al. (1995)	Whey protein concentrate	200, 600, or 800	10, 20, or 40	20		Emulsifying behavior of whey protein concentrate
Lopez et al. (1996)	Raw milk	100–400	10–60	20		Whey protein denaturation and cheese-making properties of raw milk
Buchheim and Frede (1996)	Whipped and coffee cream	100–550		10–24		Crystallization of emulsified fats
Pittia et al. (1996)	ß-LG	300, 600, or 900	5, 10, 15, or 30	20		Changes in structural and surface properties of ß-LG
Capellas et al. (1996)	Fresh goat milk cheese	400 or 500	5, 10, or 15	2, 10, or 25	*E. coli* (strain 405 CECT)	
Gervilla, Capellas et al. (1997)	Ewe's milk	200, 300, 350, 400, 450, or 500	5, 10, or 15	2, 10, 25, or 50	*Listeria innocua*	
Drake et al. (1997)	Milk	345 or 586	1, 15, or 2 1-min and 3 1-min cycles	5	*E. coli* and *Staphylococcus aureus*	Sensory quality of Cheddar cheese
Gaucheron et al. (1997)	Skim milk	250, 450, or 600	10	4, 20, or 40		Physicochemical characteristics of skim milk
Felipe et al. (1997)	Goat's milk	500	25 or 50			Whey protein in goat milk
Erkmen and Karatas (1997)	Milk	50–350	12	20	*Staphylococcus aureus* (ATC 27690)	
Adapa et al. (1997)	Skim milk	310	0.005	25		Functional properties of skim milk
Gervilla, Felipe et al. (1997)	Ovine milk	300, 400, 450, or 500	5, 10, or 15	2, 10, 25, or 50	*E. coli* 405 CECT, *Pseudomonas fluorescens* 378 CECT	
Ponce et al. (1998)	Liquid whole egg	300, 350, 400, or 450	5, 10, or 15	−15, 2, or 20	*Listeria innocua*	

emulsifying capacity and foamability compared to untreated controls, whereas the capacity for protein-protein interaction in the adsorbed layers at interfaces increased (due to an increased surface-dilational modulus and resistance to displacement by a surfactant in foams). They reported that there was a pressure-induced structural change in ß-LG, which increased its hydrophobic characteristics and aggregate formation potential (this accounted for reduced emulsifying capacity and foamability).

Capellas et al. (1996) investigated the effects of HPP on the populations of aerobic mesophiles and inoculated *E. coli* during storage of fresh goat milk cheese. They manufactured pasteurized goat milk cheese containing an added *E. coli* (strain 405 CECT) population of 10^8 cfu/g. After pressure treatment, samples were stored at 2–4°C. Microbial numbers were determined at 1, 15, 30, and 60 days after treatment. No colonies of *E. coli* were detected throughout storage and low numbers (2–3 log cfu/g) of aerobic mesophilic bacteria were consistently present. In their experiments, only three holding time periods were used and unfortunately none of these was < 5 minutes. This information would have been valuable from an industry perspective where processing that requires only a couple of minutes for complete destruction of microorganisms is attractive. Gervilla, Capellas et al. (1997) studied the effect of HPP on *Listeria innocua* 910 CECT inoculated into ewe's milk. Interestingly enough, at a temperature of 2°C, higher inactivation of *L. innocua* occurred than at room temperature when pressures between 450 and 500 MPa for 10–15 minutes were used. They reported first-order destruction kinetics for *L. innocua* and calculated D-values of 3.12 minutes at 2°C (400 MPa) and 4 minutes at 25°C (400 MPa). They commented that D-values using thermal processing at temperatures between 64 and 71.7°C are in the range of 0.95–0.09 seconds, which are much lower than D-values calculated using HPP. They noted that HPP generates reduced physicochemical modifications in milk compared with thermal processing, and that HPP at low temperatures can be used for significant inactivation of *L. innocua*. They also concluded that although fat has a thermoprotective effect, which is more dominant in ewe's milk than cow's or goat's milk, a significant reduction (7.7 log units at 400 MPa for 20 minutes at 2°C) in *L. innocua* was obtained using HPP. They recommended further studies be done to examine the baroprotective effects of ewe's milk on other microorganisms. This study was also aimed at determining D-values for inactivation of *L. innocua* using HPP, which is a requirement if HPP is to be adopted by the food industry.

Walkenstrom and Hermansson (1997) studied the microstructural (using light and transmission electron microscopy) and rheological (using dynamic oscillatory measurements and tensile tests) properties of high-pressure-treated mixed and pure gels of gelatin and WPC at pH 7.5 and 5.4. They reported that pressure-treated pure WPC gels had a higher degree of aggregation than conventionally heat-treated WPC gels, but gelatin gels remained unaffected by HPP. Further, the rheological properties of pressure-treated mixed gels indicated a higher degree of gelatin continuity than heat-treated mixed gels at pH 7.5. In contrast, at pH 5.4 the high-pressure-treated mixed gels formed a phase-separated network with a gelatin continuous phase and a discontinuous WPC phase. Not surprisingly, the rheological properties of mixed gels were the same as that of pure gelatin (independent of WPC). Drake et al. (1997) studied the effect of cycled high-pressure treatment of milk on the microbiological and sensory characteristics of Cheddar cheese. They reported the same flavor scores for pressurized and pasteurized milk cheeses but higher moisture and wet weight yields for pressurized milk cheese were associated with some texture defects. Nevertheless, they recommended HPP as an alternative to thermal pasteurization be-

fore cheese making, but they did not examine functional changes (protein denaturation) in pressurized milk cheese, which might affect its market value. Also, a comparison between the quality of static pressure-treated and cycled pressure-treated milk was not done. Gaucheron et al. (1997) investigated the combined effects of HPP on the physicochemical characteristics (lightness, Ca and P content, casein micelles, exposure of hydrophobic regions of milk proteins, and particle size) of skim milk processed with a holding time of 10 minutes. They reported that HPP produced an irreversible disintegration of casein molecules into smaller particles and caused an increase in protein hydrophobicity, casein micelle hydration, P and Ca solubilization, and ß-LG denaturation.

Felipe et al. (1997) compared the effects of HPP and thermal pasteurization on whey protein in goat's milk. They examined denaturation of the individual whey proteins using gel permeation FPLC (fast protein liquid chromatography). They found rapid aggregation of ß-LG and precipitation of disulphide- linked immunoglobulins and proteins upon pressure treatment at 25°C. The denaturation of WPC was affected differently by pressure and thermal pasteurization, while alkaline phosphatase activity in goat's milk was reduced or remained unaffected by thermal pasteurization and pressure, respectively. Adapa et al. (1997) determined the effect of HPP on the functional properties of skim milk (9% solids) due to HPP by subjecting concentrated (18% solids, using ultrafiltration) and unconcentrated milk to a pressure of 310 MPa for 0.3 seconds. This was followed by chilled storage of samples at 4°C. Stable foams and emulsions were produced only by pressurized milk, which was also more viscous. Also, L^* (lightness), a^* (red/green), and b^* (blue/yellow) values were lower in pressurized unconcentrated milk samples than controls. Creamers were made with pressurized milk and samples were reported to be stable, but their study was limited to only one level of pressure treatment. Erkmen and Karatas (1997) studied the effect of HPP on *Staphylococcus aureus* (ATCC 27690) in milk at pressures in the range of 50–350 MPa for up to 12 minutes at constant temperature (20 ± 2°C). They found no survival at pressure treatments of 350 MPa for 6 minutes and 300 MPa for 8 minutes. Also, D-values were reported as 211.8, 15.0, 3.7, and 2.6 minutes at 200, 250, 300, and 350 MPa, respectively. While these data are valuable it was unfortunate that the study was limited to use of only one temperature. Other temperatures (< 20 or > 20°C) may have resulted in total inhibition at lower pressures. Gervilla, Felipe et al. (1997) investigated the effects of HPP on *Escherichia coli* 405 CECT and *Pseudomonas fluorescens* 378 CECT strains in ovine milk by subjecting inoculated milk samples to different combinations of pressure, temperature, and time. Temperature played a very important role in inactivation of microbes as > 6 log cfu/ml reduction in microbial populations was observed at 50°C for all pressure-time combinations. They reported that the test strain of *E. coli* was more pressure resistant than the *Pseudomonas fluorescens* strain. However, they did not calculate D-values for HPP of microbial inactivation in ovine milk. Also, if shorter holding time levels (< 5 minutes) had been used, these would have been useful in determining the effects of "instant" treatment at high isostatic pressure. It is possible that most microbial inactivation occurs during the come-up time of pressurization. Their research also did not determine the influence of adiabatic heating (resulting from pressure application), which may have affected their results.

Ponce et al. (1998) studied the inactivation of *Listeria innocua* inoculated in liquid whole egg using HPP by subjecting the food to different combinations of pressures, temperature, and time. Total inhibition of *L. innocua* was not reported; however, reduction was > 5 log at 2°C for 15 minutes (450 MPa). D-values for *L. innocua* were reported as 7.35

minutes at 2°C (400 MPa) and 8.23 minutes at 20°C (400 MPa). The upper temperature limit used was 20°C, and it is possible that combinations of higher temperature and pressure (e.g., 40°C and 450 MPa) might have resulted in total inhibition of the organism. However, their approach is valuable because it documents D-values during HPP of liquid whole eggs. They also reported that the reduction of *L. innocua* was a little greater at 2 and −15°C than at room temperature (at pressures of 300 and 350 MPa). They explained this behavior as being due to the greater susceptibility of some proteins to denaturation at low temperatures. This study is one of the very few that used HPP at subzero temperatures. They also recommended work be done to pressure treat *Salmonella*-inoculated egg, since these organisms frequently contaminate egg products. The effects of different components of liquid eggs, which might act as baroprotective agents for microorganisms, need to be studied in greater depth.

Cell Sensitizer

Results indicate that high-pressure treatment can be an effective antimicrobial process, enhancing the lethality of parallel physical and chemical treatments, thereby increasing the overall efficacy of preservation processes. Earnshaw (1992) reviewed the potential applications of HPP as a cell sensitizer and mentioned that high pressure damages cell membrane structure and results in cytoplasmic leakage even at 101 MPa. Nachmanson (1995) discussed synergistic effects of HPP and modified atmospheres as well as HPP and packaging material containing zeolite (which can be used in packaging material to inhibit the growth of microorganisms). He reported a large reduction in viable numbers of yeasts (*Saccharomyces cerevisae* and *Candida tropicalis*) following treatment at 3–400 MPa when the headspace was filled with CO_2. When treatment was done using packaging material containing zeolite, there was a significant reduction of the microbial population. When the two procedures were used separately the results were relatively poor. Application of pressure with natural antimicrobials (food acids, herbs and essential oils, bacteriocins), synthetic chemicals, or modified gas atmospheres may yield another dimension for fresh food preservation by erecting a series of hurdles preventing growth by spoilage and pathogenic bacteria at levels of individual agent intervention that leave the food essentially unaffected (Earnshaw 1992).

Summary and Future Research Needs

There is no doubt that HPP represents another interesting and promising dimension for food processing not only because it inactivates microorganisms but also because it provides opportunities for development of new "value-added" food products. The need for an alternative to thermal processing as the primary means of eliminating pathogenic and spoilage microorganisms is substantial. High-pressure processing holds promise since food materials treated by this method retain their natural flavor, color, and texture without loss in vitamin or nutrient content. Furthermore, predictable changes in functional characteristics of proteins and complex carbohydrates (where little work has been done) mean that there are some exciting avenues of work in HPP treatment of foods that remain to be explored. Although a lot of research has been conducted in the area of high-pressure processing, a lot remains to be done in terms of understanding the critical limits of the process and the extent to which this might ensure appropriate treatment of food materials.

Research has been done in various research institutions, universities, and food industry R & D laboratories, yet a direct comparison of the data obtained is not possible. There can be no possibility of extensive implementation of this technology unless specific processing parameters are established for each food material treated by HPP. The possibility of commercialization of HPP also depends on its economic viability. Therefore, a detailed economic analysis of HPP needs to be done by comparing the process costs with the present costs of processing by conventional means and at the same time factoring in the added value of the improved sensory characteristics of HPP-treated foods. Some indications of investment and operational costs of HPP are available from the machine manufacturers, although perhaps not fully reliable.

Thus far no concerted effort has been made to validate HPP procedures for complete elimination of pathogenic bacteria. It is important that standard operating criteria and processing conditions be established that will ensure the reliability of HPP as an alternative to thermal processing. For example, thermal processing has had standards like D- and Z-values, holding times, and minimum temperatures developed for different foods; likewise, standards need to be set for HPP. Very few studies on the HPP inactivation of microorganisms discuss HPP effects in terms of the D-value associated with each particular strain of organism (table 12.4). More research is needed along these lines so that a database of HPP D-values for different microorganisms can be prepared. Without a well-documented database of D-values, there is no means to effectively compare the results of experiments performed with different microorganisms under different processing conditions. There is a need for comparable standards that might indicate the resistance of various microorganisms to pressure and temperature combinations used in HPP. Different D-values at constant values of temperature and pressure may be used as an indication of resistance of a particular bacterial species with respect to another species. Thus, an effort should be made to understand and derive both D-values and Z-values so that minimum processing conditions may be developed for contaminants in target foods. Since the general norm followed in the food industry is to subject food products to treatments that ensure a 12D reduction of microorganisms, to ensure that HPP is effective in inactivating microorganisms, treatments that result in 12D reduction must be another goal. Perhaps it is not surprising that no database of D- and Z-values for microbial species has been developed for HPP. It must be kept in mind that these parameters have been derived for temperature and time effects together without regard to pressures. This, therefore, does not mean that even if we have these values for various microorganisms, we can predict the precise parameters of pressure, temperature, and time required to eliminate the bacteria completely from the food by HPP. To fully validate HPP, a relationship has to be developed between the pressure of processing and the reduction in microbial population under treatment over a fixed time and temperature. The methods adopted to derive this parameter may be different but the end result must provide an effective means of predicting bacterial reductions due to HPP.

Remaining questions are variability of pressure resistance between different strains of the same microbial species; possible protecting effects of food constituents, making it important to study microbial inactivation in given foods; stressed cells and their possible recovery during chilled or ambient storage; and effects of high pressure and subzero temperature processing and effects of pressure cycling and pressure-ac exposure treatments to reduce the microbial viability. An exciting aspect of this technology is its effect on different functional characteristics of foods (starch gelatinization, protein denaturation, enzyme inactivation). Not only do these areas require further study but also HPP as a method of

Table 12.4 D-values* for different microorganisms during HPP

Microorganism	Pressure (MPa)	Temperature (°C)	D-value (min)	Reference
Clostridium pasteurianum	700	60	2.4	Maggi et al. (1995)
Clostridium pasteurianum	800	60	3.4	Maggi et al. (1995)
Citrobacter freundii	230	20	14.7	Carlez et al. (1992)
Listeria monocytogenes Scott A	414	25	2.17	Ananth et al. (1998)
Salmonella typhimurium ATCC 13311	414	2	1.48	Ananth et al. (1998)
Listeria innocua 910 CECT	400	2	3.12	Gervilla, Capellas et al. (1997)
Listeria innocua 910 CECT	400	25	4	Gervilla, Capellas et al. (1997)
Staphylococcus aureus ATCC 27690	200	20	211.8	Erkmen and Karatas (1997)
Staphylococcus aureus ATCC 27690	250	20	15	Erkmen and Karatas (1997)
Staphylococcus aureus ATCC 27690	300	20	3.7	Erkmen and Karatas (1997)
Staphylococcus aureus ATCC 27690	350	20	2.6	Erkmen and Karatas (1997)
Listeria innocua	400	2	7.35	Ponce et al. (1998)
Listeria innocua	400	20	8.23	Ponce et al. (1998)

*D-value is defined as time required to cause a decimal reduction in microbial population at a constant pressure and temperature.

storage by freezing foods (pressure-shift freezing, rapid thawing, rapid cooling) requires further work. From the methodological standpoint, it would be necessary to monitor temperatures in the food sample and pressure at different positions in the HP vessel and to use well-calibrated pressure gauges. Today, high-pressure food processors are available for batch and/or semicontinuous processes. Developing a continuous high-pressure food processor for high-viscous foods still remains an engineering challenge.

Although HPP shows promise in its ultimate usefulness for food processing, limitations with respect to difficulty in data comparison and complexity associated with understanding interactive components of the process currently limit full acceptance of the practice. The only way to fully commercialize high-pressure food processing is to find niche applications of this technology that can provide the processors and retailers with maximum profit and entry into sectors of the food industry that were untapped before.

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13 Pulsed Electric Field Technology: Effect on Milk and Fruit Juices

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Introduction

Pulsed electric field (PEF) technology has the potential to replace traditional thermal processes for the pasteurization of liquid foods such as milk and fruit juice. It is of particular interest for processing products containing heat-sensitive components such as whey proteins and immunoglubulins. Other promising applications of the technology include inactivation of enzymes and increasing the yield of juice extraction. The major advantages of PEF over thermal technologies for preservation of food are the retention of color, flavor, texture, and nutritive value of the raw (unprocessed) food and lower operational costs. Most vegetative cells can be destroyed by PEF; however, bacterial spores are much more resistant and can only be destroyed under certain circumstances. PEF can be used to extend the shelf life of milk and fruit juices, but many factors influence its effectiveness. These include the physical and chemical properties of the food, types of microorganisms present, and processing conditions. These factors need to be thoroughly understood before the technology can be widely used in an efficient and safe manner in commercial food processing.

Pulsed-energy technologies have been developed in various modes for treatment of foods to inactivate microorganisms. They utilize high energy levels in short bursts or pulses (microseconds to milliseconds) with the expenditure of average power consumption. The energy is initially generated as electrical energy and accumulated in a storage capacitor. The accumulated electrical power (many megawatts) is discharged almost instantaneously in the form of high-energy electric field pulses (PEF technology), high-intensity light pulses (pulsed light technology), or high-intensity magnetic field pulses (oscillating magnetic field technology). Of these, PEF technology has attracted by far the most attention. To date it has found little commercial application but shows considerable potential for adoption in the food processing industry.

PEF has the potential to replace, or at least complement, traditional thermal processes for the pasteurization and sterilization of liquid food products such as milk and fruit juice and for enhancing the efficiency of extraction of components from plant foods. Since most PEF operations are performed under mild conditions, often at ambient temperature, the technology offers considerable consumer benefits in terms of better quality (flavor, color, and retained nutritive value) and comparable or better safety than corresponding thermal processes. It may also offer processors benefits in terms of lower operational costs than thermal processes.

This chapter provides an overview of PEF technology and its application to milk, fruit juices, and related products. PEF has been the subject of several reviews (e.g., Barbosa-Canovas et al. 1998, 1999; Barbosa-Canovas and Zhang 2000; Jeyamkondan et al. 1999; Knorr et al. 1994; Rastogi 2003; Sampedro et al. 2005; Sitzmann 1995).

Principle of PEF Technology

Many foods conduct electricity when placed between the electrodes of an electrical circuit due to the presence of ions (Zhang, Monsalve-Gonzalez, Barbosa-Canovas et al. 1994). When the food is subjected to the electrical current, numerous phenomena occur that can contribute to destruction of microorganisms. These include ohmic heating, which results from natural electrical resistance of the food (Sastry and Barach 2001), electrolysis (Hulsheger and Niemann 1980), electroporation, the disruption of cell membranes (Sitzmann 1995), and arc discharge causing shock waves (Zuckermann et al. 2002). Although these phenomena never occur completely independently, the individual effect of each on microorganisms is determined by the manner in which the electrical energy is applied.

By applying high-voltage pulses of very short duration, with relatively long intervals between pulses, PEF technology minimizes the undesirable effects of the above phenomena; that is, the temperature increase caused by ohmic heating, the electrolytic oxidative effects encountered in electrolysis, and the disintegration of food particles caused by the shock waves produced by electric arc discharge (Hulsheger and Niemann 1980; Palaniappan and Sastry 1990; Sitzmann 1995; Zhang, Monsalve-Gonzalez, Barbosa-Canovas et al. 1994).

The pulse caused by the discharge of electrical energy from the capacitor is extremely short (1–100 μs), while the interval between discharges is comparatively large (1 millisecond to seconds) (Qin, Pothakamury et al. 1995). The number of pulses can vary from ten to one hundred. Total treatment times (number of pulses multiplied by pulse width or duration) are generally less than 1 second. The strength of the electric field that passes through the food is directly proportional to the voltage supplied across the electrodes and inversely proportional to the gap or distance between the electrodes (Barbosa-Canovas et al. 1999). PEF technology utilizes electric field strengths of 10–80 kV/cm.

PEF Equipment

The essential components of a PEF processing system are: (1) a high-voltage power supply (~30 kV); (2) an energy storage capacitor; (3) a pulse generator and switching system; (4) a treatment chamber, containing two electrodes, through which liquid food is pumped; (5) a cooling system; (6) a unit for control and monitoring of voltage, current, and electric field strength; and (7) a product delivery and packaging system.

The design of the treatment chamber is critical to the efficacy of this technology. It is essential that a uniform electric field exists throughout the cell to avoid "cold spots" and/or areas that promote electrical breakdown of the food product. The electrodes are usually 3–5 mm apart but several different treatment cell designs have been developed and patented. Common configurations are parallel plate, co-field, co-linear, and co-axial. Some have specific characteristics such as a conical-shaped electrode and insulators to prevent gas depositing within the treatment volume, which can lead to electrical breakdown of the food. Some systems operate under pressure to prevent such gas development. Multiple treatment chambers can be used to eliminate the effect of nonuniformity of electrical field strength within individual chambers.

Bactericidal Effects

The most important aspect of PEF technology in the treatment of liquid foods is its effect on bacteria. Most vegetative cells can be destroyed by the technology but bacterial spores are much more resistant and can only be destroyed under certain circumstances.

Mechanisms of Inactivation

The mechanisms through which PEF inactivates vegetative microbial cells and bacterial endospores have been studied for many years. While the mechanisms of inactivation are still not fully elucidated or understood, a number of promising theories have been postulated. It is quite conceivable that all the theorized mechanisms are involved in the complicated process of microbial inactivation by high-intensity electric fields (Zimmermann 1986).

Vegetative Bacterial Cells

Several theories exist for the destruction of bacterial cells by PEF, but they commonly describe disruption of the semipermeable cell membrane, which leads to swelling or shrinking and eventually lysis of the cell (Sale and Hamilton 1968).

Any damage to the cell membrane affects its functioning and may inhibit metabolic activities and therefore reproduction of the cell. Damage to the extent of irreversible structural changes and pore formation in the membrane, known as "electrical breakdown," can lead to osmotic imbalance and subsequent leakage of intracellular contents (Zimmermann 1986).

It has been suggested, nevertheless, that pore formation and leakage of intracellular contents is not the only mechanism of inactivation. Disruption of internal organelles and other structural changes have been reported by Barbosa-Canovas et al. (1999). Barsotti and Cheftel (1999) further state that while the observed morphological changes would affect the cells' functions and metabolism, it is unlikely that permeabilization of the membrane is the only mechanism responsible for inactivation of the microorganisms by electric pulses.

Dielectric Rupture Theory

According to the dielectric rupture theory, the cell membrane is regarded as a capacitor filled with material of a low dielectric constant (~2), while the cell cytoplasm has an electrical conductivity six to eight times greater than that of the membrane, and most liquid foods have a dielectric constant between 60 and 80 (Barbosa-Canovas et al. 1999). Due to the differences in dielectric constants (i.e., the low conductivity of the cell membrane compared with much higher conductivities in the suspension liquid and cell cytoplasm), free charges of opposite signs accumulate at the inner and outer surfaces of the cell membrane, causing a tiny transmembrane potential of about 10 mV (Jeyamkondan et al. 1999). While free charges accumulate at both the surfaces, they do not exist in high concentrations inside the membrane's phospholipid bilayer structure (Barsotti and Cheftel 1999; Zimmermann 1986).

When a high-voltage electric field is applied to cells suspended in a fluid, ions inside the cell move along the field until they are restrained by the membrane. This causes an increase in the voltage potential across the membrane of the cell, equal to the potential induced by the electric field plus the original transmembrane potential. The charges generated on the membrane surfaces have been shown to be of opposite charge, thereby attracting each other to the extent of compressing the membrane and reducing its thickness. Furthermore, as the thickness of the membrane decreases, the attractive forces between opposite charged ions become stronger (Barbosa-Canovas et al. 1999; Jeyamkondan et al. 1999). The compression of the membrane is naturally opposed by the membrane's

viscoelastic properties, but in a well-conducting fluid these restoring forces cannot match the increase in electrocompressive force that accompanies the decreasing membrane thickness with increasing strength of the applied electric field (Barsotti and Cheftel, 1999; De Jong and Van Heesch 1998).

Once a critical transmembrane voltage (V_c) is exceeded, the electrocompression increases to a point where breakdown or pore formation is induced at a given location on the membrane. This is because any local perturbations on the membrane surface will grow spontaneously if the transmembrane potential is of sufficient intensity to overcome the opposing restoring forces (Zimmermann 1986). Typical V_c values are close to 1 V, or at least in the range of 0.75V–1.25V (Sensoy et al. 1997). In terms of PEF process parameters, an external electric field of 1–10 kV/cm corresponds to a transmembrane potential of 1 V (Castro et al. 1993).

The dielectric rupture effect may be reversible or irreversible, depending on the intensity of the electric field (how much the applied voltage exceeds V_c) and the number of pulses delivered (Simpson et al. 1999). Reversible pores may be repaired within seconds or minutes, such that when the external electric field is removed, the membrane reverts from a conducting state to regain its original electrical properties and permeability (Zimmermann 1986). But if the duration of the 1–10 kV/cm electric field pulse is longer than 10–15 milliseconds, or the number and size of pores become large relative to the membrane surface, irreversible breakdown occurs (Castro et al. 1993).

The formation of reversible pores significantly increases the cells' vulnerability to subsequent stresses, such as the simultaneous application of other treatments like heat, high hydrostatic pressure, or ultrasonication (Simpson et al. 1999).

Several factors favor the inactivation either by enhancing the initial perforation or subsequent expansion of pores. Higher electric field strengths and pulse durations cause greater perforation and expansion, while treatment fluids with low ionic strengths favor pore expansion. Cell size is also important, as cells with a larger surface area are prone to more areas of compression and subsequent poration (Barbosa-Canovas et al. 1999).

Electroporation Theory

The "electroporation" theory describes how the functionality of the cell membrane may be destroyed by disturbing its ordered structure. The primary targets are the constituent dipolar molecules, principally phospholipids and proteins (Castro et al. 1993).

In aqueous solutions, a phospholipid bilayer is formed spontaneously with the fatty acids inverted toward one another and the polar portions exposed to the aqueous external environment (Brock et al. 1994). Due to its net electric charge, the membrane's lipid bilayer is also affected by electric fields. According to Glaser et al. (1988), lateral thermal fluctuations of lipid molecules cause the spontaneous formation of reversible pores within the membrane, which typically have a wall of hydrocarbon lipid tails. These are termed *hydrophobic* pores.

As expansion of the hydrophobic pores continues under an applied electric field, the pore radius increases to a point where "pore inversion" occurs. This refers to the reorientation of the phospholipid molecules toward a lower energy configuration, to form *hydrophilic* pores (Barbosa-Canovas et al. 1999). Hydrophobic pores predominate below a pore radius of 0.3–0.5 nm, but above this size inversion is energetically enhanced and hydrophilic pores with a radius of 0.6–1.0 nm are formed (Barsotti and Cheftel 1999).

Hydrophilic pores conduct current, resulting in localized joule heating (Simpson et al. 1999). This, in turn, causes thermal transformation of the lipid bilayer from a rigid gel structure to a liquid crystalline structure. This can impair the semipermeability of the membrane and increase its permeability to ions. Or, if pores exceed a certain radius, they will grow indefinitely to result in mechanical breakdown of the membrane (Barbosa-Canovas et al. 1999; Jeyamkondan et al. 1999). According to Glaser et al. (1988), an increase in membrane conductivity during and after breakdown results from the formation of these hydrophilic pores. Since the conductivity of the membrane is a nonlinear function of voltage, the voltage will influence the number, the mean size, and the conductivity of the pores. The voltage difference required across the cell membrane for such breakdown of the phospholipid bilayer is reportedly 150–500 mV (De Jong and Van Heesch 1998).

Intact membranes do not allow free diffusion of polar molecules but instead regulate the uptake and concentration of solutes within the cell (regardless of the solute concentration outside the cell) through the action of membrane transport proteins (Madigan et al. 2000). These proteins span the bilayer such that they have surfaces exposed on both the inside and outside of the cell, and their opening and closing occurs due to small transmembrane potentials of about 50 mV (Jeyamkondan et al. 1999). These protein channels are extremely sensitive to transmembrane potentials or electric fields, and many voltage-sensitive protein channels will open before the rearrangement of membrane phospholipids and formation of hydrophobic pores take place (at transmembrane potentials of 150–500 mV) (Barsotti and Cheftel 1999; De Jong and Van Heesch 1998). When a high electric field is applied, however, protein channels will not only open but also experience much greater current than they are designed to conduct during normal metabolic activities (Tsong 1991). As a result of this current, the proteins forming the channels become denatured due either to localized joule heating or direct electric modification of functional hydroxyl, carboxyl, sulfhydryl, or amino groups (Simpson et al. 1999).

Vegetative Yeast Cells

The effect of PEF on yeast, especially strains of *Saccharomyces cerevisiae*, has been investigated in a similar manner to the effect on bacteria. The theories of membrane permeabilization and disruption and leakage of intracellular contents appear to apply to the inactivation of yeast cells as they do to vegetative bacteria (Grahl and Markl 1996; Mizuno and Hori 1988; Qin et al. 1998), although one published study reported that bacteria and yeast do not exhibit the same membrane characteristics when inactivated in electric fields (Hulsheger et al. 1983).

Morphological examination by electron microscopy of yeast cells subjected to PEF has revealed a range of structural changes. Sitzmann (1995) stated that PEF-treated *S. cerevisiae* cells in salt solution appeared quite different from untreated cells, the high-voltage pulses causing little "craters" on the cell surface. Mizuno and Hori (1988) observed that *S. cerevisiae* cells in deionized water appeared punctured by high-voltage pulses, but many were not punctured and still appeared normal. Grahl and Markl (1996), after examining the morphology of PEF-treated *S. cerevisiae* cells, concluded that pulsed electric fields caused the formation of small pores in the cell membrane. Their experiments on the disintegration of yeast cells showed that the cells did not burst under high-voltage pulses, but the small pores allowed the release of small cell components. Castro et al. (1993) observed the leakage of *S. cerevisiae* intracellular contents to a much greater extent, reporting that the PEF-

treated yeast cells appeared like deflated balloons compared with untreated cells. This was supported by Jin et al. (1998), who used a high-magnification microscope interfaced with a high-speed image system to observe real-time breakage of the membranes of yeast cells and release of intracellular components by PEF treatment.

Bacterial Endospores

Bacterial endospores are much more resistant to PEF than vegetative cells. The mechanisms and kinetics of inactivation of spores are, however, not well understood. The different structure, composition, and electrical properties of endospores compared with vegetative cells suggest the effect of electric fields on their functionality must also be different.

It has been determined that electrical conductivity is considerably less in endospores than in vegetative cells, due to the fact that ions outside the spore core are mobile, but ions within the core are not (Marquez et al. 1997). Consequently, only about 40% of the spore volume can be penetrated by electric fields (Carstensen et al. 1971). The higher inactivation rates observed by Marquez et al. (1997) when the temperature of the system was raised from 5–10°C to 25°C might be due to the faster leakage of mobile ions from extracted (decoated) spores. The increase in average kinetic energy of mobile ions at higher temperatures would hasten their movement and therefore enhance the rate of leakage from the cell. A higher temperature also increases the motion of the solvent molecules in both the core and the surrounding cortex so that the molecules could more readily migrate from one electrode to the other.

PEF-inactivated spores studied under scanning electron microscopes have been observed to suffer structural changes similar to those of thermally inactivated spores (Jin et al. 1998; Su et al. 1996). Marquez et al. (1997) observed that PEF damage to *Bacillus cereus* spores apparently took on two forms: some spores suffered visible holes due presumably to pulse shots, while others appeared to be enlarged and completely destroyed.

Factors Affecting the Bactericidal Effects of PEF

Electric Field Strength

As described above, the transmembrane potential across a bacterial cell increases with the electric field strength applied and a critical potential (V_c) must be reached before cell membranes are damaged by PEF (Sensoy et al. 1997). At low field strengths, sublethal damage may occur in the membrane, but it self-repairs at the cessation of the PEF treatment. However, for all cells, when the field strength reaches a critical level (E_c), irreversible damage begins to occur. Greater damage, and hence greater bacterial destruction, is inflicted on the cell as the electric field strength is increased.

Suitable electric field strengths able to be employed through PEF processing are limited to a range dependent on E_c for a specific cell type, and the composition/properties of the suspension liquid. When the electric field strength is smaller than the E_c of the bacteria present, the energy delivered to the food is not effective in inactivating bacteria and only contributes to unnecessary heating of the food (Qin, Pothakamury et al. 1995). At excessive field strengths, arcing in the treatment fluid and dielectric breakdown may result (Jayaram et al. 1993).

The relationship between electric field strength and microbial inactivation is not linear.

Jayaram et al. (1992) reported that with increases of electric field strength greater than E_c, larger numbers of *Lactobacillus brevis* cells became inactivated, but the rate gradually declined.

In most cases, electric field strength is the dominant factor for microbial inactivation, with higher field strengths being more bactericidal than longer treatment times, even though critical treatment times must also be reached for inactivation to occur (Jeantet et al. 1999; Simpson et al. 1999). Electrode distance is inversely proportional to electric field strength, but wave pulse-form can also change with electrode distance, and this has a separate influence on critical field strength, as discussed later (Ho et al. 1995).

Other factors such as treatment temperature, type of cells targeted, and fluid properties can also influence the critical electrical field strength needed for bacterial inactivation (Ho et al. 1995; Sensoy et al. 1997).

Treatment Time

PEF treatment time is the product of the number of pulses applied and the pulse width or pulse duration. In the case of an exponentially decaying pulse, the pulse width has been defined as the time needed to decrease the voltage to 37% of its peak value (Vega-Mercado et al. 1997). Pulse widths in the order of nanoseconds to milliseconds have been described, and the time lapse between pulses is typically much longer than the width of each pulse (Bruhn et al. 1998; Zhang et al. 1995).

Both critical field strengths (E_c) and critical treatment times (T_c) need to be met to inactivate bacteria, but adjusting field strength has a greater impact on the lethality of PEF than adjusting treatment time. Jeyamkondan et al. (1999) stated that the inactivation rate of microorganisms is directly proportional to the applied electric field strength but proportional to the logarithm of treatment time. The critical treatment time also depends on the electric field strength applied, especially the extent to which the field strength exceeds E_c. Grahl and Markl (1996) state that for electric field strengths more than 1.5 times higher than E_c, the critical treatment time seems to be constant.

Increased pulse widths required lower E_c to give higher inactivations, though it must be noted that larger pulse durations may cause undesirable heating in the food (Barbosa-Canovas et al. 1999). Glaser et al. (1988) state that an increase in the width of each pulse leads to a proportional increase in the number of pores formed in the membrane, and also that the radii of pores increase during the pulse.

An increase in the number of pulses applied also results in greater inactivation, but like electric field strength, the rate of inactivation does not increase linearly with number of pulses. It has been found for both *S. cerevisiae* and *L. brevis* cells that most inactivation occurred with the first three to five pulses, but as more pulses were applied the inactivation rate did not increase accordingly (Jayaram et al. 1993; Zhang, Monsalve-Gonzalez, Barbosa-Canovas et al. 1994).

The number of pulses or pulse width may be more important than the total treatment time for inactivating microorganisms, as Barsotti and Cheftel (1999) explain that the lethal effect of ten square pulses of 2 μs each appears to be greater than one pulse of 20 μs.

Shape of the Pulse Wave

Electric field pulses may be applied in a number of shapes or forms such as square, exponentially decaying, oscillatory, and instant charge reversal ("spiked"), as well as being

either monopolar or bipolar. Several authors have concluded that square wave pulses are more lethal to bacteria than exponentially decaying pulses (Qiu et al. 1998; Simpson et al. 1999; Zhang, Monsalve-Gonzalez, Qin et al. 1994). Although square wave pulses require more complex circuitry systems, they are also more energy efficient (Zhang, Monsalve-Gonzalez, Qin et al. 1994).

Oscillatory pulses are considered the least efficient for microbial inactivation (Barbosa-Canovas et al. 1999) because they prevent continuous exposure of the cells to a high-intensity electric field for an extended time (Jeyamkondan et al. 1999).

Bipolar pulses are caused by a reversal in polar orientation of the applied electric field and are more lethal than monopolar pulses. This is because a sudden reversal of the electric field orientation changes the direction of charged molecules on the cell membrane. The alternating stress produced in the membrane causes structural fatigue, which increases the susceptibility of the membrane to electrical breakdown (Jeyamkondan et al. 1999). Bipolar pulses also minimize energy utilization and reduce deposition of solids on electrode surfaces and electrolysis of the food (Barbosa-Canovas et al. 1999).

"Spiked" waves, which undergo instant charge reversal, differ from bipolar pulses in that there is no relaxation time between the pulses of opposite polarity. Instant charge reversal may reduce the critical electric field strength, due to the alternating electrical stress on cell membranes resulting in structural fatigue (Ho et al. 1995). Marquez et al. (1997) found that application of electric pulses with instant charge reversal decreased the viability of *B. subtilis* and *B. cereus* endospores compared with treatment without instant charge reversal.

Treatment Temperature

A major advantage of PEF technology is its ability to reduce the microbiological load of foods without significant heating. Many authors have achieved substantial inactivation of both microorganisms and enzymes in food at temperatures below 50°C (Ho et al. 1997; Marquez et al. 1997; Vega-Mercado et al. 1995). However, different PEF conditions are often necessary for different products to maintain suitably low temperatures. For example, Qin, Pothakamury et al. (1995) found fresh apple juice, raw skim milk, and beaten eggs all required three steps of PEF exposure of between three and seven pulses (different for each product) to keep temperatures below 45°C, 55°C, and 53°C, respectively.

It has been shown that a synergistic effect exists between applied electric fields and elevated temperatures. At constant electric field strength, sensitivity and inactivation of microorganisms increase with an increase in product temperature (Simpson et al. 1999). This may be related to the phase transition of phospholipid molecules in the membrane's lipid bilayer, from a rigid gel structure to a liquid-crystalline phase. The associated reduction in bilayer thickness may make the membrane more susceptible to breakdown at a relatively high temperature and low field strength (Reina et al. 1998). For example, Reina et al. (1998) observed greater reductions of *L. monocytogenes* in milk treated by PEF at 50°C than at 25°C. Zhang et al. (1995) observed greater PEF inactivation of *E. coli* in skim milk at 20°C than at 7°C, but an additional temperature increase to 33°C did not affect the inactivation rate. Such results suggest that the temperature influence on membrane breakdown under PEF may depend on the microorganism tested as well as the media used (Reina et al. 1998).

While it has been proposed that moderate heating (60°C) be used in combination with PEF to maximize its lethality (Simpson et al. 1999), this may have adverse consequences depending on the food product being treated and its sensitivity to heat. The application

of heat may negate the greatest advantage of PEF to industry, its non-thermal nature, and possibly affect both product quality and the amount of energy input required. Marquez et al. (1997) observed a marked reduction in survivability of bacterial spores using PEF at 25°C instead of 5–10°C (at all combinations of electric field strength and pulse number). This demonstrates that temperatures of 50–60°C are not necessary for effective inactivation of certain endospores, which are typically the most resistant types of cell (Sitzmann 1995).

Microbiological Factors

Type of Microorganism

Types of microorganisms differ in their sensitivity to PEF; generally, yeasts are more sensitive than bacteria, rod-shaped organisms are more sensitive than cocci, and Gram negative bacteria are more sensitive than Gram positive (Jeyamkondan et al. 1999).

These properties are related to specific characteristics such as cell size and membrane structure. Larger cells such as yeast have a greater membrane surface area where charges may accumulate under applied electric fields. This would help in developing a larger potential difference across the membrane and make it more susceptible to electrical breakdown (Barbosa-Canovas et al. 1999). Membrane characteristics that may influence sensitivity in an electric field include surface properties and elasticity (Knorr et al. 1994; Martin et al. 1997).

Simpson et al. (1999) noted that variations in sensitivity to several preservation treatments occurred between different strains within a species. It was therefore hypothesized that such variations may exist for PEF. However, Evrendilek et al. (1999) found no difference in sensitivity between *E. coli* O157:H7 and *E. coli* 8739 strains.

Growth Stage of Microorganisms

Cell and membrane properties of microorganisms differ at different stages of growth, and sensitivity to applied electric fields is highest during the logarithmic phase and lowest in the stationary growth phase (Pothakamury et al. 1996; Sensoy et al. 1997).

Barbosa-Canovas et al. (1999) explained the high sensitivity of cells in the logarithmic growth phase by their state of high proliferation. During reproduction, the area between the mother and daughter cells as well as the sensitive parts of the cell envelope are more susceptible to the applied electric field.

Treatment Medium Parameters

Numerous chemical and electric properties of suspending/treatment media can affect the biological changes induced in microbial cells by PEF (Simpson et al. 1999). These include the medium's conductivity, ionic strength, and pH; the presence of particles or gas bubbles; and the addition of antimicrobials (Barbosa-Canovas et al. 1999).

Conductivity and Ionic Strength

The conductivity of a medium refers to its ability to conduct electric current and increases as a consequence of increased ionic strength (Barbosa-Canovas et al. 1999). Martin et al.

(1997) found lower inactivation of *E. coli* in skim milk than in buffer solutions after a treatment with the same field intensity and number of pulses. It was proposed that the complexity of skim milk composition and its higher conductivity provided the protective effect on *E. coli* against PEF.

Jeyamkondan et al. (1999) state that lowering conductivity of the medium increases the difference in ionic concentration between the cytoplasm and suspending fluid. This can weaken the membrane structure by increasing the flow of ionic substances across the membrane. According to Barbosa-Canovas et al. (1999), foods with high conductivities are difficult to work with because they generate smaller peak electric field strengths across the treatment chamber.

Medium Composition

Liquid foods are primarily composed of water, with lesser amounts of nutrients such as proteins, carbohydrates, triglycerides, vitamins, and minerals. The presence and concentration of certain compounds can influence the sensitivity of microbial cells to PEF (Zhang et al. 1995).

Reina et al. (1998) studied the effect of PEF treatment and milk fat content on the survival of *L. monocytogenes* and found no significant differences between whole and skim milk. Xanthan gum in the treatment medium creates a protective layer for cells and necessitates higher field strengths for destruction (Ho et al. 1995). On the other hand, the high osmotic pressures exerted by sucrose solutions reduced the field strength required for cell lysis (Ho et al. 1995).

Dissolved ions in the treatment medium also affect microbial sensitivity to PEF. The presence of Na^+ and K^+ in the medium does not seem to influence inactivation, but Ca^{2+} and Mg^{2+} have been shown to induce a protective effect against PEF (Barbosa-Canovas et al. 1999).

pH

Lowering of pH has been shown in many cases to increase the efficacy of PEF. This is of particular interest for the treatment of fruit juices that have a naturally low pH (Aronsson and Ronner 2001; Jia et al. 1999; Mermelstein, 1998; Qiu et al. 1998).

Jeyamkondan et al. (1999) also reported that PEF was more effective as pH was reduced from neutral to acidic. This enhanced the damaging effects on the cytoplasm once membrane poration occurred. Barbosa-Canovas et al. (1999) explained that low pH (~3.5), due to the addition of organic acids such as sorbate and benzoate, induced the loss of cell membrane integrity during PEF treatment and allowed entry of undissociated acids into the bacterial cell.

When *E. coli* O157:H7 was PEF treated in a 10% glycerol solution, inactivation was enhanced synergistically by lowering the pH from 6.4 to 3.4 using benzoic or sorbic acid (Liu et al. 1997). Similar results have been reported for skim milk and liquid eggs when the pH was adjusted with organic acids (Fernandez-Molina et al. 2001; Gongora-Nieto et al. 1999).

In a study by Garcia et al. (2005), a comparison of the resistances of *B. subtilis*, *L. monocytogenes*, *S. aureus*, *E. coli*, *E. coli* O157:H7, *S.* Senftenberg, and *Y. enterocolitica* was conducted using the same PEF treatment. The authors found that the relative resist-

ances of these pathogens were dependent on the pH of the medium. At pH 7, the order of increasing resistance was *E. coli*, *Y. enterocolitica*, *S. aureus*, *E. coli* O157:H7, *S.* Senftenberg, *B. subtilis*, and *L. monocytogenes*. At pH 4, the resistance order changed dramatically. The most sensitive pathogen was *S. aureus*, followed by *Y. enterocolitica*, *B. subtilis*, *L. monocytogenes*, *E. coli*, *S.* Senftenberg, and *E. coli* O157:H7. The authors concluded that the pH of the treatment medium, rather than the bacterial type (Gram positive or Gram negative) or the shape of the organism (cocci or rod), was the determining factor of the resistance (Garcia et al. 2005).

In contrast, Sensoy et al. (1997) concluded that the critical breakdown potential was influenced by medium temperature, but not pH. Smith et al. (2002) found no extra inactivation of the microflora in raw milk when treated with PEF plus acidification with hydrochloric acid, when compared with PEF alone. Also, Evrendilek and Zhang (2001) found that exposing *E. coli* O157:H7 to pH 3.6 before PEF treatment caused less inactivation than exposing it to pH 5.2 or 7.0. Adaptation of *E. coli* to the acid stress appeared to increase its survivability during PEF treatment.

Increasing the pH of the medium beyond neutral was also reported to increase inactivation by PEF. Jeantet et al. (1999) reported that inactivation of *Salmonella* was greater at pH 9 than at pH 7 and 8 and concluded that the higher pH induced additional stress (Jia et al. 1999; Mermelstein, 1998; Qiu et al. 1998) on the cell and increased its susceptibility to PEF treatment.

Antimicrobials and Alanine

The combination of the antimicrobial compound nisin with PEF treatment has an additive effect on inactivation of bacteria (Calderon-Miranda et al. 1999a; Terebiznik et al. 2000). PEF application followed by nisin exposure caused an additive inactivation of *Listeria innocua* in liquid egg at low PEF intensity but a synergistic interaction when the intensity of PEF or nisin concentration was increased (Calderon-Miranda et al. 1999b). A synergistic inactivation of Gram positive and Gram negative bacteria by PEF in combination with nisin and/or lysozyme has been observed in several studies (Iu et al. 2001; Jagus et al. 1999; Liang et al. 2002; Pol et al. 2000; Smith et al. 2002). A PEF process combining high-voltage pulses (60 kV/cm and eighty pulses of 2 μs width), moderate heating (60°C), and lysozyme inactivated *B. subtilis* spores by up to 4 log cycles (Barsotti and Chefetel 1999). The stress caused to the cell by PEF may facilitate access to the cytoplasmic membrane by nisin or lysozyme, which increases the size or extends the lifetime of pores (Pol et al. 2000; Smith et al. 2002). Conversely, it has been suggested that the action of lysozyme is probably to disturb or destabilize the outer coating and cortex of spores, thus making them more sensitive to electric pulses (Pagan et al. 1998).

Since PEF alone has little effect on bacterial spores (Grahl and Markl 1996; Knorr et al. 1994; Pagan et al. 1998; Sitzmann 1995), some researchers have investigated strategies for initiating spore germination to produce vegetative cells that are much more sensitive to PEF (Barbosa-Canovas et al. 1998; Knorr et al. 1994). Su et al. (1996) used this concept to inactivate > 95% of *B. subtilis* spores when PEF was applied to a saline spore suspension containing 0.01% of the germinating agent L-alanine. Similarly, Barbosa-Canovas et al. (1999) reported a doubling of the inactivation rate of bacterial spores by a PEF process of 30 kV/cm for 1.8 ms when 0.01% L-alanine was added to the treatment medium.

Gas Bubbles and Particles

The presence of gas bubbles and particles in the treatment medium not only limits the maximum field strength that can be applied (Zhang, Monsalve-Gonzalez, Barbosa-Canovas et al. 1994) but may also lead to nonuniform treatment and operational and safety problems. The dielectric breakdown of foods is of particular relevance to the industrial success of PEF technology. It is characterized by a bright luminous spark, a large electrical current in a narrow channel, impulsive pressure through the liquid, and pit formation on the electrodes (Barbosa-Canovas et al. 1999). The presence of gas or solid "impurities" substantially enhances the local electric field strength (due to differences in dielectric properties), so that breakdown takes place at gas-liquid and liquid-solid interfaces more readily than in homogenous liquids (Zhang, Monsalve-Gonzalez, Barbosa-Canovas et al. 1994).

To limit the presence of gas bubbles (air or liquid vapor), the medium can be deaerated prior to treatment or the treatment can be performed under pressure. In some situations, cooling the fluid after filling of the treatment chamber (to increase the solubility of gas in the liquid) can be employed. Use of pulses of duration shorter than 1 μs also minimizes the chance of dielectric breakdown (Zhang, Monsalve-Gonzalez, Barbosa-Canovas et al. 1994).

PEF pasteurization of liquid foods containing particles is currently not feasible, due to the possibility of dielectric breakdown and nonuniformity of treatment, as well as the limitations of treatment chamber design and pumps for handling particulates (Barbosa-Canovas et al. 1999).

Combination with Other Non-thermal Technologies

There is now considerable interest in combining non-thermal technologies to exploit possible syngergistic effects (Ross et al. 2003). Two of the most promising technologies to combine with PEF are high pressure and ultrasonication.

High pressure has been found to either increase or decrease the effectiveness of PEF depending on the treatment conditions. In one study, pressurization was found to protect the bacterial cell membranes from permeabilization by simultaneous PEF treatment despite the fact that either treatment used separately would cause damage to the membrane (Knorr 2001). On the other hand, high pressure can be used to initiate germination of spores, which, on outgrowth into vegetative cells, are sensitive to PEF. However, Pagan et al. (1998) found that high-pressure-germinated spores, without an intermediate holding step to allow outgrowth, were not sensitized to PEF below 40°C although they were sensitive to heat inactivation in this state.

Jin et al. (1998) found a synergistic effect between PEF and ultrasonication. While PEF alone at an electric field strength of 30–40 kV/cm and 2–3 ms treatment time only inactivated *B. subtilis* spores by about 95% (i.e., a 1.3-log reduction), a 4-log reduction was obtained when PEF was combined with ultrasonication. Su et al. (1996) also reported that ultrasonication enhanced the inactivation of *B. subtilis* spores to PEF.

Effect on Pathogenic Bacteria

The effect of PEF on pathogenic bacteria is of major significance when the technology is being considered as a non-thermal alternative to pasteurization for low-acid foods such as milk. Pasteurization of milk at 72°C for 15 seconds has been used very successfully to elim-

inate pathogenic bacteria and render milk safe. These heating conditions are set in order to destroy the most heat-resistant bacterium likely to contaminate milk, *Coxiella burnetti.* Testing for the adequacy of milk pasteurization is achieved with an enzyme, alkaline phosphatase, which has inactivation kinetics very similar to those of *Coxiella burnetti.* Unfortunately, to date, a corresponding simple test for the adequacy of a PEF treatment in destroying the most PEF-resistant pathogenic organism in milk has not emerged. This is largely because sufficient research on the effect of PEF on pathogens has not been conducted.

The important parameters of PEF treatment, such as electric field strength, number and shape of pulse waves, treatment duration, medium composition, medium acidity, treatment cell design, and temperature of the treatment, influence the effectiveness of the treatment in destruction of pathogenic microorganisms as they do for nonpathogenic bacteria. As a consequence, comparison of the PEF sensitivities and resistances of different pathogens from the available literature is difficult because different combinations of the parameters have been used. An example for such variation is the study of Garcia et al. (2005) presented in the "pH" section above.

This section presents the response of six important pathogenic bacteria to PEF treatments according to specific factors such as treatment medium composition, bacterial growth phase, and temperature. It also discusses the nonlethal effect of PEF treatment exhibited by some organisms. A collation of the maximum and minimum log reductions reportedly achieved by PEF on these pathogens appears in table 13.1.

Escherichia coli

E. coli is the most studied of all the pathogenic bacteria treated with PEF. Low electric field strength, as low as 2.5 kV/cm, is reported to cause reversible electroporation of the cell membrane of *E. coli* without inactivating it (San Martin et al. 2001). At higher field strength, 24 kV/cm, with ten pulses at 20–40°C, leakage of cell contents, as well as loss of the cells' ability to plasmolyse (shrinkage of the cell contents away from the cell wall) in hypertonic medium was reported in *E. coli* (Sale and Hamilton 1967).

The killing effect of PEF on *E. coli* was more profound at lower pH. It was suggested that the enhanced effect of low pH was due to the ability of *E. coli* to maintain its cytoplasmic pH near neutrality. The acidity of the medium may influence the cells' permeability and the rate of H^+ transport (Aronsson et al. 2004; Vega-Mercado et al. 1996). Lowering water activity, however, increased the resistance of *E. coli* to PEF (Aronsson et al. 2004).

E. coli in the logarithmic growth phase is more resistant to PEF than when it is in the lag phase. Inactivations of 86% and 28% were reported for lag phase and logarithmic phase, respectively, in milk (Sampedro et al. 2005).

E. coli, stressed by exposure to low pH or low temperature, appeared to have increased resistance to PEF. For example, the application of PEF at 30 kV/cm immediately after preexposure of *E. coli* to pH 3.6 produced a 2.75-log reduction while the same treatment applied after 6 hours of exposure produced a 1.28-log reduction. Preexposure to pH 5.2 and 7.0 for up to 6 hours before PEF did not influence the sensitivity of *E. coli*. Exposure of *E. coli* to 4°C for 6 hours and then subjecting it to PEF treatment of 30 kV/cm reduced the inactivation from 3.6- to 2.7-log reductions. Similar results were observed with pretreatment at 40°C. These results indicated that adaptation to acidic and low- or high-temperature (but sublethal) conditions made *E. coli* more resistant to PEF (Evrendilek and Zhang 2003).

Table 13.1 Summary of effects of PEF on pathogenic bacteria*

	Lowest Log Reduction Reported			Highest Log Reduction Reported		
Pathogen	LR	Treatment Condition	Source	LR	Treatment Condition	Source
E. coli	0.5	15–20 kV/cm, 21–24°C, pH 7	Alvarez et al. 2004; Aronsson et al. 2004	8 9	60 kV/cm, 60°C, 210 μs, UHT milk PEF energy of 97 kJ/L, 16 pulses, SMUF	Jung et al. 2002 Zhang et al. 1995
Listeria monocytogenes	1	25 kV/cm, 25°C, 600 μs, skim milk or 30 kV/cm, 25°C, 32–50 pulses	Reina et al. 1998; Fleischman et al. 2004	6	36 kV/cm, 61°C, 25 pulses or 40 kV/cm, 66°C, 20 pulses, pH7	Sepulveda, Gongora-Nieto et al. 2005
Salmonella spp.	< 0.1	50 kV/cm, 500 Hz *S.* Enteriditis	Sampedro et al. 2005	6.5	90kV/cm, 55°C, 50 pulses, + nisin (27.5U/ml) and lysozyme (690U/ml) *S.* Typhimurium	Liang et al. 2002
Staphylococcus aureus	2.2	9.25 kV/cm	Hamilton and Sale 1967	6	40 kV/cm, 64 pulses	Zhang, Chang et al. 1994
Yersinia enterocolitica	0.8	22 kV/cm, 2 μs pw, 800 μs, water activity 0.93	Alvarez, Raso, Sala, and Condon et al. 2003	6	22 kV/cm, 2 μs pw, total energy 3500 kJ/kg	Alvarez, Raso, Sala, and Condon et al. 2003
Mycobacterium paratuberculosis	—	—	—	5.3-5.9	30 kV/cm, 2,500 pulses, 50°	Rowan et al. 2001

LR = log reduction, SMUF = simulated milk ultrafiltrate, pw = pulse width, IC = initial cell count.
*Lowest and highest log reductions reported.

Cultivation temperatures, however, affected the sensitivity of *E. coli* to PEF differently. After PEF treatment at 50°C, *E. coli* cells grown at 20 and 42°C showed a 3-log reduction compared to a 2-log reduction of cells cultivated at 30 and 37°C (optimum growth temperatures). The authors concluded that cells grown at the extremes of their growth temperature range were more sensitive to PEF than those grown in the optimum temperature range (Ohshima et al. 2002).

Sublethal injury, indicated by higher counts on nonselective media compared to those on selective media, of *E. coli* after PEF treatment has been reported by some researchers (Ravishankar et al. 2002; Selma et al. 2004). These authors argued that although the use of a selective medium enhanced the growth of the target bacteria, the ingredients contained in these media that inhibit the growth of other bacteria may also inhibit the growth of injured target bacteria (i.e., injured *E. coli*). Therefore, counts on the selective medium only may overestimate the effectiveness of the treatment. Significantly prolonged lag phases followed by normal growth rates were also found for PEF-treated *E. coli*. This suggests that PEF produced sublethal injury since lag phase elongation is related to repair and adaptation (Selma et al. 2004).

Listeria monocytogenes

The most significant factor in enhancing the effectiveness of PEF on *L. monocytogenes* is temperature. This effect has been observed in a number of studies (Alvarez et al. 2004; Fleischman et al. 2004; Sepulveda, Gongora-Nieto et al. 2005). Sepulveda, Gongora-Nieto et al. found that the number of pulses and the electric field intensity were less significant in bacterial inactivation than the treatment temperature. The effectiveness of PEF treatments increased as the temperature was increased to 55°C. At this temperature, there was a dramatic increase in the inactivation rate of *Listeria*. It was suggested that at and above this temperature there was a thermal effect in addition to the PEF effect. Stanley (1991) reported a temperature-related phase transition of phospholipid molecules in cell membranes, which changed from a packed gel-like structure to a looser liquid-crystalline phase at higher temperatures, reducing the thickness and the mechanical resistance of the cell membrane (Stanley 1991) . The results at 55°C reported by Sepulveda, Congora-Nieto et al. (2005) suggest such a phase transition in the cell membrane. The authors argued that thinning of the cell walls due to a thermal effect would make the cells more susceptible to disruption by PEF. In a study comparing the effect of PEF and heating, Wouters et al. (1999) found that under similar heating times and temperatures, PEF greatly increased the effect of heat on *L. innocua*. After 13.2 seconds of treatment of 22–24 kV/cm, 55–62.5°C, > 6-log reduction was obtained, while after heating at 55–62.5°C for 13.2 seconds without PEF, the log reduction was < 0.9.

Unlike the situation with some other bacteria, the fat content in milk did not protect *L. monocytogenes* from PEF. In a treatment of 22 kV/cm for 800 μs, the same log reduction was obtained regardless of fat content (1.5 or 3.5%) (Alvarez et al. 2000).

Sublethal injury and recovery of injured *L. monocytogenes* have been studied using both selective and nonselective media for enumeration. However, no difference in viable counts on selective and nonselective agar was reported, suggesting that PEF treatments inactivated the cells without, or with only little, sublethal injury (Reyns et al. 2004; Russell et al. 2000). Simpson et al. (1999) found leakage of UV-absorbing substances from the treated cells at electric field strengths as low as 10 kV/cm, which was not lethal to the bacteria, suggesting that pore resealing during or soon after PEF treatment enabled the bacteria to

survive. The authors obtained similar results for *S. typhimurium* (Simpson et al. 1999). *S. typhimurium* has been found to be more sensitive to PEF than *L. monocytogenes* (Reyns et al. 2004).

Salmonella *spp.*

The composition of the treatment medium was found to influence the effect of PEF on *Salmonella*. When *S. typhimurium* in distilled water, HEPES-KOH pH 7 buffer, or Tris-HCl buffer was exposed to 300 monopolar square waveform pulses of 2 μs at 26.7 kV/cm and 25°C, the percentage viable cells of the initial cells determined 1.5 hours after treatments were 9, 0.2, and 0.05%, respectively, for the three media above. After 24 hours incubation at 25°C, the survival rates decreased to 0.9, 0.09, and 0.04%, respectively (Reyns et al. 2004). However, unlike other pathogens under the combined effect of PEF and acidity, *Salmonella* was found to be more resistant at acidic pH than at neutral pH (Alvarez et al. 2000).

Similar to *E. coli*, *Salmonella* cells in the lag phase were found to be more susceptible to PEF than cells in the exponential growth phase (Sampedro et al. 2005).

No sublethal injury or recovery of injured cells was detected in PEF-treated *Salmonella* using selective and nonselective growth media. The authors concluded that the effect of PEF on *S. typhimurium* was either inactivation or no effect, with no transition stage of sublethal injury (Reyns et al. 2004; Russell et al. 2000).

Staphylococcus aureus

Inactivation of *S. aureus* was reported to be enhanced by increased electrical field strength (Hamilton and Sale 1967; Sampedro et al. 2005) and increased treatment duration (Evrendilek et al. 2004). No recovery of *S. aureus* was detected using a selective medium plating technique. In fact, in a shelf-life study of skim milk ultrafiltrate, PEF-treated samples showed that, by the second week of refrigerated storage, there was a significant reduction of *S. aureus*, which was not observed in the untreated sample. This might be due to the increased sensitivity of the cells to cold temperature after treatment. *S. aureus* was found to be more resistant to PEF in milk ultrafiltrate than other microorganisms such as *E. coli* (Qin, Pothakamury et al. 1995)

Yersinia enterocolitica

PEF treatment of 22 kV/cm, 2 μs pulse width was applied to *Y. enterocolitica* (Alvarez, Raso et al. 2003). As treatment time increased from 0 to 800 μs, inactivation increased gradually to 4.5 log cycles from an initial count of 10^6 cfu/ml. However, unlike the other bacteria, the bactericidal effect on *Y. enterocolitica* was independent of pH, pulse width, and frequency of pulses. Three different pH conditions of 7.0, 5.4, and 3.8 were tested at 22 kV/cm; after any treatment duration, the inactivation rates were the same for all samples, achieving a 4-log reduction after 800 μs. Similarly, changing the pulse width from 0.4 to 15 μs or the pulse frequencies from 1 to 5 while keeping other conditions constant produced the same level of inactivation. However, the inactivation was strongly influenced by electric field strengths, total treatment time, and total energy supplied. As the energy increased from 0 to 3,500 kJ/kg, the killing effect reached 6 log cycles. However, there were

maximum field strength thresholds at different energy levels. Above these thresholds, the inactivation of *Y. enterocolitica* was independent of the electric field strength and only dependent on the energy supplied; the energy required to achieve a given inactivation decreased with increases in electric field strength (Alvarez, Raso et al. 2003)

Mycobacterium paratuberculosis

M. paratuberculosis has attracted considerable attention because of its possible link to Crohn's disease and differing opinions about the effectiveness of high-temperature-short-time (HTST) pasteurization. Rowan et al. (2001) treated *M. paratuberculosis* in peptone water and sterilized milk with PEF at 30 kV/cm (2,500 pulses) at 50°C and found log reductions of 5.3 and 5.9, respectively. Heating alone to 50°C for 25 minutes caused a 0.01-log reduction only. PEF at temperatures lower than 50°C resulted in less lethality.

Effect on Enzymes

Enzyme Inactivation

The destruction of enzymes in foods is another prime concern in food preservation. Food spoilage enzymes can be naturally present in food materials or produced from microorganisms (Ho et al. 1997). As enzymes are proteins, they can be denatured and inactivated by thermal treatments (Nagodawithana and Reed 1993). Temperature sensitivity of enzymes is related to enzyme type (specific structure) and the water content of the system, as water is required to facilitate the unfolding of proteins during thermal denaturation (Nagodawithana and Reed 1993). The secondary and tertiary structures of enzymes are stabilized by weak noncovalent forces (e.g., hydrogen bonds, van der Waals forces, hydrophobic interactions, and salt bridges). Changing the strength of any such forces by PEF may lead to denaturation of enzyme structure and hence inactivation of the enzymic activity (Barbosa-Canovas et al. 1999). The sensitivities of enzymes are dependent on their nature and on the settings of the PEF treatments applied (e.g., electric field strength, pulse width/treatment time).

Ho et al. (1997) found that thirty pulses of 2 μs width at 13–87 kV/cm caused 70–85% inactivation of lipase, glucose oxidase, and α-amylase, 30–40% inactivation of peroxidase and polyphenol oxidase, but only 5% inactivation of alkaline phosphatase. Grahl and Markl (1996) found that PEF at 21.5 kV/cm caused 60% inactivation of lipase but alkaline phosphatase and lactoperoxidase were not affected by these conditions. With seventy pulses at 18.8 kV/cm, Yang et al. (2004) obtained a reduction in the activity of alkaline phosphatase by 65%. Ho et al. (1997) concluded that differences in secondary and tertiary structures play an important role in enzyme sensitivity to PEF.

Vega-Mercado et al. (1995) inactivated plasmin (milk alkaline protease) by 90% at 15°C using fifty pulses at both 30 and 45 kV/cm. They proposed that the inactivation resulted from oxidation of key components in the enzyme, induced by the electric fields. However, Yeom et al. (1999) investigated the mechanism of PEF on papain and concluded that although oxidation of the active site did occur, the major cause of inactivation was structural change in the enzyme, related to loss of its α-helix (secondary) structure.

In general, it has been accepted that the main mechanisms of enzyme inactivation by PEF include oxidative reactions and conformational changes on the tertiary structure of the protein (Barbosa-Canovas et al. 1998). In the case of enzymes present in microorganisms,

localized joule heating of cell membrane components due to PEF may cause thermal denaturation of membrane-bound enzymes (Simpson et al. 1999).

Significant enzyme inactivation generally requires a higher voltage than destruction of vegetative microbial cells, suggesting that some enzymes used in food processing may remain viable under PEF treatment while microbial control is achieved (Giner et al. 2000; Ho et al. 1997). However, pulse width and possibly waveform may be more important than electric field strength in inactivating enzymes (Ho et al. 1997).

Enzyme Stimulation

Some enzymes in food have been found to undergo stimulation as a result of PEF treatment. Although enzyme inhibition occurred once a particular electric field strength was reached, the activities of lysozyme and pepsin were enhanced by PEF (Ho et al. 1997). This effect may have been due to a number of factors:

1. The creation of more active sites or increasing the size of existing sites.
2. Charge reversal of pulses, causing greater enzyme-substrate interaction due to induced oscillatory motion of enzymes.
3. The pulses might decrease activation energy of the reaction due to proximity and orientation effects (i.e., bringing together of reactants in correct relative orientation for reaction at a faster rate).

Milk and Milk Products

Microbial Inactivation and Shelf Life

Significant reductions of non-sporeforming bacteria in milk are possible with PEF treatment (Fernandez-Molina et al. 2000; Grahl and Markl 1996; Knorr et al. 1994; Michalac et al. 1999; Qin, Pothakamury et al. 1995; Raso et al. 1998; Smith et al. 2002). Furthermore, PEF treatments result in less flavor degradation of milk than equivalent heat treatments, and no chemical or physical changes in milk fat, protein integrity, and casein structure (Michalac et al. 1999; Qin, Pothakamury et al. 1995). Hence, PEF may even be used to preserve heat-sensitive dairy products such as whey protein concentrate (Grahl and Markl 1996).

As discussed in the previous sections, several factors, particularly electric field strength, treatment time, pulse width and frequency, shape of the pulse wave, and temperature influence the bactericidal effect of PEF. Unfortunately, different workers have used different conditions and some have not reported all the treatment parameters used. Therefore, comparison of the various reports of PEF treatment of milk and milk products is difficult. In a collation of these reports, Sampredo et al. (2005) showed a range of effects on bacteria in milk. In general the log reductions reported were ≤ 4. A summary of the highest reported reductions achieved with PEF, without added antimicrobial agents or temperatures > 50°C, of selected bacteria in milk and milk products is given in table 13.2.

Qin, Pothakamury et al. (1995) reported an extension of raw milk shelf life to 2 weeks at refrigeration temperature using PEF (two steps of seven pulses, and one step of six pulses at 40 kV/cm). Sensory panelists could not detect a difference between the PEF-treated and heat-pasteurized milk.

Longer shelf life can be obtained with a combination of heat and PEF. A shelf life of

Table 13.2 Highest reported levels of microbial inactivation in milk and milk products by PEF

Microorganism	Treatment Conditions	LR	Source
E. coli	PEF energy of 97 kJ/L, 16 pulses, SMUF	9	Zhang et al. 1995
Lactobacillus brevis	22 kV/cm, 45–50°C, 20 pulses, 20 μs	4.6	Zhang, Chang et al. 1994
Listeria monocytogenes	50°C, 30 kV/cm, 400 pulses	4	Bendicho, Barbosa-Canovas et al. 2002
	22 kV/cm, 35°C, 800 μs, pH 3.8	5.1	Alvarez, Pagan et al. 2003
Saccharomyces cerevisiae	4.7 kV/cm, < 5 μs	4	Grahl and Markl 1996
Salmonella Dublin	18 kV/cm	4	Vega-Mercado et al. 1997
Staphylococcus aureus	16 kV/cm, 60 pulses, 300 μs	3–4	Pothakamury et al. 1995
Streptococcus thermophilus	25 kV/cm	2.5	Vega-Mercado et al. 1997
Pseudomonas genus	60 kV/cm, 50°C, 210 μs	8	Sampedro et al. 2005

LR = log reduction; SMUF= simulated milk ultrafiltrate.

15–22 days was achieved with raw skim milk treated for 30 pulses of 30–50 kV/cm at 70–80°C (Fernadez-Molina et al. 2001). When PEF was applied to pasteurized milk its shelf life was extended to 60–78 days compared to 2–3 weeks shelf life of the milk treated with pasteurization alone (Sepulveda, Gongora-Nieto, Guerrero and Barbosa-Canovas 2005).

Sensory and Nutritional Quality

The vitamin content including thiamine (B1), riboflavin (B2), cholecalciferol (D), and tocopherol (E) of PEF-treated milk remained unchanged after treatments of up to 400 μs at field strengths from 18.3 to 27.1 kV/cm. The ascorbic acid content in milk was slightly affected. After PEF treatment of 22.6 kV/cm for 400 μs, 93% of the ascorbic acid was retained (Bendicho, Estela et al. 2002). This agrees closely with the 90% retention reported by Grahl and Markl (1996) and is significantly higher than the retentions after thermal treatments of low-temperature, long-time pasteurization at 63°C for 30 minutes (49.7%) and HTST pasteurization at 75°C for 15 seconds (86.7%) (Bendicho, Estela et al. 2002). The same authors also reported that the vitamin A content of the treated milk was not significantly affected.

Other chemical aspects of milk that have been reported to be unaffected after PEF treatments at field strengths from 20 to 80 kV/cm are pH and titratable acidity (Jung et al. 2002; Michalac et al. 1999); fat content (Cortes et al. 2005; Dunn 1995; Qin, Pothakamury et al. 1995); fat and protein integrity, starter growth, rennet clotting yield, cheese production, and calcium distribution (Dunn 1995; Michalac et al. 1999); and color, moisture, and particle sizes (Michalac et al. 1999).

Li et al. (2003) reported that treatment up to 35 kV/cm for 73 μs showed no effect of the bovine milk IgG in a protein-enriched soymilk, indicating the potential of PEF in treating products with bioactive components. Flavored milk, yogurt, and yogurt drink treated with PEF and heat also showed increased shelf life, lower counts of yeasts and molds, and no changes in sensory scores (Evrendilek et al. 2001; Yeom et al. 2004).

Effect on Enzymes

Vega-Mercado et al. (1995) achieved a 90% reduction of plasmin activity in a simulated milk ultrafiltrate by PEF at 30 kV/cm. Plasmin is a very heat-stable enzyme and can cause

bitterness and gelation defects in UHT milk (Datta and Deeth 2001). Its destruction by the non-thermal PEF is of particular interest.

Grahl and Markl (1996) achieved a 60% reduction of milk lipase activity by PEF treatment at 21.5 kV/cm. However, the latter treatment caused no inactivation of alkaline phoshatase and lactoperoxidase. At 18.8 kV/cm, seventy pulses reduced the activity of alkaline phosphatase by 65% (Yang et al. 2004).

Inactivation by PEF of proteases and lipases produced by psychrotrophic microorganisms such as *Bacillus* and *Pseudomonas* is of great interest because these enzymes are heat stable and some remain active even after high-temperature treatment. Lipases cause rancid flavor in milk, while proteases degrade casein, causing bitterness, decreasing cheese yield, and affecting milk stability during heating and subsequent storage. Bendicho, Estela et al. (2002) and Bendicho et al. (2003) achieved 62% inactivation of lipase after eighty pulses of 27.4 kV/cm, while the maximum levels of protease inactivation after 35.5 kV/cm, 866 μs, 111 Hz were 57.1% in whole milk and 81.1% in skim milk. The inactivation increased with field strength, treatment time, input energy, and pulse frequency and was dependent on the composition of milk. The maximum inactivation achieved in skim milk was higher than that of whole milk (81.8% vs. 57.1%). The author suggested that the fat in milk protected the enzyme by making it structurally more stable (Bendicho et al. 2003).

Fruit and Vegetable Juices

Microbial Inactivation and Shelf Life

Several studies have been conducted on the use of PEF on fruit juices (table 13.3). A majority have used a combination of PEF and heat. This takes advantage of the synergistic effects of the two treatments to produce a fresh-like product with minimal loss of nutritional and physiological value. Heinz et al. (2003) recommended a specific energy input of 40 kJ/kg and an initial temperature of 55°C for optimum results. After the treatment with twenty pulses of 65 kV/cm at 50°C, Wu et al. (2005) found that 10–50% of the microbial reductions were due to thermal inactivation. The low pH of fruit juice is a major factor favoring microbial inactivation by PEF.

PEF treatment of juices can significantly increase their shelf life. Reported shelf-life extensions for orange juice are up to 28 days at 4°C (80 kV/cm for twenty pulses, without aseptic packaging) (Hodgins et al. 2002), 112 days (35 kV/cm for 59 μs) (Ayhan et al. 2001, 2002), and 196 days (40 kV/cm for 97 μs) (Min, Jin, Min et al. 2003); for apple juice, up to 67 days (35 kV/cm for 94 μs) (Evrendilek et al. 2000); and for tomato juice, up to 112 days (40 kV/cm for 57 μs) (Min, Jin, and Zhang 2003). PEF at 30 kV/cm for 240 μs was reported to be as effective on yeasts and molds, major spoilage organisms for juices, as heat treatment at 90°C for 1 minute (Jia et al. 1999).

Sensory and Nutritional Quality

A benefit of PEF over heat treatment is preservation of ascorbic acid content and the retention of flavor in juices (Hodgins et al. 2002). The combination of PEF (80 kV/cm) and thermal treatment up to 50°C was reported to have little or no effect on the vitamin C content of orange juice, which is sensitive to heat—less than 80% retention after thermal pasteurization (Torregrosa et al. 2006; Wu et al. 2005). Vitamin C was also retained in apple juice and cider after PEF treatment (Evrendilek et al. 2000). The vitamin C of PEF-treated

Table 13.3 The effects of PEF on the microflora of juices*

Product	Microorganism	Conditions	LR	Reference
Apple juice	*Aspergillus niger*	34 kV/cm, IT 55°C, 40 kJ/kg, IC 4.5 log	4.3	Heinz et al. 2003
	E. coli	34 kV/cm, IT 55°C, 40 kJ/kg, IC 7 log	6.2	
		90 kV/cm, IC 42°C, 10 pulses, + cinnamon/nisin	6–8	Iu et al. 2001
	Lactobacillus plantarum	22 kV/cm, 1,000 μs, IC 6 log	5.8	Gomez et al. 2005
	Listeria enterocolitica	28 kV/cm, < 35°C, IC 6 log	6	
	L. rhamnosus	34 kV/cm, IT 55°C, 40 kJ/kg, IC 7 log	4.9	Heinz et al. 2003
	L. innocua	34 kV/cm, IT 55°C, 40 kJ/kg, IC 5.5 log	4.3	
	Rh. rubra	34 kV/cm, IT 55°C, 40 kJ/kg, IC 6 log	6.5	
	Saccharomyces cerevisiae	35 kV/cm, IT 34°C, 25 μs	6	Qin, Chang et al. 1995
	Natural flora	33 k/cm, IT 50°C, IC 6.6 log	3.1	Liang et al. 2006
Grape juice	*A. niger*	34 kV/cm, IT 55°C, 40 kJ/kg, IC 4.5 log	4.6	Heinz et al. 2003
	E. coli	34 kV/cm, IT 55°C, 40 kJ/kg, IC 7 log	6.4	
	L. rhamnosus	34 kV/cm, IT 55°C, 40 kJ/kg, IC 6.3 log	4.6	
	Rh. rubra	34 kV/cm, IT 55°C, 40 kJ/kg, IC 6 log	5.4	
Orange Juice	*E. coli*	30 kV/cm, IT 30°C, IC 9 log, 6–7 pulses	5.5–6.6	McDonald et al. 2000
	Leuconostoc mesenteroides	30 kV/cm, IT 30°C, IC 9 log, 6–7 pulses	2–6	
	L. innocua	30 kV/cm, IT 30°C, IC 9 log, 6–7 pulses	5–5.5	
	Saccharomyces cerevisiae	35 kV/cm, 25 μs	6	Qin, Chang et al. 1995
		35 kV/cm, IC 8 log, 1,000 μs	5.1	Elez-Martinez et al. 2004
	Salmonella Typhimurium	90 kV/cm, 55°C, 50 pulses	5.9	Liang et al. 2002
	Natural flora	40 kV/cm, IT 45°C, IC 6 log, 97 ms	5.8–6	Min, Jin, Min et al. 2003
		35 kV/cm, 59 μs	7	Yeom et al. 2002
Tomato Juice	Natural flora	40kV/cm, 57μs, IC 5–6 log	6	Min, Jin, and Zhang et al. 2003
Cranberry juice	*Byssochlamys fulva* canidiospores	36.5 kV/cm, 22°C, 3.3 μs pw, 2 pulses	5.9	Wouters et al. 2001
	Yeasts and molds	51 kV/cm, 22°C, 3.3 μs pw, 2 pulses	3.8	
	Natural flora	40 kV/cm, 3.3 μs pw, 150 μs	3.8	

LR = log reduction, IT = initial temperature; IC = initial count, pw = pulse width.
*The highest inactivation reported.

juice was also found to be more stable than that in heat-treated samples, having a half-life of up to 60 days, 2 to 5 times longer, depending on the PEF treatment and storage temperature (Min, Jin, Min et al. 2003; Torregrosa et al. 2006), and as bioavailable for human absorption as that of the fresh orange juice (Sanchez-Moreno et al. 2004).

PEF-processed tomato juices possessed smaller and more uniform-sized particles compared to thermally treated juice. The two treatments produced samples with the same °Brix and pH value, but significantly higher overall acceptability by sensory evaluation was observed in PEF-processed tomato juice (Min, Jin, and Zhang et al. 2003); they were also not distinguishable from the untreated sample (Min and Zhang 2003).

PEF-treated orange juice also had better texture, flavor, and overall acceptability than thermally processed juice. It had a significantly higher content of the flavor compounds D-limonene, α-pinene, myrcene, and valencene, compared to thermally treated and untreated samples (Ayhan et al. 2002; Min, Jin, Min et al. 2003). PEF-treated tomato juice also showed higher contents of volatile flavor compounds after treatment, and these remained higher during the first 60 days of storage, compared to thermal treatment. Ayhan et al. (2002) suggested this increase in volatiles was due to further release of flavor compounds from the pulp into the aqueous phase.

Jia et al. (1999) reported some loss of volatile flavor compounds in PEF-treated orange juice, with the loss increasing with treatment time. However, the maximum loss was 9.7% compared to 10–41.7% from the heat-processed orange juice.

PEF-treated orange juice showed less browning and a brighter color during storage at 4°C compared to a heat-treated sample; this might be due to the higher retention of vitamin C (Min, Jin, Min et al. 2003). PEF also caused less browning in tomato juice, with a much slower browning rate during storage, decreasing little over 60 days. PEF-treated tomato samples were redder than heat-treated samples and similar to the untreated sample (Min and Zhang 2003). Reports on the color of PEF-treated apple juice are conflicting: Ortega-Rivas et al. (1998) reported color fading after two to sixteen pulses at 50–466 kV/cm, while Evrendilek et al. (2000) observed no color loss after 94 μs at 35 kV/cm in PEF-treated apple juice and cider.

Effect on Enzymes

The enzymes that have attracted most attention are pectinmethylesterase (PME), polyphenoloxidase, and lipoxygenase. PEF combined with moderate heat (< 50°C) caused 90–92.7% reduction of PME activity in orange juice, which was sufficient to prevent development of cloudiness (Hodgins et al. 2002; Yeom et al. 2002). In tomato, where it is responsible for thinning tomato sauce, PME was reduced by 94% after treatment with four hundred pulses at 24 kV/cm (Giner et al. 2000).

PEF treatment decreased polyphenoloxidase activity (which causes browning) in apple cider by 25–33% (Liang et al. 2006), by 97% in apple extract, and by 72% in pear extract (Giner et al. 2001). The activity of lipoxygenase in tomato, which is responsible for the destruction of essential fatty acids and development of off-flavors, was reduced by up to 90% by PEF treatment of 30 kV/cm at 50°C for 60 seconds (Min, Min and Zhang 2003).

Extraction

PEF induces plant tissue damage, which facilitates the expulsion of liquid from the interior of cells and increases the efficiency of juice extraction. The mechanism of the dam-

age is still unclear. While some researchers have found that the cell wall structure of the tissue remains intact (Lebovka et al. 2004a), others have found evidence of cell wall damage due to increased tissue porosity (Bazhal; Ngodi, and Ragavan 2003; Bazhal, Ngodi, Ragavan and Nguyen 2003). Lebovka et al. (2004b) suggested that PEF causes non-thermal rupture of cellular membranes, which reduces or eliminates the turgor component of the plant cells.

PEF treatment when combined with mechanical pressing resulted in significantly increased yield and decreased discoloration of apple juice, especially when PEF was applied after a precompression period. Apart from increasing tissue damage and diffusion of moisture, the combined treatment reduced cell resealing (Bazhal et al. 2001). The high efficiency of the treatment was achieved at electric field strengths of 0.5 to 1 kV/cm (Lebovka et al. 2003).

The addition of PEF and mild thermal treatment increased textural damage in carrots, potatoes, and apples, allowing high efficiency of juice extraction by mechanical compression (El-belghiti and Vorobiev 2005; Lebovka et al. 2004a, 2004b). El-belghiti and Vorobiey (2005) found that the solute yield of carrots increased as the electrical energy supplied was increased up to a threshold value of 9 kJ/kg. Carrot juice yield increased from 30 to 50% to 70 to 80% under the influence of PEF, compared to control treatment (Knorr et al. 1994). PEF was also found useful in the processing of coconut, giving a 20% increase in yield of coconut milk, as well as 50% increase of protein and 58% increase of fat content, after treatment of twenty pulses at 2.5 kV/cm (Ade-Omowaye et al. 2001). By applying a field strength of 0.68 kV/cm, the liquid extracted from potato tissue was significantly increased—50–65% yield compared to 40–50% for mechanical pressing alone (Chalermchat and Dejmek 2005)

Juice yield from sugar beets increased significantly, up to 88%, after being treated with PEF (Praporscic et al. 2004). Pretreatment of sugar beets with PEF also resulted in higher sugar yield, by about 25%, than the normal hot-water extraction method (El-belghiti and Vorobiev 2004; Jemai and Vorobiev 2003), increased the dry matter content of the pulp from 15 to 25%, and reduced sucrose loss from 0.6 to 0.5% (Rastogi 2003). Rastogi concluded that PEF allows maximum permeabilization at low energy input and short treatment times.

Conclusions

PEF technology has considerable potential for use in the food industry. Its major application is in non-thermal inactivation of vegetative microorganisms in foods. It causes little or no change in color, flavor, and nutritive value, changes often associated with thermal treatments. Other applications such as inactivation of enzymes and increasing the yield of juice extraction also show promise for improving product quality and increasing processing efficiency.

PEF is effective for extending the shelf life of milk and fruit juices, but the suitability of foods that may be processed by PEF is a limiting factor. It is largely restricted to liquid foods that can endure high electric fields and is not suitable for fluids containing particulates or gases, without pretreatment. PEF may also be very useful for treatment of products containing heat-sensitive components such as whey proteins and immunoglubulins.

Many factors related to the processing conditions, types of microorganisms, and properties of the treatment medium affect the efficacy of PEF treatment. A greater understanding of these factors in specific applications is required before the technology can be widely used in an efficient and safe manner in commercial food processing.

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