Effects of food processing on disease agents

Roberto A. Buffo and Richard A. Holley

1 Introduction

Food-processing technology has evolved substantially since its industrial birth in the 1800s with the initial development of pasteurization and other thermal processes for packaged foods. However, it was not until many years later, in the 1930s, that safe canning processes were developed. In the 1940s, processes for frozen foods were introduced. In the 1950s, vacuum-packed and modified-atmosphere packaged foods became available, and the technology revolutionized the meat industry, changing it from carcass distribution to vacuum-packed and boxed cuts, which allowed a five-fold shelf-life extension. Modified atmospheres were successful in storage of fresh fruits and vegetables, and later for the packaging of cut produce. In the 1980s, aseptic food processing and packaging became a commercial reality, along with food irradiation (gamma and e-beam). The 1980s and 1990s witnessed the application of a variety of new thermal processes (microwave and radiofrequency treatments, and ohmic- and inductive-heat...
technologies). Also, a number of non-thermal processes came into commercial use, with some still under study (pulsed X-rays, high-pressure processing, pulsed electric fields, pulsed light, UV light, magnetic fields and ultrasound). Advantages and limitations of these new processing technologies were recently outlined (Davidson and Harrison, 2002), and some new information is included in this chapter.

In this chapter the focus is on foodborne pathogens, although, for clarification, effects of processing on spoilage organisms are mentioned as necessary. The emergence of previously unrecognized organisms as significant contributors to foodborne illness in humans raises questions with respect to their origin and whether modern manufacturing practices associated with mass processing have provided unique opportunities for their survival. These organisms include *Campylobacter*, *Escherichia coli* O157:H7 and *Listeria monocytogenes*, and the protozoan *Cyclospora*.

The food-processing industry has become less reliant upon final product inspection and more dependent upon control of adequate processes to ensure the safety of food (HACCP). Accurate estimates of bacterial susceptibility to lethal steps used in food processing are essential, as the goal is to eliminate foodborne pathogens during processing. Better refrigeration of cooked cured meats and pasteurization of dairy products have reduced incidents of foodborne illness caused by *Staphylococcus aureus*; other organisms contaminating product at packaging have replaced this threat. Refrigeration of perishable products, such as minimally processed products and ready-to-eat prepared products, may select for the psychrotroph *Listeria*, at the same time the consolidation of industrial operations into larger plants with greater geographic distribution of product with an extended shelf-life has had an influence upon the size of foodborne illness outbreaks.

During the 1970s and the 1980s shelf-stable foods were developed using a combination of inhibitory treatments (salt, nitrite, temperature), which interacted to inhibit pathogens at lower individual levels than needed if each treatment was used alone. The system has worked well and is generally accepted. However, these multiple treatments may have contributed to the development of microbial resistance through a generalized stress response (GSR), which may contribute to salmonellosis.

Many challenges remain to produce safe and nutritious food with an adequate shelf-life. Low-salt, -sugar or -fat formulations may change water activity ($a_w$) or pH, and may provide new niches in foods for pathogens. Processes used for the manufacture of dry sausage and aged Cheddar cheese from thermized (unpasteurized) milk have failed to eliminate *E. coli* O157:H7 and S. Enteritidis, respectively. Many vegetables and sprouts cannot be freed from pathogens once contaminated. Alternative approaches are needed to eliminate such threats.

## 2. High-temperature preservation

### 2.1 History and present practices

Credit for discovering the value of heat as a preservative agent goes to the French chef, distiller and confectioner Nicholas Appert; he held the view that the cause of food spoilage was contact with air, and that the success of his technique was due to the exclusion of air from the product. This view persisted for another
50 years, sometimes with disastrous consequences, until Louis Pasteur established the relationship between microbial activity and putrefaction (Adams and Moss, 1995).

The use of high temperatures for processing of foods is the gold standard against which all other food-preservation technologies are evaluated. Thermal exposures (time–temperature combinations) have been developed that result in pathogen destruction with minimal changes in the functional characteristics of the food. High temperatures are used either to pasteurize or to sterilize foods, and each has commercial applications (Jay, 1996).

### 2.1.1 Pasteurization

Pasteurization (e.g. pasteurization of milk) implies the destruction of disease-producing, non-spore-forming organisms. The following equivalent heat treatments will eliminate organisms such as *Mycobacterium tuberculosis* and *Coxiella burnetti*:

- 63 °C for 30 minutes (low temperature, long time, LTLT);
- 72 °C for 15 seconds (high temperature, short time, HTST);
- 89 °C for 1 second;
- 90 °C for 0.5 seconds;
- 94 °C for 0.1 seconds; and
- 100 °C for 0.01 seconds (Jay, 1996).

Packaged products treated with mild heating combined with chilled storage are known as REPFEDs (i.e. refrigerated processed foods of extended durability) or cook-chill products, including ‘*sous vide*’ meals (i.e. foods mildly heated within vacuum packs). For these chilled pasteurized foods specific attention is given to the control of non-proteolytic *Clostridium botulinum*, because of its ability to grow at chilled temperatures as low as 3 °C. Typically, an additional hurdle is necessary to ensure safety (Notermans *et al.*, 1990; Mossel and Struijk, 1991; Graham *et al.*, 1997). The hurdle concept is discussed later in this chapter.

### 2.1.2 Sterilization

Sterilization results when there is destruction of all viable organisms, as measured by an appropriate technique. The concept of commercial sterility has been applied to retorted or canned food when no viable organisms can be detected by the usual methods, or when the number of survivors is so low as to be non-significant under normal conditions. The term *appertization* (after Nicholas Appert) has been suggested for use as a replacement for the term ‘commercially sterile’, on the grounds that sterility is not a relative concept (Jay, 1996; Adams and Moss, 2000).

A major technological development in heat processing has been the use of ultra-high temperature, short-time treatments (UHT-ST), which involve rapid heating to temperatures of about 140 °C, holding for several seconds, and then rapidly cooling, to produce foods that are safe and shelf-stable at ambient temperatures. These processes provide opportunities for use of a variety of flexible packaging materials, and a large range of products is available (Lewis, 1993). UHT-ST treatment permits continuous operation, and is applied before packaging (Jelen, 1982). It is widely used for fruit juices, dairy products, creams and sauces and, more recently, for low-acid soups, including particulate-containing products (Rose, 1995).
2.2 Thermal destruction

Thermal processes are neither uniform nor instantaneous; a series of fundamental concepts have been developed to describe them (Jay, 1996):

- The Thermal Death Time (TDT) is the time needed to kill a given number of organisms at a specified temperature. Of lesser importance is the Thermal Death Point (TDP), defined as the temperature needed to kill a given number of microorganisms in a fixed time – usually 10 minutes.
- The $D$ value (Decimal Reduction Time) is the time required to destroy 90% of the organisms. Mathematically, the $D$ value is represented by the slope of the logarithm of survivors plotted against time (see Tables 18.1 and 18.2; Adams and Moss, 1995).
- The $z$ value is the number of degrees (Celsius or Fahrenheit) required to change the $D$ value by a factor of ten; $z$ values thus provide information on the destruction rate at different temperatures, allowing for the calculation of equivalent thermal processes at different temperatures.
- The $F$ value is the equivalent time at 250 °F (121 °C) of all temperatures to which food is exposed during a heat process. The integrated lethal value of heat received at the coolest point in a container (generally close to the center) during processing is designated as $F_0$ and represents the capacity of a heat process (expressed as minutes at 250 °F or 121 °C) to reduce the number of $C. botulinum$ spores.
- The 12-$D$ concept refers to a process that reduces the probability of survival of proteolytic $Clostridium$ botulinum$ spores by a factor of $10^{12}$.

These thermal destruction parameters assume that the effects of heat on microorganisms are constant and unaffected by the heating rate in the sub-lethal temperature.

<table>
<thead>
<tr>
<th>Table 18.1 Heat resistance of vegetative bacteria</th>
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<tr>
<td><strong>Species</strong></td>
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<tr>
<td><strong>$D$ value (min. at temperature)</strong></td>
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<tr>
<td><strong>$z$ value (°C)</strong></td>
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<tr>
<td>$70^\circ$C</td>
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<td>--------------------------------------------------</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
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<td>0.1</td>
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<tr>
<td>1.1</td>
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<tr>
<td>Escherichia coli</td>
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<td>0.1</td>
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<tr>
<td>4.0</td>
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<tr>
<td>Lactobacillus plantarum</td>
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<td>4.7–8.1</td>
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<td>5.0–8.3</td>
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<tr>
<td>Listeria monocytogenes</td>
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<tr>
<td>Pseudomonas aeruginosa</td>
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<tr>
<td>1.9</td>
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<tr>
<td>1.0–2.0</td>
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<tr>
<td>Pseudomonas fluorescens</td>
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<td>Salmonella spp.</td>
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<tr>
<td>440$^a$</td>
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<td>0.02–0.25</td>
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<tr>
<td>2.6–6.1$^a$</td>
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<td>0.3–2.0</td>
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<td>6.0$^b$</td>
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<tr>
<td>6.8–13.0$^c$</td>
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<tr>
<td>Salmonella Senftenberg</td>
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<td>816$^a$</td>
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<tr>
<td>0.056</td>
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<tr>
<td>6.0$^b$</td>
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<tr>
<td>6.8–13.0$^c$</td>
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<tr>
<td>Salmonella Typhimurium</td>
</tr>
<tr>
<td>0.2–2.0</td>
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<td>4.5–7.8</td>
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<td>4.5</td>
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<tr>
<td>Staphylococcus aureus</td>
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<tr>
<td>From Adams and Moss, 1995; Farkas, 2002.</td>
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<tr>
<td>$^a$ In milk chocolate</td>
</tr>
<tr>
<td>$^b$ In skim milk</td>
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<tr>
<td>$^c$ In heart infusion broth.</td>
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range. However, the rate of temperature elevation affects the subsequent isothermal death of several vegetative bacteria (Mackey and Derrick, 1986).

The assumption that the reduction in log numbers of survivors proceeds in a linear manner with time has served the food industry and regulatory agencies well. For bacterial spores, a good fit is generally observed. However, with milder thermal processes that use low processing temperatures significant deviations from linearity have been observed, including ‘shoulder’ and/or ‘tailing’ of the curves; therefore under these circumstances, $D$ and $z$ values cannot be accurately determined. Various explanations for these phenomena have been offered (Han, 1975; Cerf, 1977; Perkin et al., 1980; Gould, 1989). In recent years mathematical models have included normal distribution of heat sensitivity and logistic functions, which are better able to predict thermal inactivation at relatively low heating rates (Cole et al., 1993; Stephens et al., 1994).

### 2.3 Heat resistance

#### 2.3.1 Mesophilic organisms

Damage to DNA is the most probable key lethal event occurring when vegetative cells or spores are subjected to heat. Preventing spore germination is also important. Deviations from linearity in thermal death kinetics of vegetative cells indicate that there is a multiplicity of target sites, including the cytoplasmic membrane, key enzymes, RNA and the ribosome. This damage is cumulative rather than instantly lethal (Adams and Moss, 2000).

Heat resistance of microorganisms is related to their optimum growth temperatures. Psychrophilic and psychrotrophic organisms are the most heat sensitive, followed by mesophiles and thermophiles. Gram-positive bacteria tend to be more heat resistant than Gram-negatives, with cocci being more resistant than rods. Yeasts and molds tend to be fairly heat sensitive, and both yeast ascospores and asexual spores of molds are only slightly more heat resistant than their vegetative counterparts (Jay, 1996).

| Table 18.2  Heat resistance of bacterial endospores |
|------------|-----------------|-----------------|-----------------|-----------------|
| **Species** |**$D$ value (min at temperature)** |**$z$ value (˚C)** |
|----------|-----------------|-----------------|-----------------|-----------------|
| Bacillus cereus | 5.0 | | |
| Bacillus coagulans | 0.01–0.1 | | |
| Bacillus steatorrhemophilus | 4.0–4.5 | 3000 | 7.0 |
| Bacillus subtilis | 11.0 | | |
| Clostridium botulinum types A & B | 0.1–0.2 | | |
| Clostridium botulinum type E | > 1 s | 0.1–0.3 | |
| Clostridium butyricum | 0.1–0.5 | | |
| Clostridium perfringens | 0.3–20.0 | 10.0–30.0 | |
| Clostridium sporogenes | 0.1–1.5 | 9.0–13.0 | |
| Clostridium thermosaccharolyticum | 3.0–4.0 | 12.0–18.0 | |
| Desulfotomaculum nigrificans | 2.0–3.0 | | |

From Stumbo et al., 1983; Farkas, 2001.
The heat resistance of bacterial endospores is primarily related to their ability to maintain a very low water content in the central DNA-containing protoplast (Adams and Moss, 1995; Jay, 1996).

Exposure of vegetative cells to temperatures that induce a heat shock improves resistance to higher temperatures (Mackey and Derrick, 1986). Synthesis of a family of proteins called heat-shock proteins (HSP), in response not only to heat shock but also to chemical or physical stresses, occurs in most organisms (Schlesinger et al., 1982; Auffray et al., 1995; Boutibonnes et al., 1995). Many of the HSP inducers produce protein damage (Hightower, 1991), and it has been proposed that the signal for HSP induction is protein denaturation (Parsell and Sauer, 1989). For foods heated relatively slowly, the possibility of an increase in heat resistance of vegetative cells during heating must be taken into account to establish safe heat treatments (Farkas, 2002).

2.3.2 Thermophilic organisms
Thermophilic organisms have unique physiological characteristics that confer thermal tolerance.

The enzymes of thermophiles can be divided into three groups according to heat sensitivity (Jay, 1996). The base composition of rRNA has been shown to affect thermal stability. Pace and Campbell (1967) found that the microbial G-C content of rRNA tended to increase and the A-U content to decrease with increasing maximal growth temperatures. The increased G-C content makes for a more stable structure through more extensive hydrogen bonding. However, there seems to be no differences between thermophiles and mesophiles regarding the structure of DNA and mRNA.

Thermophilic growth is associated with a predominance of saturated lipids (Marr and Ingraham, 1962). Brock (1967) stated that thermophilic growth is linked to the nature of cellular membranes, much more so than to the properties of specific macromolecules or cytoplasmic organelles, and lethal injury may be primarily due to the melting of lipid constituents of the cell membrane, with resulting leakage of essential constituents and subsequent death (Jay, 1996).

2.4 Bacterial growth in canned foods
Bacterial growth in canned foods may result from under-processing permitting the survival of bacterial spores, or from contamination of the can content with spore-formers and non-spore-formers due to leakage through the seams (Jay, 1996). The extent of heat processing required depends largely on acidity: the more acidic a product, the milder the process needed (Adams and Moss, 2000). Thus, canned foods are classified based on acidity values (Adams and Moss, 1995; Jay, 1996).

2.4.1 Low-acid foods (pH > 4.6)
Examples are meat and marine products, milk, vegetables (e.g. corn and lima beans), and mixtures of meat and vegetables. Toxin production by surviving proteolytic *C. botulinum* may occur; these products must therefore undergo cooking sufficient to ensure 12 log₁₀ reductions of the spores of this pathogen.
2.4.2 Acid foods (pH 3.7–4.0 to 4.6)
These foods are mostly fruits, such as pears, tomatoes and figs, often spoiled by thermophilic organisms. *Clostridium botulinum* cannot grow in acid foods.

2.4.3 High-acid foods (pH < 3.7–4.0)
This group includes fruits and brined or fermented vegetable products (e.g. grapefruit, rhubarb, sauerkraut and pickles). They are generally spoiled by non-spore-forming mesophiles, yeasts, molds and/or lactic acid bacteria.

Cans must be cooled rapidly after processing to prevent spoilage by thermophiles. (Adams and Moss, 2000).

Leakage is the most common cause of microbial spoilage in canned foods. During processing, cans are subjected to physical stress. The negative pressure created inside the can during the rapid cooling stage may lead to microorganisms in the cooling water being sucked inside through any small defect in the seam. To prevent this contamination, the outside of cans must remain clean, and chlorinated water has to be used to cool them (Adams and Moss, 2000).

2.5 Examples of heat resistance of pathogens

Veeramuthu *et al*. (1998) studied the thermal inactivation of two pathogens, *E. coli* O157:H7 and *Salmonella* Senftenberg, in ground turkey thigh meat, and examined the thermal denaturation of endogenous enzymes for their potential use as time–temperature indicators. The study was based on the premise that the USDA Food Safety and Inspection Service may amend cooking regulations to require that any thermal process used for poultry products be sufficient to cause a 7-*D* reduction in *Salmonella* spp. Bacteria counts were determined and muscle extracts assayed for residual enzyme activity or protein denaturation. *S*. Senftenberg had higher *D* values at all temperatures. The *z* values of *E. coli* were 6.0–5.7 °C; those of *S*. Senftenberg were 5.6–5.4 °C. Lactate dehydrogenase (LDH) was the most heat-stable enzyme at 64 °C. LDH, triose phosphate isomerase (TPI), acid phosphatase and immunoglobulin G had 10-fold activity reduction values of 3.8, 5.8, 6.3 and 8.6 °C, respectively. Temperature dependence of TPI was most similar to that of *S*. Senftenberg, suggesting it might be used to monitor adequacy of processing if a performance standard based on this pathogen is implemented.

*D* values and *z* values were determined for *L. monocytogenes* Scott A in raw ground pork by Ollinger-Snyder *et al*. (1995). *D* values were 108.81, 9.80 and 1.14 minutes at 50 °C, 55 °C and 60 °C, respectively, when heating in ground pork without soy hulls, and 113.64, 10.19 and 1.70 minutes, respectively, when heating in ground pork with soy hulls added; *z* values were 5.45 °C and 5.05 °C in ground pork prepared with and without soy hulls, respectively. Assuming that ground pork naturally contains ca. $10^2$ *L. monocytogenes* cells per gram, and that safety can be assured with a 4-*D* *Listeria* cook (i.e. reducing the population by four orders of magnitude), this study indicated that ground pork should be heated to an internal temperature of 60 °C for at least 4.6 minutes without soy hulls and for at least 6.8 minutes with added soy hulls.
In beverage manufacture, cider and apple juice may be stored for a short time at ambient temperature before pasteurization. Storage time and temperature may affect the subsequent thermotolerance of bacteria in these beverages. Ingham and Uljas (1998) examined thermotolerance of two *E. coli* O157:H7 strains at 61 °C after storage in pH 3.4 apple cider or apple juice. Both strains exhibited biphasic survivor curves. Strain ATCC 43894 was consistently more thermotolerant than strain ATCC 43889, with 33–153% greater $D$ values derived from the linear portion of each survivor curve. Prior storage at 21 °C for 2 or 6 hours hastened thermal destruction of both strains in apple cider, but the effect was not statistically significant. However, when apple juice was tested, prior storage at 21 °C for 2 hours significantly decreased the thermotolerance of strain ATCC 43889, but not that of strain ATCC 43894. Storage at 21 °C for 6 hours in apple juice caused a decrease of 2.1 and 0.5 log$_{10}$ CFU/ml in populations of strain ATCC 43889 and strain ATCC 43894, respectively. Experiments with filtered apple cider showed that the presence of filterable pulp enhanced the thermotolerance of both strains. These authors concluded that short-term (≤6 hours), room-temperature storage of pH 3.4 filtered apple cider or apple juice may enhance lethality of subsequent pasteurization.

Grijspeerdt and Herman (2003) determined a series of inactivation curves for *Salmonella* Enteritidis in boiled eggs using different conditions of time and temperature. The inactivation curves consistently showed an initial slow decline in bacterial numbers at lower temperatures, after which a very rapid inactivation took place. Results suggested that *S. Enteritidis* is more resistant to a slower heating process.

The safety of REPFEDs with respect to non-proteolytic *C. botulinum* is a central issue. Lindström *et al.* (2001) evaluated ‘mild’ and ‘enhanced’ heat treatments in relation to survival of type B spores in *sous vide* processed ground beef and pork cubes. At a reference temperature of 85 °C and a $z$ value of 7.0 °C, the ‘mild’ treatment ran for 0–2 minutes and the ‘enhanced’ treatment for 67–515 minutes. Two concentrations of nisin were tested for the ability to inhibit growth and toxin production by non-proteolytic *C. botulinum* in the same products. A total of 96 samples were heat processed and analyzed for the presence of the pathogen, and for botulinum toxin after storage for 14–28 days at 4° and 8 °C. Predictably, after ‘mild’ processing all the samples of both products showed botulinal growth, and one ground beef sample became toxic at 8 °C. The ‘enhanced’ heat process resulted in growth but not toxin production by *C. botulinum* in one ground beef sample in 21 days at 8 °C. No growth was detected in the pork cube samples. The ‘enhanced’ processing of both products resulted in higher sensory quality than that corresponding to the ‘mild’ one. Nisin did not inhibit growth of *C. botulinum* in either product; growth was detected at 4 °C and 8 °C, and ground beef became toxic within 21–28 days at 8 °C. Aerobic and lactic acid bacterial counts were reduced by the addition of nisin at 4 °C. The study demonstrated that the mild processing temperatures commonly utilized in *sous vide* technology do not eliminate non-proteolytic *C. botulinum* type B spores. The heat treatment needs to be carefully evaluated for each product to ensure product safety in relation to non-proteolytic *C. botulinum*. 
3 Low-temperature preservation

3.1 History and terminology

As early as the eleventh century BC the Chinese had developed ice houses as a means of storing ice through the summer months, and by the nineteenth century the cutting and transporting of natural ice had become a significant industry in Europe and the Americas in areas blessed with a cold climate (Adams and Moss, 1995). Mechanical methods of refrigeration and ice making were first patented in the 1830s. Shipment of chilled meat from North America to Europe was started in the 1850s, and by the 1890s the technology had been refined to the extent that shipping chilled and frozen meat from North and South America and Australia to Europe was a large and profitable enterprise. Presently, a highly sophisticated cold chain allows the distribution of all kinds of food products around the world (Adams and Moss, 1995).

There are three distinct temperature ranges used for low-temperature storage of foods. Chilling temperatures are those between the usual refrigerator (5–7 °C) temperatures and the slightly higher temperatures (10–15 °C) that are suitable for the storage of certain vegetables and fruits such as cucumbers, potatoes and limes. Refrigeration temperatures are those between 0 °C and 7 °C. Freezing temperatures are those at or below −18 °C. Growth of microorganisms is generally prevented at freezing temperatures, although some of them can and do grow slightly below 0 °C but at an extremely slow rate (Jay, 1996).

Bacterial strains able to grow at or below 7 °C are more common among Gram-negative than Gram-positive genera. Growth at temperatures below 0 °C is more likely for yeasts and molds than for bacteria. Among pathogenic bacteria, Listeria, Yersinia and non-proteolytic C. botulinum have the lowest minimum growth temperatures. Salmonella spp. have higher minimal growth temperatures than Staphylococcus aureus (Jay, 1996).

The range of chilled and refrigerated foods available has increased in recent years, as traditional products such as fresh meat, fresh fish and dairy products have been joined by a huge variety of new items, including complete meals, prepared salads and delicatessen foods, dairy desserts and the like. The development and almost general availability of an efficient cold chain from manufacturing to consumption has played a key role in satisfying this market need (Adams and Moss, 2000).

3.2 Freezing, chilling, and frozen storage

Freezing should not be regarded as a means of destroying foodborne microorganisms. The extent to which microorganisms lose viability upon freezing differs from strain to strain, and depends on the rate of freezing, the nature and composition of the food in question, and the length of time of frozen storage (Georgala and Hurst, 1963).

Quick freezing is done by lowering the temperature of foods to about −20 °C within 30 minutes. Small intracellular ice crystals are formed. Microorganisms undergo a rapid thermal shock (with no time for low temperature adaptation or blocking of suppression of metabolic activity), and there is only a brief exposure to adverse
concentrations of solutes. Slow freezing takes place when the desired temperature (≤ −4 °C) is achieved within 3–72 hours. This is the type of freezing that occurs in the home freezer. Microorganisms are exposed for much longer to increased concentrations of solutes. There is no thermal shock effect, and this may allow for gradual adaptation to increasing concentrations of solutes and ‘injury recovery’ among survivors. It is important to emphasize that if the transition 0 to −4 °C is traversed quickly then small ice crystals are formed, whereas if the transition occurs slowly large ice crystals are formed; the latter scenario causes a higher lethality than the former (Jay, 1996; Adams and Moss, 2000). Ingram (1951) made three key observations related to the response of microorganisms freezing: (1) a sudden mortality occurs immediately on freezing; (2) the cells surviving immediately after freezing die gradually when stored in the frozen state; and (3) the decline in numbers is relatively rapid at temperatures just below freezing, especially about −2 °C, but less so at lower temperatures, and especially below −20 °C.

Jay (1996) has summarized the metabolic events that occur when cells freeze. During frozen storage, the death of survivors is fast initially and then slows gradually until the survival level stabilizes. The death rates tend to be lower than during the freezing process; survival seems to be related to the unfrozen, very concentrated residual solution formed by freezing. The concentration and composition of this residual solution may change during the course of storage, and the size of the ice crystals may increase, especially at fluctuating storage temperatures. In general, there is less loss of viability during frozen storage when the storage temperature is static rather than fluctuating. The lower the temperature of frozen storage, the slower the death rate of survivors. Gram-positive microorganisms survive frozen storage better than Gram-negative ones (International Commission on Microbiological Specifications for Foods, 1980).

Though psychrotrophs can grow in chilled foods, they do so relatively slowly. For example, the generation time for a Pseudomonas species isolated from fish was about 6.7 hours at 5 °C compared with 26.6 hours at 0 °C (Adams and Moss, 2000). Gill (1995) found that with fresh meat stored at 0 °C, the attainable shelf-life was only 70 % of that achievable at −2 °C (just at the freezing point of meat). This decreased to 50 %, 30 % and 15 % at 2 °C, 5 °C and 10 °C, respectively. Since chilling is not a bactericidal process, the use of raw materials of good microbiological quality and hygienic handling are key requirements for the production of safe chilled foods. Mesophiles that survive cooling, albeit in an injured state, can persist in the food for extended periods, and may recover and resume growth if the temperature becomes favorable at a later time. Thus, chilling will prevent an increase in the pathogenic risk from mesophiles, but will not assure their elimination (Adams and Moss, 2000). Psychrotrophic organisms can be found among yeasts, molds, and Gram-negative and Gram-positive bacteria. However, they all share the property of being inactivated at moderate heating temperatures, possibly because of excessive membrane fluidity at higher temperatures.

### 3.3 Thawing

The fate of microorganisms in food that has been frozen is partially determined by their ability to survive subsequent thawing. Three key factors should be considered (Fennema et al., 1973).
The time–temperature combination during thawing is potentially detrimental to quality and safety. During thawing, the temperature rises rapidly to near the melting point and remains there throughout the course of thawing (which can be lengthy), thus allowing considerable opportunity for chemical reactions, recrystallization, and microbial growth if the process is slow enough.

Most frozen-food processors advise against the refreezing of foods once they have been thawed. Although this is mostly related to textural, flavor and nutritional qualities, the microbiology of thawed frozen foods is important. Some textural changes associated with freezing would seem to allow the invasion of surface microorganisms into deeper tissues, thus facilitating microbial growth. Thawing causes the release of enzymes such as nucleases, phosphatases, glycosidases and others, which may degrade macromolecules and generate simpler compounds that are more readily utilized by the microbial flora.

3.4 Cold resistance

Psychrophiles and psychrotrophs possess physiological mechanisms related to their ability to grow at low temperatures.

3.4.1 Cold-adapted enzymes and slower metabolic rates
The cold-adapted enzymes are more thermolabile than their mesophilic counterparts, so that at quite moderate temperatures (40–50 °C) they become too flexible, lose catalytic efficiency and eventually denature.

3.4.2 Lipid composition and membrane functionality
The usual lipid content of most bacteria is between 2 % and 5 %, most or all of which is located in the cell membrane. Even though different cold-adapted bacteria may have similar low-temperature growth ability, they will almost certainly have quite different fatty acyl composition. Differences will be influenced by phylogenetic distinctions and different metabolic capabilities, and by specific protein–lipid interactions. For example, Salmonella adapt to temperature almost entirely by changing lipid unsaturation, whereas Listeria, which contain predominantly branched fatty acyl chains, modify the anteiso/iso-branched ratio and the acyl chain length. Bacillus spp. use a combination of unsaturation and changes in branching pattern (Russell, 2002).

A second aspect of membrane structure is the need to preserve the bilayer (lamellar) structure and prevent the formation of non-bilayer phases such as hexagonal arrangements, which destroy the selective permeability properties of the membrane (Russell, 1989).

3.4.3 Cold shock and cold adaptation
Cold shock occurs when many cells of mesophilic bacteria die upon the sudden chilling of a suspension of viable cells grown at mesophilic temperatures. It is generally a property of Gram-negative but not of Gram-positive bacteria. However, spoilage organisms within the genera Lactobacillus and Pseudomonas and pathogens within the genera Escherichia, Listeria, Salmonella and Yersinia exhibit this response as well.
According to Rose (1968), cold shock results from the sudden release of cell constituents following cold damage to membrane lipids, with the consequent development of holes in the membrane.

Cold acclimation depends on the ability to synthesize stress proteins, usually called cold-shock proteins (CSP). Table 18.3 summarizes the major functions of CSP related to blocking deleterious cold-shock effects (Russell, 2002). The importance of the ribosome in sensing temperature changes is evident, as is the fact that the cellular function most sensitive to cold shock is the initiation of translation (Graumann and Marahiel, 1996, 1997). The regulation of CSP synthesis occurs at several levels, both transcriptional and translational, involving protein and mRNA stabilities. Significantly, compared to mesophiles, in a cold-shock event involving psychrotrophs there is no concomitant suppression of the expression of the ‘housekeeping’ genes that encode enzymes of central metabolic pathways. Thus, growth lag times are likely to be shorter or non-existent. Moreover, the number of CSP and the extent of their synthesis depend on the depth of the cold shock (Gounot and Russell, 1999; Hébraud and Potier, 2000). In addition, changes in lipid composition and synthesis of CSP are functionally linked, because balanced growth requires coordination of intracellular and extracellular events as well as those occurring within the membrane matrix (Hoch and Silhavy, 1995).

<table>
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### 4 Multiple intervention technology

#### 4.1 Introduction

An essential task in the food industry is to maintain the quality and safety of foods during extended periods of storage. All foods deteriorate in quality at some specific rate following harvest, slaughter or manufacture. This quality deterioration is caused by a wide range of reactions, including physical (e.g. moisture migration to and from the environment or between the components of a composite food), chemical (e.g. rancidity due to oxidation), enzymatic (e.g. lipolysis leading to rancidity) and microbiological (spoilage and/or growth of pathogenic microorganisms) reactions.
While the aim of effective food preservation is to control all forms of quality deterioration, the overriding priority is to minimize the potential for the occurrence and growth of food spoilage and pathogenic microorganisms (Leistner and Gould, 2002).

Thus, preservation technologies are essentially aimed at inactivation of microorganisms or the delay or prevention of microbial growth. These preservative factors have been called ‘hurdles’. This a less than fortunate term, because it may imply that microorganisms, in order to prosper, must overcome one hurdle at a time – thus giving the impression that the effect of several hurdles is the sum of the individual effects. However, this is not the case; because of interactions (or synergisms), the total effect may be more like the product of individual effects. An example is a factorial experiment on the preservation of canned cured meats where significant interactions were demonstrated for nitrite, sodium chloride and heat treatment (Riemann, 1963). The hurdle concept was originally introduced by Leistner (1978), and has since then become generally accepted; the term will therefore also be used in this text. There are six fundamental hurdles: temperature (high or low), water activity ($a_w$), acidity (pH), oxidation–reduction potential ($E_h$), preservatives (e.g. nitrites, sorbates, sulfite), and competitive microorganisms (e.g. lactic acid bacteria) (Leistner, 2000). However, the full list of possible hurdles is much more extensive, including natural antimicrobials, vacuum and modified atmosphere packaging, and alternative processing techniques, which are covered in later sections of this chapter.

In industrialized countries, the hurdle technology approach is currently of most interest for minimally processed foods that are mildly heated or fermented, and for establishing the microbial stability and safety of foods being developed in response to consumer demands for ‘healthier’ foods with less fat, sugar or salt. In developing countries, hurdle technology is applied to produce foods that remain stable, safe and tasty when stored at ambient temperature. Impressive success has been achieved in Latin America with the development of minimally processed, high-moisture fruit products.

### 4.2 Basic concepts

Food preservation implies generating an environment that inhibits the growth or causes the death of microorganisms in food. The fundamental biological concepts related to hurdle technology are as follows.

#### 4.2.1 Homeostasis

Homeostasis is the stability of reactions in the cytoplasm of organisms. If hurdles in foods significantly disturb the homeostasis of microorganisms, they will not multiply – that is, they will remain in the lag phase and may even die before homeostasis is re-established. Thus, food preservation is achieved by disturbing the microbial homeostasis either temporarily or permanently (Leistner, 2000).

#### 4.2.2 Metabolic exhaustion

Microorganisms respond to stress by activating repair mechanisms to re-establish homeostasis and overcome the hostile challenge. By doing this, they may use up their
energy resources and die as they become metabolically exhausted. Essentially, an ‘autosterilization’ phenomenon occurs (Leistner, 1995a).

### 4.2.3 Stress reactions

Some bacteria become more resistant or even more virulent under stress as a result of generating shock proteins. This may interfere with safe food preservation, but the activation of genes for the synthesis of these ‘novel’ proteins has an energy cost. Thus, if different stresses are received simultaneously, microorganisms may become metabolically exhausted at an accelerated rate (Leistner, 1995a).

### 4.2.4 Multi-target preservation

This concept refers to the goal of an effective but mild food preservation approach, based on the fact that – as mentioned earlier – different hurdles in a food might not have just an additive antimicrobial effect but may also act synergistically (Leistner, 1978, 1995b). A synergistic effect could be achieved if the applied hurdles affected different targets within the microbial cell (e.g. the membrane, DNA, enzyme systems, pH). If so, restoration of homeostasis and activation of stress-shock proteins becomes more difficult (Leistner, 1995b). This means that it is more effective to employ several hurdles of small intensities than one inhibitory factor of larger intensity (Leistner, 1994). This approach may also minimize losses of product quality.

### 4.3 Survey of fundamental hurdles

Of the six fundamental hurdles, four are covered here: pH, $a_w$, $E_h$ and competitive microorganisms. Temperature was discussed in the previous section on temperature preservation (high and low), whereas preservatives will be covered in the following sections on antimicrobial agents (natural and synthetic).

#### 4.3.1 Acidity

Most microorganisms grow best at pH values around 7.0 (range 6.6–7.5); few grow below pH 4.0. Bacteria, especially pathogenic bacteria, tend to be more sensitive than molds and yeasts to low pH. Such foods as fruits, soft drinks, vinegar and wines have pH levels below that at which bacteria normally grow. Thus, the excellent keeping quality of these products is essentially due to pH. Not accidentally, fruits undergo primarily mold and yeast spoilage, because these organisms are able to grow at pH < 3.5. On the other hand, most meats and seafood have a final ultimate pH of about 5.6 and higher, which makes them susceptible to bacteria as well as mold and yeast spoilage. Vegetables tend to have higher pH values than fruits, and as such they are also prone to bacterial spoilage (Jay, 1996).

Some foods are characterized by inherent acidity. Others owe their low pH to the action of certain microorganisms, which is called biological acidity; this type of acidity is found in fermented dairy products, sauerkraut and pickles. Meats are more highly buffered than vegetables, owing to their higher protein content (Jay, 1996).

An adverse pH affects at least two essential characteristics of the metabolism of a microbial cell: the functioning of the enzymatic systems and the transport
of nutrients into the cell. The cytoplasmic membrane is relatively impermeable to H\(^+\) and OH\(^-\) ions in order to maintain a constant internal pH. Key compounds such as DNA and ATP require neutrality. Thus, when placed in an acid environment the cell must either keep H\(^+\) from entering or expel H\(^+\) ions as they enter in order to avoid a fatal alteration in its homeostasis. With respect to the transport of nutrients, the bacterial cell tends to have a negative charge. Therefore, non-ionized nutrients can enter the cell, whereas ionized ones cannot. At a neutral or alkaline pH organic acids do not enter, but in an acidic environment these compounds are non-ionized and can enter the negatively charged cell. In addition, the ionic character of ionizable groups is affected on either side of neutrality, resulting in increasing denaturation of membrane and transport enzymes (Jay, 1996).

4.3.2 Water activity

Water activity is defined as the ratio of the water vapor pressure over food to the vapor pressure over pure water at the same temperature. In mathematical terms, 
\[ a_w = \frac{p}{p_0} \]
where \( p \) is the vapor pressure of the solution and \( p_0 \) the vapor pressure of the solvent (water). This concept is related to relative humidity (RH), as 
\[ RH = 100 \times a_w \]

Most fresh foods have \( a_w \) values > 0.99. In general, bacteria require higher \( a_w \) values than fungi, and Gram-negative bacteria require higher \( a_w \) values than Gram-positive bacteria. Most spoilage bacteria do not grow below \( a_w = 0.91 \), whereas spoilage molds can grow at levels as low as 0.80. The lowest reported value for bacteria of any type is 0.75 for halophilic (‘salt-loving’) organisms. Xerophilic (‘dry-loving’) molds and osmophilic (‘barophilic’) yeasts can grow at \( a_w \) values of 0.65 and 0.61, respectively (Jay, 1996).

Three important relationships have been identified among \( a_w \), temperature and nutrition (Morris, 1962): at any temperature, the ability of microorganisms to grow is reduced as the \( a_w \) is lowered; and the \( a_w \) range allowing growth is greatest at the optimum growth temperature and with optimum nutrients.

Curing by the addition of salt, conservation by the addition of sugar, the addition of other solutes (such as glycerol) and the removal of water from food by drying, as well as the immobilization of water by freezing, all lead to a reduction in water activity (Leistner and Gould, 2002). The general effect of lowering \( a_w \) below the optimum is an increase of the lag phase of growth and a decrease of the growth rate and size of the final microbial population. These effects result from adverse influences of lowered water availability on all metabolic activities, because all cellular chemical reactions take place in an aqueous environment. Lowering the water activity causes osmotic stress in microorganisms, and microorganisms respond by intracellular accumulation of compatible solutes. Bacteria can accumulate substances such as glutamate, glutamine, proline, alanine, sucrose and trehalose; whereas fungi produce polyhydric alcohols such as glycerol and arabitol (Jay, 1996). Halophilic bacteria are able to operate under low \( a_w \) conditions by virtue of their ability to accumulate potassium chloride (Brown, 1964).

The so-called intermediate moisture foods (IMF) rely on a lowered \( a_w \) as the main factor for microbial stability. IMF are characterized by moisture contents of around 15–50 % and \( a_w \) values of between 0.60 and 0.85, which protects against growth of
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pathogenic bacteria. Traditionally, the lowered $a_w$ values are achieved by withdrawal of water by desorption or adsorption, and/or the addition of permissible additives such as salts and sugars. More recently other ingredients (called humectants) have been utilized, including glycerol, sorbitol and glycols (Jay, 1996). To control water by adsorption, food is first dried (often freeze-dried) and then subjected to controlled rehumidification until the desired composition is achieved. By desorption, the food is placed in a solution of higher osmotic pressure so that the desired $a_w$ is reached at equilibrium (Robson, 1976). Although identical $a_w$ values may be achieved by these two methods, IMF produced by adsorption are more inhibitory to microorganisms than those produced by desorption (Sloan et al., 1976).

Gram-negative bacteria will not proliferate within the $a_w$ range of IMF products. This is also true for most Gram-positive bacteria, with the exception of cocci, some spore-formers and lactobacilli. Molds and yeasts have the ability to grow in the IMF $a_w$ range. Ultimately, antimicrobial activity results from interaction among the lowered $a_w$, the pH, the $E_h$, added preservatives (e.g., sorbates, benzoates and even some of the humectants), a competitive microflora, low storage temperatures, and the heat process applied during manufacturing (Jay, 1996).

4.3.3 Redox potential

The oxidation–reduction (or redox) potential ($E_h$) of a substrate is defined as the ease with which it gains or loses electrons. When an element or compound gains electrons it becomes reduced, whereas when it loses them it becomes oxidized. Oxidation also results from the addition of oxygen to the substrate. A substance that readily gives up electrons (i.e. that is readily oxidized) is a good reducing agent. A substance that readily takes up electrons (i.e. that is readily reduced) is a good oxidizing agent. When electrons are transferred from one compound to another, a potential difference is created between them. The more highly oxidized a substance is, the more positive will be its electrical potential; the more highly reduced a substance is, the more negative will be its electrical potential. When the concentration of oxidant and reductant is equal, a zero potential exists (Jay, 1996).

Aerobic microorganisms (e.g. Bacillus) require positive $E_h$ values for growth, whereas anaerobic microorganisms (e.g. Clostridium) require negative $E_h$ values. Some aerobic bacteria actually grow better under slightly reduced conditions; these organisms are called microaerophiles (e.g. Lactobacillus and Campylobacter). Other bacteria are able to grow under either aerobic or anaerobic conditions, and are called facultative anaerobes (e.g. Staphylococcus and Salmonella). Most molds encountered in food are aerobic, while most yeasts are facultative anaerobes (Jay, 1996).

Microorganisms affect the $E_h$ of their environments during growth, just as they do pH. This is particularly true for aerobes, which can lower the $E_h$ of their environment. As aerobes grow the oxygen is depleted, resulting in the lowering of $E_h$. However, growth is not slowed due to the ability of the microbial cell to make use of $O_2$-donating or $H_2$-accepting substances in the medium. The result is that the medium becomes poorer in oxidizing and richer in reducing substances (Morris, 1962). Microorganisms can reduce the $E_h$ of their surrounding medium by producing
metabolic by-products such as H$_2$S, which has the capacity to lower E$_h$ to $-300$ mV. Because H$_2$S reacts readily with O$_2$, it will accumulate only in anaerobic environments (Jay, 1996).

Most plant and animal foods have a low E$_h$ in their interiors because of the presence of reducing substances, such as reducing sugars and ascorbic acid in plants and sulfhydryl groups in meats. As long as the cells respire and remain active, they tend to balance the E$_h$ system at a low level. Thus fresh fruits, vegetables, and meats will have aerobic conditions at and near the surface. Plant foods, especially plant juices, tend to have E$_h$ values of 300–400 mV, and aerobic bacteria and molds are the common cause of spoilage of products of this type. Solid meats can support the growth of aerobic slime-forming bacteria on the surface while simultaneously permitting growth of anaerobic bacteria in the interior (Genigeorgis and Riemann, 1979; Jay, 1996).

Food-processing operations can change the E$_h$ of foods by destroying or altering reducing and oxidizing substances and/or allowing oxygen to diffuse into the tissues. As E$_h$ changes as a result of biochemical changes in food and/or microbial metabolism, new species of microorganisms may succeed those that initiated growth.

4.3.4 Competitive microorganisms

In general, microbial interference is a phenomenon of non-specific inhibition or destruction of one species of microorganisms by others in the same environment, due to competition for nutrients, competition for attachment/adhesion sites, rendering the environment unfavorable, or a combination of these (Jay, 1995). It is known that the background biota needs to be numerically larger than the organism to be inhibited, and that the interfering biota are generally not homogeneous; however, the specific roles that individual species play are often unclear (Jay, 1996).

An example of interference is the inhibition or killing of a spectrum of food spoilage and/or pathogenic organisms by mixed cultures of lactic acid bacteria. Bacteriocins, pH depression, organic acids, hydrogen peroxide, diacetyl and possibly other products affect the inhibition (Jay, 1996). These compounds and their corresponding mechanisms of action are discussed in section 8.

Microorganisms that can be added to a food product to effect preservation have been designated protective cultures by Holzapfel et al. (1995). Lactic acid bacteria represent the largest and most important group in this category, and have the following properties: they present no health risks, they provide beneficial effects beyond the interference phenomenon, and they have no negative impact on sensory properties.

4.4 Examples of application

Kanatt et al. (2002) developed a number of ready-to-use, shelf-stable, intermediate-moisture (IM) spiced mutton and spiced chicken products, with a combination of reduced moisture, vacuum packaging and irradiation. The $a_w$ of the products was reduced to about 0.80 either by grilling or by hot-air drying. These IM products were vacuum-packed and subjected to gamma radiation at 0–10 kGy. There was a dose-dependent radiation reduction in total viable counts and in numbers of *Staphylococcus* spp. IM meat products that did not undergo radiation showed visible
mold growth within 2 months; products irradiated at 10 kGy showed an absence of viable microorganisms and retained high sensory acceptability for up to 9 months at ambient temperatures.

Marin et al. (2002) developed methods to prevent fungal growth of common contaminants on bakery products, including the genera *Eurotium*, *Aspergillus* and *Penicillium*. A factorial design was used to test logarithmically increasing levels of calcium propionate, potassium sorbate and sodium benzoate (0.003 %, 0.03 % and 0.3 %), pH (4.5, 6 and 7.5) and $a_w$ (0.80, 0.85, 0.90 and 0.95). Potassium sorbate at 0.3 % was found to be the most suitable preservative in combination with the common levels of pH and $a_w$ in Spanish bakery products. Sub-optimal concentrations led to an enhancement of fungal growth. None of the preservatives had a significant inhibitory effect at neutral pH.

Uyttendaele et al. (2001) studied the growth and survival of *E. coli* O157:H7 exposed to a combination of sub-optimal factors in a red meat medium (beef gravy). Hurdles were temperature (22 °C, 7 °C and −18 °C), pH (4.5, 5.4 and 7.0) and lactic acid. Prolonged survival was noted as the imposed stress became more severe, and as multiple growth factors became sub-optimal. At 7 °C or −18 °C, survival was prolonged at the more acid pH; and at a pH of 4.5, greater survival was observed at 7 °C than at 22 °C. The addition of lactic acid instead of HCl to reduce pH to 4.5 resulted in a more rapid decrease of the pathogen. However, high survival was observed in beef gravy, pH 5.4 at −18 °C (simulation of frozen meat), with an unsatisfactory reduction from log 3.0 to log 1.3 after 43 days, and in beef gravy at pH 4.5 with 5 % NaCl and 7 °C (simulation of a fermented dried meat product kept refrigerated). There was less than 1 log reduction in 43 days. These results suggest that preservatives may inhibit multiplication but induce prolonged survival of *E. coli* O157:H7.

Terebiznik et al. (2002) investigated the effect of nisin combined with pulsed electric fields (PEF) and $a_w$ reduction by NaCl on the inactivation of *E. coli* in a simulated milk ultrafiltrate medium. The reduction of $a_w$ from 0.99 to 0.95 by NaCl, without any other hurdle, did not affect *E. coli* viability. A reduction in PEF effectiveness occurred when the NaCl concentration was increased because of an increase in conductance, which reduced the pulse decay time. In cells subjected to PEF, nisin activity was decreased – probably due to the non-specific binding of nisin to cellular debris or the emergence of new binding sites in or on cells. However, the lethal effect of nisin was re-established and further enhanced when $a_w$ was reduced to 0.95. A synergistic effect was evident when low-intensity PEF was applied. Decreasing $a_w$ to 0.95 and applying PEF at 5 kV/cm (a non-lethal intensity when no other hurdle is used) with the further addition of nisin (1200 IU/ml) resulted in a 5-log reduction of the bacterial population.

Application of hurdle technology has largely been based on good judgment as well as trial and error. Although this empirical approach has proved successful in practical food design, food engineers question the lack of a mathematically and statistically sound base (Leistner and Gould, 2002). McMeekin et al. (2000) emphasized that a very sharp cut-off often occurs between conditions permitting growth and those preventing growth, allowing combinations of factors that deliver long-term stability and safety to be defined precisely and modelled (see also section 11, on predictive bacteriology).
5 Fermentation and safety

5.1 History and principles

Fermentation is one of the oldest methods of food processing. Foods such as bread, beer, wine and cheese originated in ancient times; and the principles involved in their manufacturing have hardly changed (Nout, 2001). There are many benefits associated with fermentation. It can preserve food (i.e. increase shelf-life), improve digestibility, enrich food nutritionally, and enhance taste and flavor. Furthermore, fermentation has the potential to enhance food safety by removing toxic components and controlling pathogens. Thus, it makes a significant contribution to human nutrition, particularly in developing countries where economic problems are a major barrier to ensuring food safety (e.g. the absence of freezing and/or refrigeration facilities) (Motarjemi et al., 2001).

The microbes involved in fermentation include molds (mycelial fungi), yeasts (unicellular fungi) and bacteria. Microbial enzymes break down carbohydrates, lipids, proteins and other food components, thus improving food digestion and increasing bioavailability. Substances of microbial origin found in fermented foods include organic acids, alcohols, aldehydes, esters and many others. The presence of living microbial cells such as in non-pasteurized yogurt may have advantageous effects on the intestinal microflora and, indirectly, on human health (Nout, 2001).

In the strict sense, fermentation refers to a form of anaerobic energy metabolism. Nevertheless, in the context of fermented foods microbial growth and metabolism can also take place under aerobic conditions. Mold-related fermentations provide a typical example. There is a huge variety of fermented foods worldwide. Those of plant origin are derived from a variety of raw materials of different chemical composition and biophysical properties: cereals and potatoes having high starch contents; legumes and oil seeds rich in proteins; fruits containing high concentrations of reducing sugars; and green vegetables, carrots, tomatoes, olives and cucumbers of high moisture content. Most fermentative processes of vegetables and cereals are due to the action of lactic acid bacteria, often in combination with yeasts; other bacteria such as Bacillus spp., or mycelial fungi such as Rhizopus and Aspergillus spp., are equally important in the fermentation of legumes and oil seeds. Foods of animal origin are highly perishable products, and fermentation has long been an effective method for prolonging their shelf-life. Lactic acid bacteria are the main organisms carrying out the fermentation of animal products, although halotolerant bacteria and yeasts are also involved in fermenting fish and seafood (Nout, 2001).

Fermentations can be distinguished according to the physical state under which they take place. In liquid fermentations, the microorganisms are suspended while a mixing device is used to ensure homogeneity. Control of temperature and levels of dissolved oxygen can be achieved with immersion coolers or heaters, and aeration. In solid-state fermentations, even though the particulate matter contains sufficient water to allow microbial growth, water is not in the continuous phase; gas (air) is, but it is a poor heat conductor, and solid-state fermentations tend to develop gradients not only of temperature but also of gas composition. Controls of homogeneity and
mixing systems are thus more complex than in liquid fermentations. There are also intermediate situations, such as shredded vegetables or olives, whose fermentations are carried out in brine; these are called immersed liquid fermentations (Nout, 2001).

5.2 Lactic acid bacteria

Lactic acid bacteria predominate in the majority of fermented foods. Their growth and metabolism inhibit the normal spoilage flora and bacterial pathogens through either bacteriostatic or bactericidal action. With toxigenic pathogens, the bacteriostatic action can effectively ensure safety (assuming that initial pathogen numbers are below those necessary to produce illness). With infectious pathogens, bacteriostatic action can be insufficient because the infectious dose of some is very low. In this case, bactericidal action is indispensable, and complete elimination of risk will also depend on other factors such as the type of pathogen considered and its initial numbers and physiological state (Adams, 2001).

Lactic acid bacteria form a group of Gram-positive, non-spore-forming, fermentative anaerobes that are often aerotolerant. They produce most of their cellular energy as a result of sugar fermentation. In the case of hexoses, this can proceed by one of two pathways (Axelsson, 1998): homofermenters ferment hexoses by the Embden–Meyerhof–Parma glycolytic pathway, yielding almost exclusively lactic acid; and heterofermenters produce less acid overall as a mixture of lactic acid, acetic acid, ethanol and carbon dioxide, using the 6-phosphogluconate/phosphoketolase pathway.

Antimicrobial activity by lactic acid bacteria derives from low pH, organic acids, bacteriocins, carbon dioxide, hydrogen peroxide, diacetyl, ethanol and reuterin, as well as nutrient depletion and overcrowding. The specific modes of action of these antimicrobial agents are described in section 7 of this chapter. The predominant actions are the production of organic acids and pH reduction, with the others contributing to the aggregate effect, particularly by ensuring the successful early dominance of lactic acid bacteria (Adams, 2001).

5.3 Control of microbial hazards in fermented foods

5.3.1 Bacteria

Factors to be considered are decontamination of raw materials; prevention of product contamination by cleaning, disinfection and zoning (mainly segregation of raw from finished product); controlled fermentation and ripening processes (primarily related to temperatures); and whether the product will be consumed without terminal heating (Beumer, 2001).

Prevention of contamination of raw materials with pathogenic microorganisms is a primary safety measure. However, total removal of pathogens often becomes a hopeless task, considering that pathogens are frequently present in soil as well as in surface water and the gut of animals (and are therefore also found on the surfaces of fruits and vegetables or embedded in plant tissue). To minimize risks, pretreatment of raw materials against pathogens is helpful. For example, washing in clean water can eliminate up to 90% of pathogens; cleaning and disinfection of surfaces and equipment
and proper hygiene practices by plant personnel are also important. Another possibility is heat treatment (e.g. pasteurization), typically used for milk. If pasteurization is not possible, a post-process pasteurization step may be necessary to eliminate pathogens in the final product – for example in fermented sausages – although this may have serious effects on sensory quality (Beumer, 2001).

Zoning or segregation of process steps helps to contain contamination and should be part of an HACCP plan. As applied to fermentation processes, there should be separate areas for the storage of the raw materials, the preparation of raw materials (i.e. washing, cutting and adding ingredients), the fermentation process per se, the filling of packages, and the storage of final products. A typical example of an area requiring strict hygiene is the room where starter cultures are prepared for the lactic fermentation of milk, where potential dangers are contamination and the infection of cultures with bacteriophages (Beumer, 2001). In meat processing, the use of the same area of the plant for slicing raw fermented sausages and dry-cured ham containing high levels of viable lactic acid bacteria can result in contamination of cooked, vacuum-packed, cured meat products, and accelerate their spoilage. Every effort should therefore be made to ensure that separate slicing areas for these different product types (raw and cooked) are established.

The following are the major pathogenic bacteria and their association with fermented foods.

### 5.3.1.1 Aeromonas

Aeromonas are found in water (including sewage) and in food products that have been in contact with contaminated waters, such as seafood and vegetables (see Chapter 10). Aeromonas hydrophila is the most important pathogen (Roberts et al., 1996).

In general, Aeromonas do not seem to be a serious risk in well-produced fermented foods. For example, the addition of Aeromonas to skim milk during lactic fermentation and to yogurt resulted in a sharp decrease in pathogen numbers (Aytac and Ozbas, 1994; Ozbas and Aytac, 1996). Spanish fermented sausages (longaniza and chorizo) contained Aeromonas in numbers between 1.0 and 4.5 log₁₀ CFU/g; the hygienic states of the factories significantly influenced their incidence and numbers. However, aeromonads were rapidly inactivated during the early stages of manufacture regardless of initial contamination (Encinas et al., 1999).

### 5.3.1.2 Campylobacter species

Campylobacter species, mainly Campylobacter jejuni, Campylobacter coli and Campylobacter lari, cause severe gastroenteritis (Roberts et al., 1996; see Chapter 7).

Although the infective dose of Campylobacter is relatively low compared to that of other pathogenic microorganisms, Campylobacter transmission by fermented foods is regarded as not significant, since the organisms do not grow below 30 °C and are sensitive to freezing, drying, low pH and sodium chloride. This has been confirmed by artificial contamination of fermented products such as yogurt and salami, where the numbers of the pathogen decreased rapidly (Northolt, 1983; Morioka et al., 1996).
5.3.1.3 Vibrios
Among vibrios, *Vibrio cholerae* and *Vibrio parahaemolyticus* are the most important pathogenic species. These are found mostly in coastal marine waters, and are therefore associated with shellfish and other marine animals (Roberts *et al.*, 1996; see Chapter 5). Vibrios are not acid tolerant, and are inhibited by lactic acid bacteria isolated from fermented fish products (Ostergaard *et al.*, 1998). This has also been observed in pickles and squid shiokara that were artificially contaminated with *V. parahaemolyticus* (Yu and Chou, 1987; Wu *et al.*, 1999).

5.3.1.4 Bacillus cereus
*Bacillus cereus* causes both foodborne infection characterized by diarrhea and intoxication characterized by vomiting, after ingestion of the toxin cereulide. As a spore-former, *B. cereus* is ubiquitous and may be found in cereals and spices (Roberts *et al.*, 1996; see Chapter 15).

The behavior of *B. cereus* has been studied in fermented products such as tempeh, sauce-based fermented salads, and fish sausage. There was initial growth in all cases, but in products where pH decreased due to lactic acid bacteria activity, inhibition correlated directly with the degree of acidity. In tempeh, where no lactic acid fermentation is involved, *B. cereus* numbers reached $10^8$ CFU/g, although soaking of the soybeans and subsequent acidification below pH 4.5 stopped its growth (Nout *et al.*, 1989; Aryanta *et al.*, 1991; Bonestroo *et al.*, 1993).

5.3.1.5 Clostridium botulinum
Botulism is a neuroparalytic syndrome caused by the botulinum neurotoxin. The organism responsible, *Clostridium botulinum*, is ubiquitous, and spores are widely distributed in the soil, shores and bottom deposits of lakes and coastal waters, and in the intestinal tracts of fish and animals (Roberts *et al.*, 1996; see Chapter 13).

Most outbreaks of foodborne botulism have been related to inadequate processing of vegetables, fish and meats (e.g. home processing). Nitrites inhibit this pathogen and are a safety factor in the production of cured and fermented meats (Beumer, 2001).

5.3.1.6 Staphylococcus aureus
*Staphylococcus aureus* produce several heat-stable enterotoxins. *S. aureus* is present on the skin and mucous membranes of warm-blooded animals, including humans. Contamination of cooked or ready-to-eat food products by a colonized person followed by storage at ambient temperatures is often implicated in outbreaks (Roberts *et al.*, 1996; see Chapter 14).

Fermented sausages and raw milk cheeses have been associated with *S. aureus* outbreaks, but in general the organism is regarded as a poor competitor, and its growth in fermented foods is typically associated with a failure of the normal fermenting flora (Bacus, 1986; Johnson *et al.*, 1990; Nychas and Arkoudelos, 1990). González-Fandos *et al.* (1999) investigated survival and toxigenesis of *S. aureus* in Spanish-type dry sausages (chorizo and salchichón). *Lactobacillus curvatus* in combination with dextrose and relatively low fermentation temperatures ($< 20 ^\circ C$) was an effective anti-staphylococcal agent during fermentation. Portocarrero *et al.* (2002) inoculated
S. aureus in fresh hams that were then cured, equilibrated to ≥ 2.5 % NaCl, cold- or non-smoked, and aged. Their results indicated that higher salt contents and lower a_w values played a decisive role in controlling the growth and toxin production of the pathogen.

5.3.1.7 Listeria monocytogenes
Listeria monocytogenes is ubiquitous, and is a human and animal pathogen. Approximately 30 % of cases of listeriosis are perinatal, with 20–25 % of those cases being fatal for the newborn. Most other cases occur in immuno-compromised persons, with a death rate varying from 30 % to 50 % (Roberts et al., 1996; see Chapter 9).

Listeria is not particularly acid-tolerant, but in fermented products where a mold-ripening step is involved, the rise in pH can allow surviving cells to resume growth (Beumer, 2001). Products such as home-made sausages and mold-ripened cheeses have been associated with listeriosis (Farber et al., 1993; Nissen and Holck, 1998).

5.3.1.8 Enterobacteriaceae
Enterobacteriaceae are typical components of the fecal flora of animals, which is the reason for the use of some members as hygiene indicators for processed foods (Beumer, 2001).

Salmonella is often found on raw meat. Widely distributed in the environment, salmonellae are present in the gut of infected animals and humans, and are shed in the feces (see Chapter 3).

Meat, milk, poultry, and eggs are the main vehicles for Salmonella transmission, and fermented foods derived from these raw materials, including salami and cheeses, have been occasionally associated with Salmonella outbreaks (Leyer and Johnson, 1992; Beumer, 2001). Sauer et al. (1997) studied a number of cases of human salmonellosis caused by S. Typhimurium associated with Lebanon bologna. The pathogen might have survived the fermentation process used by the manufacturer due to its high numbers in the raw meat (> 10^4 CFU/g). Stricter process controls in the manufacture of semi-dry fermented sausages were suggested. Inhot et al. (1998) inoculated a six-strain cocktail of S. Typhimurium into pepperoni batter. Fermentation and drying resulted in about a 3.0-log_10 reduction in numbers of the pathogen, and subsequent vacuum storage at ambient temperature was more lethal than refrigerated storage.

Shigella is not a natural inhabitant of the environment (see Chapter 10). Person-to-person transmission due to poor personal hygiene and the consumption of contaminated water or foods washed with contaminated water cause infection (Roberts et al., 1996). The rapid decrease in Shigella numbers at pH < 5.0 indicates that this microorganism is in fact a minor risk in fermented foods (Nout et al., 1989; Kunene et al., 1999).

Yersinia enterocolitica present in raw milk, seafood and raw pork has been associated with foodborne infections (see Chapter 8). Survival of Yersinia has been tested in fermenting milk and yogurt. Although there was some growth during the first few hours of the fermentation process, numbers fell below detection levels after completion of fermentation and a 4-day storage period (Ozbas and Aytac, 1996; Bodnaruk and Draughon, 1998).
E. coli O157:H7, because of its acid resistance, can survive the fermentation process. In recent years there have been a number of outbreaks involving fermented foods where this pathogen has been found responsible (Beumer, 2001; see Chapter 6).

The fate of various E. coli O157:H7 strains during the fermentation and storage of diluted cultured milk drink fermented with Lactobacillus casei spp. casei and Lactobacillus delbrueckii ssp. bulgaricus was investigated by Chang et al. (2000). All strains of the pathogen grew rapidly in skim milk and reached a maximum population of ca. 8.0–9.0 log\textsubscript{10} CFU/ml after 24 hours. However, populations declined as cultivation proceeded further. Viable cells of E. coli O157:H7 were reduced to non-detectable levels in the non-sugar-added cultured drink. Sugar extended the survival period according to the pathogen strain and the amount of sugar added to the system.

Dineen et al. (1998) studied the persistence of E. coli O157:H7 as a post-pasteurization contaminant in fermented dairy products, its ability to compete against commercial starter culture in fermentation systems, and its survival in the yogurt production process. These authors concluded that E. coli O157:H7 is a serious potential health hazard in the case of post-processing entry into fermented dairy products, and that commercial starter cultures differ widely in their ability to reduce E. coli O157:H7 numbers in fermentation systems. The pathogen, inoculated at 10\textsuperscript{5} CFU/ml in the starting milk, did not survive the yogurt manufacturing process after curd formation.

Faith et al. (1998) prepared beef jerky batter with fat contents of about 5 and 20 % and inoculated it with a five-strain mix of E. coli O157:H7 at 10\textsuperscript{8} CFU/g. Pathogen numbers were determined in both the raw batter and the strips formed from it after a number of drying processes of different lengths and temperatures. There was no direct correlation between the moisture-to-protein ratio of the final product and the viability of the pathogen. However, higher fat contents, longer drying times and lower drying temperatures increased the viability of E. coli O157:H7.

Studies to determine the fate of E. coli O157:H7 during the production and storage of fermented dry sausage were conducted by Glass et al. (1992). A commercial sausage batter inoculated with 4.8 × 10\textsuperscript{4} E. coli O157:H7 per g was fermented to pH 4.8 and dried until the moisture-to-protein ratio was ≤ 1.9:1. The sausage chubs were then vacuum-packed and stored at 4 °C for 2 months. The organism survived but did not grow during fermentation, drying or subsequent storage, and had decreased by about 2 log\textsubscript{10} CFU/g by the end of the storage period. The importance of using beef containing low populations of or no E. coli O157:H7 in sausage batter was stressed, because when initially present at 10\textsuperscript{4} CFU/g, this organism could survive fermentation, drying and storage of sausage regardless of whether a starter culture was used.

5.3.2 Fungi
A number of species of fungi can produce relatively low molecular weight metabolites that are toxic to humans and domesticated animals and are called mycotoxins (see Chapter 16). Their biosynthesis has been associated with pre-harvest production by fungi that are obligate endophytes of plants, plant pathogens, or members of the flora responsible for the decay of plant materials. However, the highest concentrations of
these toxic metabolites are produced by fungi growing on post-harvest commodities that are stored under inappropriate conditions. Considering that a number of fermented foods involve a mold-ripening stage, and that some of these molds are known to be toxigenic, the safety implications with regard to fermented foods are evident (Moss, 2001).

5.3.2.1 Aflatoxins
Aflatoxins are a family of complex heterocyclic metabolites regarded as acutely toxic, carcinogenic and immunosuppressive. They have been found in a wide range of tropical and subtropical products, such as figs, pistachio and Brazil nuts, spices, peanuts and maize. The most important of these commodities used as a raw material for fermented foods is maize, but there have also been reports of low concentrations of aflatoxins in rice and wheat.

Although aflatoxins are produced by a small number of species within the genus *Aspergillus* (e.g. *Aspergillus flavus* and *Aspergillus parasiticus*), they are widespread and have several routes of contamination (Moss, 2001). Direct contamination occurs through mold spoilage of stored products or pre-harvest growth by endophytic association of an aflatoxin producer with plants such as maize or groundnuts, followed by some kind of stress on the growing crop (primarily drought); this is followed by passage through the food chain into animal products such as milk, following the consumption of contaminated feed by farm animals.

Aflatoxins in milk can resist cold storage, heat treatment or spray drying; and they are not completely degraded by the fermentation processes used in the manufacture of cheese, cream or butter (Yousef and Marth, 1989). However, they may be detoxified by the action of *Lactococcus lactis*, which is able to degrade them into harmless compounds (El-Nezami *et al*., 1998). Alternatively, aflatoxins can be removed from the gastrointestinal tract by probiotics, using strains of *Lactobacillus* and *Propionibacterium* (Ahokas *et al*., 1998); however, the most effective strategies for limiting human exposure to aflatoxins are to avoid contamination in the first place or to use a chemical process (e.g. ammoniation) to degrade them irreversibly in animal feeds (Riley and Norred, 1999).

5.3.2.2 Ochratoxin A
Ochratoxin A is produced by *Penicillium verrucosum* and by a number of *Aspergillus* species, especially *Aspergillus ochraceus*, in temperate climates. It is most common in cereals such as barley, oats, rye and wheat, but has also been found in maize, coffee, cocoa, dried vine fruits, wine and beer (Moss, 1996; Pittet, 1998). Ochratoxin A is relatively thermostable and is not destroyed by most food processes. It is nephrotoxic and possibly carcinogenic (De Groene *et al*., 1996).

Like aflatoxins, ochratoxin A can also pass through the food chain and may be found in meat products, especially pork, but does not seem to be secreted effectively in cow's milk (Krogh, 1987; Valenta and Goll, 1996). There have been several reports of its presence in meat products such as kidneys, liver and even sausages, due to transfer from animal feeds. However, its presence in moldy meat products such as smoked pork or sausages is much more serious (Kuiper-Goodman and Scott, 1989).
5.3.2.3 **Patulin**
Patulin is produced by a number of species of *Penicillium, Aspergillus* and *Byssochlamys*, but in the context of human foods, the most important species is *Penicillium expansum*. This mold is especially associated with a soft rot of apples, and the natural occurrence of patulin in commercial apple juice was reported as long ago as 1972. It is toxic to mammals, forming sarcomas (Preita et al., 1994; Moss, 2001).

It is known that patulin disappears during fermentation of apple juice to cider using the yeast *Saccharomyces cerevisiae*; but most importantly, a patulin-free cider depends on the quality of the apple juice used in its manufacture (Harwig et al., 1973; Moss, 2001).

5.3.2.4 **Fumonisins**
Fumonisins are produced by *Fusarium moniliforme* and related species within *Fusarium*. They are associated with esophageal carcinoma in humans and mostly present in maize and maize products (e.g. polenta, corn flakes and popcorn). In fact, fumonisins are remarkably widespread in corn-based products and can occur at high concentrations (Rheeder et al., 1992; Pittet, 1998; Moss, 2001).

Fumonisins are relatively stable to elevated temperatures, and survive a range of cooking, baking and frying processes (Jackson et al., 1997). They can also survive the alkaline process used in the manufacture of tortillas in Central and South America (Scott and Lawrence, 1996).

5.3.3 **Viruses**
Viruses differ significantly from bacteria because of the fact that they are obligate intraacellular parasites and must replicate exclusively within an appropriate living host cell (see Chapter 11). Thus, the source of all viruses is an infected being shedding infectious particles in its immediate environment. Transmission to another host can be direct, as in person-to-person spread (e.g. through aerosols created by vomiting), or indirect, involving some other agent as a carrier for the virus (Carter and Adams, 2001).

Foods are contaminated with viruses as a result of the distribution of fecal-derived and/or vomit-derived viruses through the environment, eventually contaminating the food or water of another potential host. This process can occur directly, as in the case of an infected food handler passing the pathogen to food immediately before consumption, or it could be the result of a long distribution process, moving virus from fecal material to supplying waters. Effectively water, either directly ingested or used as an ingredient or washing agent during food processing, is the chief vehicle for disseminating enteric viruses. It can carry viruses to plants in the fields through irrigation, and to the most significant vehicle of foodborne viral diseases, molluskan shellfish. Viruses can survive well in water, assisted by high protein, calcium and magnesium contents. These conditions are typical of sewage and sewage-contaminated waters (Carter and Adams, 2001).

There are few data related to the survival of viruses in fermented foods, which complicates the assessment of controlling foodborne viral diseases through fermentation. Nevertheless, some general inferences can be drawn (Carter and Adams, 2001). The type of food on which the fermented product is based is an important factor. Shellfish
are obvious high-risk materials because they often live in sewage-contaminated estuarine waters and concentrate available viruses in their tissues. Thus, fermented foods prepared from organisms that consume shellfish, such as octopus or scavenging crabs, may be indirectly contaminated. Vegetable produce may be contaminated on the surface (through handling, washing or spraying with contaminated water), or deep within the tissues (e.g. resulting from the uptake of viruses contaminating irrigation waters). Surface contamination can be removed by careful peeling.

5.3.4 Parasites
Typically, parasitic infections are acquired by eating food products that are either raw or incompletely cooked, or poorly preserved (see Chapter 12). Most of those infections are preventable if proper processing is applied to destroy the pathogens. However, many infections are commonly associated with eating habits that have been in practice for generations (Taylor, 2001). There are few reports of parasite-related diseases following the ingestion of fermented foods. One example is the infection with *Giardia*, a protozoan parasite, occurring as the result of consumption of contaminated cheese dip. Fermentation alone and the physicochemical conditions associated with it may not be sufficient to prevent the transmission of foodborne parasites. Thus, potentially infected material should be avoided wherever possible or, alternatively, subjected to freezing or some form of heat treatment (Taylor, 2001).

6 Food packaging

6.1 Introduction
Modified atmosphere for food packaging (MAP) includes vacuum packaging, gas flushing, and naturally respiring products, and involves the use of special permeable films, and controlled-atmosphere packaging (CAP). In CAP the product is continually exposed to a constant mixture of gases, while in gas-flushing or gas-packaging the particular gas mixture desired is flushed only once at the time of packaging into an evacuated or non-evacuated environment surrounding the food (Farber, 1991).

Oxygen, nitrogen and carbon dioxide are the three main gases used. Oxygen (O₂) will stimulate the growth of aerobic bacteria and can inhibit the growth of strictly anaerobic bacteria, although there is a very wide variation in the sensitivity of anaerobes to oxygen. Oxygen is very important in MAP meats to maintain myoglobin in its oxygenated form, oxymyoglobin, which is the form that most consumers associate with fresh red meat. Nitrogen (N₂) is an inert, tasteless gas that displays little or no antimicrobial activity. Because of its low solubility in water, the presence of N₂ in a MAP food can the prevent pack collapse that can occur when high concentrations of carbon dioxide are used. In addition, nitrogen, by displacing oxygen in the pack, can delay rancidity and also inhibit the growth of aerobic microorganisms.

Carbon dioxide (CO₂) is both water- and lipid-soluble, and is mainly responsible for the bacteriostatic effect on microorganisms in MA environments. Not only does CO₂ have biostatic activity; it is also known to have an inhibitory effect on product
respiration. Although the specific way in which CO$_2$ exerts its bacteriostatic effect is unknown, the overall effect on microorganisms is an extension of the lag phase of growth and a decrease in growth rate during the logarithmic phase (Farber, 1991). CO$_2$ acts on a bacterial cell by alteration of cell membrane function, including nutrient uptake and absorption, by the inhibition of enzymes. Penetration of bacterial membranes may lead to intracellular pH changes; and changes in the physicochemical properties of proteins (Daniels et al., 1985; Dixon and Kell, 1989). The inhibitory effects of CO$_2$ on microorganisms in a culture medium or food depend on many factors, including its partial pressure and concentration, the volume of headspace, the water activity, the type of microorganism, the microbial growth phase and the growth medium. The storage temperature of a CO$_2$-MAP product should be kept as low as possible, because the solubility of CO$_2$ decreases dramatically with increasing temperature. Thus, improper temperature control will usually eliminate the beneficial effects of an elevated CO$_2$ concentration (Farber, 1991).

CAP may be done by the introduction at packaging of a sufficient volume of gas in the package in such a way that the concentration of gas in the headspace does not change during storage. An example is the use of 100 % CO$_2$ in master packs of fresh pork or beef, where the volume ratio of CO$_2$ to the total volume of meat in the package is > 2 : 1. During storage, the gas volume decreases as CO$_2$ dissolves in the meat, but there is sufficient CO$_2$ present to maintain the CO$_2$ concentration at 100 % in the main package headspace. At appropriate storage temperatures, this approach has yielded shelf-lives of > 10 weeks for pork and beef (Jeyamkondan et al., 2000). While such systems have been successfully used for intercontinental transport of fresh beef, more common systems use CO$_2$, O$_2$ and N$_2$ alone or combined. Nitrogen oxide, sulfur dioxide and even carbon monoxide are other gases that have potential (Farber, 1991).

6.2 The use of MAP in food products

6.2.1 Red meats
Carbon dioxide, or combinations of CO$_2$ with O$_2$, can effectively extend the shelf-life of meats. The closer the temperature to 0 °C, the higher the CO$_2$ concentration and the lower the number of bacteria, the longer the shelf-life extension is (Clark and Lentz, 1969). When dealing with MAP of red meats, there are four areas of concern: the control of bacterial pathogens and spoilage microorganisms; the maintenance of meat color; the control of weight loss; and the development of meat tenderness (Farber, 1991).

Consumers relate the red color of meat to its freshness, attributing color changes to bacterial spoilage or to old animals. Color deterioration has been identified as the major limiting factor for the marketability of fresh red meats (Shay and Egan, 1987), and is known as ‘loss of bloom’ (Cross et al., 1986). The brown color is due to myoglobin oxidation to form metmyoglobin, which signals approaching staleness; however, under vacuum or anoxic atmospheres, the red color is changed due to lack of oxygen. This has no correlation with freshness or bacterial spoilage, but rather with the transition from oxymyoglobin to deoxymyoglobin (i.e. the reduction of the heme moiety of oxymyoglobin, yielding a purplish red color). Such meats will
normally ‘bloom’ to the desirable red color after 30 minutes exposure to air. Nevertheless, until consumers are educated regarding the color of meat in vacuum and MA packages, these types of packaging should not be used for the retail-ready sale of meats (Jeyamkondan et al., 2000).

*Pseudomonas, Moraxella, Psychrobacter* and *Acinetobacter* species are the main spoilage bacteria of aerobically stored, chilled, fresh red meats, and are generally inhibited by concentrations of 20 % CO2 or greater. Gram-positive microorganisms, such as *Lactobacillus* spp. and *Brochothrix thermosphacta*, are usually resistant to inhibition by CO2. Thus, a shift from an initial Gram-negative aerobic spoilage flora to a predominantly Gram-positive facultative anaerobic microflora dominated by *Lactobacillus* spp. occurs in meat during MA storage or MAP (Newton and Gill, 1978; Holley et al., 2003). Lactic acid bacteria are capable of continuously pumping out CO2 from inside the cells to the environment, thereby maintaining metabolic balance. Because this process is rather energy consuming, the growth rate of lactic acid bacteria is fairly low (Jeyamkondan et al., 2000) and the by-products of the metabolism of lactobacilli are inoffensive compared to the typical proteolytic spoilage odors produced by *Pseudomonas* spp. Depending on initial levels of sanitation, facultative Gram-negative organisms such as *Aeromonas* or *Shewanella* may be problematic in MAP-stored fresh meats (Newton and Gill, 1978; Holley et al., 2004).

Potential pathogens in meat stored under anaerobic conditions and at low temperatures are *Yersinia enterocolitica, Listeria monocytogenes* and *Aeromonas hydrophila*, which can grow at low temperatures. However, when 100 % CO2 and a storage temperature of −1.5 °C are simultaneously applied according to the hurdle concept, none of these pathogens can grow (Farber, 1991).

A study on the behavior of *L. monocytogenes* and *L. innocua* in raw minced beef packaged under MA was carried out by Franco-Abuín et al. (1997). Three gas atmospheres were tested with various CO2 concentrations: 100 % CO2, 65 % CO2, 25 % O2, 10 % N2, and 20 % CO2 with 80 % O2. The 100 % CO2 atmosphere was the most effective for the inhibition of growth of both species. Microbial inhibition was influenced by pH, but low pH values were not the most important factor in the inhibition of *Listeria*; instead, it was the direct effect of CO2. Water activity values did not change during storage, and none of the gas mixtures was bactericidal.

Tsigarida et al. (2000) studied the effect of aerobic, MAP (40 % CO2, 30 % N2, 30 % O2) and vacuum packaging (VP) on the growth and survival of *L. monocytogenes* on sterile and naturally contaminated beef fillets in relation to film permeability and oregano essential oil. The dominant microorganisms and the effect of endogenous flora on the growth and survival of *L. monocytogenes* were dependent on the type of packaging film. The pathogen increased whenever *Pseudomonas* sp. dominated, that is, aerobic storage and MAP/VP in O2 high-permeability film, suggesting that this spoilage group might be able to enhance the growth of *Listeria*. *B. thermosphacta* constituted the major proportion of the total microflora in MAP/VP. Within the O2 low-permeability film, no growth of *L. monocytogenes* was detected. The addition of 0.8 % (v/w) oregano essential oil resulted in an initial reduction of 2–3 log cycles of the majority of the bacterial population. Lactic acid bacteria and *L. monocytogenes* showed the most extensive decrease in all gaseous environments, but there was limited growth of *L. monocytogenes* in MAP/VP regardless of film O2 permeability.
The growth and virulence of pathogenic *Yersinia enterocolitica* were investigated by Bodnaruk and Draughon (1998) on high (pH > 6.0) and normal (pH < 5.8) pH pork packaged in MA and stored at 4 °C. MAs used in the study were vacuum packaging and saturated CO₂. Pork was packaged in a high gas-barrier packaging film and examined over a 30-day period. Numbers of *Y. enterocolitica* on the lean surface of high pH pork slices increased by about 2.7 log CFU/cm² when vacuum packaged and stored at 4 °C for 30 days. Storage of inoculated normal-pH pork in 100 % CO₂ resulted in *Y. enterocolitica* remaining in the lag phase over the storage period. Virulence of the pathogen was maintained in 25–35 % of isolates following storage for 30 days at 4 °C in vacuum- and CO₂-packaged meats, and was not affected by pH.

### 6.2.2 Poultry

The organisms most often associated with foodborne diseases involving poultry include *Salmonella* spp., *Staphylococcus aureus*, *Clostridium perfringens*, *Bacillus cereus* and *Campylobacter*. *Campylobacter jejuni* and *Salmonella* spp. (which may be able to survive in a MAP product) and *L. monocytogenes* and *A. hydrophila* may, because of the extended storage lives of the MAP products, have additional time to grow to potentially high numbers (Farber, 1991). Although *C. perfringens* may be able to survive better in some MA as compared to air, it would not be able to grow at the chill temperatures commonly used for MAP products. Thus, it would not be much of a health hazard in a MAP product unless the product was temperature abused (Labbe, 1989). On the contrary, *Campylobacter* is a cause for constant concern because, although not a psychrotroph, the infective dose is low.

To determine the role of packaging and storage conditions on the survival of *Campylobacter jejuni* on chicken, the virulent strain *C. jejuni* 81116 was inoculated onto chicken skin pieces and stored at different temperatures and under various packaging conditions (air, N₂, CO₂, and VP). *C. jejuni* remained viable at −20 °C and −70 °C. The pathogen could also withstand repeated freeze–thaw cycles. The importance of packing *C. jejuni*-free chicken was stressed (Lee *et al.*, 1998).

### 6.2.3 Seafood and fish

Seafood and fish, unlike other muscle foods, are very susceptible to both microbiological and chemical deterioration. The major spoilage organisms found on spoiled seafood and fish include *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Flavobacterium*, *Photobacterium*, *Aeromonas*, *Shewanella*, *Carnobacterium* and *Cytophaga* species.

*L. monocytogenes* has been identified as a significant contaminant in smoked fish, particularly salmon. For example, Domínguez *et al.* (2001) analyzed about 170 samples of smoked fish and 182 samples of pâté for sale in retail outlets and supermarkets in the nine provinces of Castille and León (Spain) for the prevalence of this pathogen and other *Listeria* spp. *L. monocytogenes* was isolated from 22.3 % of the smoked fish samples and 5.4 % of the pâté samples. Of these, 52 % of the former but only 10 % of the latter contained *L. monocytogenes* at > 100 CFU/g. According to Rørvik (2000), raw salmon is not an important source of *L. monocytogenes*, and contamination of smoked salmon occurs during processing and storage. Thus, the main issue for producers is to prevent colonization of the processing environment...
and spread of the bacteria to products. This should be achieved by the systematic implementation of Good Manufacturing Practices, together with HACCP.

From a microbiological safety standpoint, the organism of greatest concern when dealing with MAP seafood and fish products is the non-proteolytic *C. botulinum* type E. The restricted growth of the normal spoilage bacteria by MAP may actually enhance the proliferation of *C. botulinum*. In fact, *C. botulinum* type E is a natural seafood and fish contaminant that can grow at temperatures as low as 3.3 °C (Farber, 1991). Particularly critical are seafood and fish products that are eaten without terminal heating— for example, cooked peeled shrimp and smoked salmon. The health risks associated with eating MAP smoked fish products may be high because of the general contamination of fish with botulinal spores that can survive smoking, fewer competing microorganisms, and consumption without further cooking. However, spoilage and/or safety problems may be regarded as the result of insufficient salting, gross temperature abuse or heavy initial contamination with botulinal spores (Hauschild, 1989).

Cai *et al.* (1997) investigated the production of toxins by *C. botulinum* type E in MA-packaged channel catfish. Samples of catfish were inoculated with 3–4 log spores/g of a mixed pool of four strains of the pathogen, and packaged with an oxygen-barrier bag with a MA of 80% CO₂, 20% N₂, or in a master bag with the same MA. Packaged fish were stored at either 4 °C and sampled at intervals over 30 days or at 10 °C and sampled at intervals over 12 days. Under abusive storage conditions of 10 °C, *C. botulinum* toxin was detected on fish from each package type by day 6. At 4 °C, toxin production was detected by day 18. No toxin was found in the master bags held continuously at 4 °C. Spoilage preceded toxin production for samples stored at 4 °C for each type of packaging, whereas at 10 °C spoilage and toxin production coincided.

The growth and toxigenesis of *C. botulinum* type E in vacuum-packaged, unprocessed, raw, pickled or cold-smoked rainbow trout stored at slightly abusive temperatures were studied by Hyytia *et al.* (1999). In unprocessed fish there was only a 2-log increase in type E cell numbers at the time the toxicity first occurred after 2 weeks of storage at 8 °C. Neither growth nor toxin production was observed in raw pickled fish with a NaCl concentration of 6.7% (w/v) during 6 weeks of storage at 6 °C. In cold-smoked fish with a NaCl level of 3.2% (w/v), toxic samples were detected after 3 and 4 weeks of storage at 8 °C and 4 °C, respectively, with no increase in type E cell count.

Challenge studies were carried out by Lyver *et al.* (1998) to evaluate the safety of raw and cooked seafood nuggets inoculated with 10³ CFU/g of *L. monocytogenes*. Nuggets were packaged in air or 100% CO₂, with and without an oxygen-absorbent agent, and stored at 4 °C or 12 °C. Headspace O₂ decreased to < 1% (v/v) in most samples, while headspace CO₂ ranged between 1% and 100%, depending on the packaging conditions and storage temperature. Most products maintained an acceptable appearance throughout storage, but nuggets stored at 12 °C developed sharp, acidic odors by day 28. *Bacillus* spp. and lactic acid bacteria numbers increased to 10⁵ and 10⁷ CFU/g respectively in raw nuggets, while only *Bacillus* spp. reached 10⁴ CFU/g in cooked nuggets by 28 days. With the exception of nuggets packaged in 100% CO₂ with or without an absorbent, numbers of *L. monocytogenes* increased to approximately 10⁷ CFU/g in the uncooked product stored at both 4 °C and 12 °C after 28 days.
6.2.4 Vegetables

With the increased popularity of fresh produce in Europe and North America over the last 25 years, there has been a parallel, significant increase in the number of incidents and cases of foodborne illness from fresh fruit, juice, and vegetable consumption. Vehicles have included tomatoes, strawberries, raspberries, lettuce, sprouts (alfalfa and other species and varieties), parsley, basil, apple cider, orange juice, cantaloupe, watermelon and salads (potato, pea, garden fruit). Organisms implicated have included viruses (hepatitis A), protozoan parasites (*Cyclospora*, *Cryptosporidium*, and *Giardia*), and bacteria (*Salmonella* spp., *E. coli O157:H7*, other enterotoxigenic *E. coli*, *Shigella*, *C. botulinum*, and *Listeria*). The change in frequency of illness from these sources has been so substantial that there are now almost as many outbreaks of foodborne illness from these products as there are from meat products. Many of these incidents arise from the use of poor-quality water during irrigation or washing after harvest, and from the growth of pathogens on cut produce surfaces when offered for retail sale without appropriate refrigeration. Of continuing concern is the growth of pathogens on produce with an extended shelf-life under MAP (Guan and Holley, 2003).

Demand for fresh, convenient, minimally processed vegetables has led to an increase in the quantity and variety of products available to the consumer. MAP, in combination with refrigeration, is increasingly being employed to ensure the quality and shelf-life of ready-to-use vegetables. The nature of these products and the storage conditions have presented microorganisms with new vehicles. Psychrophilic pathogens and those capable of maintaining infectious potential are of particular concern. *L. monocytogenes*, *A. hydrophila*, *C. botulinum*, *Salmonella* spp. and *Shigella* spp. are among the main ones with respect to vegetables (Francis et al., 1999).

The effect of initial head spaces of air and 5% CO₂, 95% N₂ on the microflora of tomato salad (i.e. lactic acid bacteria, pseudomonads and yeasts) was studied at 4 °C and 10 °C by Drosinos et al. (2000). Lactic acid bacteria were the predominant organisms in all samples. The pH dropped during storage, particularly at 10 °C. The concentration of different organic acids, such as lactic, acetic, formic and propionic acids, increased in all samples stored under MAP conditions at both temperatures. The spoilage of tomatoes stored under 5% CO₂, 95% N₂ was delayed, as indicated by changes in texture, color and odor compared with those samples stored in air. When the salad was inoculated with *S. Enteriditis*, the pathogen survived at both temperatures but did not grow regardless of the packaging system used.

Francis and O’Beirne (1998) used a solid-surface model system to study the effects of gas atmospheres encountered in MAP of minimally processed lettuce on the survival and growth of *L. monocytogenes* and competing microorganisms. The effects of increasing CO₂ levels from 5% to 20%, of 3% O₂, and of 80–95% N₂ were determined. CO₂ concentrations of 5–10% had no inhibitory effect on pure cultures of *L. monocytogenes*. Growth and inhibitory activities against *Listeria* or *Enterobacter cloacae* and *E. agglomerans* were inversely related to the concentration of CO₂. In contrast, the growth and anti-listerial activities of *Leuconostoc citreum* increased with elevated CO₂ concentrations. In the low O₂ atmosphere, *L. monocytogenes* grew considerably better in the presence of indigenous microflora of lettuce than when in
pure culture. These results indicated that the gas atmospheres present within MA packages of minimally processed vegetables might affect the interactions between the pathogen and the natural competitive microflora sufficiently to enhance \textit{L. monocytogenes} growth.

González-Fandos et al. (2001) studied the potential of \textit{L. monocytogenes} to grow in mushrooms packaged in perforated and non-perforated polyvinylchloride (PVC) films and stored at 4 °C or 10 °C. CO\textsubscript{2} and O\textsubscript{2} contents inside the package, aerobic mesophiles, psychrotrophs, \textit{Pseudomonas} spp., fecal coliforms, anaerobic spores and \textit{L. monocytogenes} were determined. Mushrooms packaged in non-perforated film and stored at 4 °C had the most desirable quality parameters (texture, development stage, and absence of fungi). \textit{L. monocytogenes} was able to grow at 4 °C and 10 °C in inoculated mushrooms packaged in perforated and non-perforated films, between 1 and 2 log units during the first 48 hours. After 10 days of storage, populations of \textit{L. monocytogenes} were higher in mushrooms packaged in non-perforated films stored at 10 °C. It was concluded that MAP followed by storage at 4 °C or 10 °C extended shelf-life by maintaining an acceptable appearance, but allowed the growth and survival of \textit{L. monocytogenes}.

The survival of \textit{C. botulinum} in MA-packaged, fresh, whole ginseng roots was investigated by Macura et al. (2001). Ginseng roots were packaged in medium-barrier O\textsubscript{2} transmission films in air. Anaerobic conditions developed during 2 °C, 10 °C and 21 °C storage, but most rapidly at the elevated temperatures. \textit{C. botulinum} challenge tests were performed for 10 °C and 21 °C samples. At 10 °C, the botulism toxin was recorded after 14 weeks of storage, before all product was spoiled and rendered unfit for human consumption. At 21 °C, the product spoiled before it came toxic. The authors recommended that commercial production of MAP fresh ginseng should not be contemplated until the safety of the packaged product can be ensured.

The survival and growth of \textit{E. coli} O157:H7 and \textit{L. monocytogenes} during storage at 4 °C and 8 °C on ready-to-use (RTU) packaged vegetables (lettuce, rutabaga, dry coleslaw mix, soybean sprouts) were studied by Francis and O’Beirne (2001). The vegetables were sealed with oriented polypropylene packaging film, and MA was developed in packs during storage due to produce respiration. Survival and growth patterns were dependent on vegetable type, package atmosphere, storage temperature and bacterial strain. \textit{E. coli} O157:H7 generally survived and grew better than \textit{L. monocytogenes}. Storage at 4 °C enabled survival of both pathogens on all products throughout the storage period.

During the last 20 years, an ever-increasing demand for RTU vegetables has led to a continuous growth in the quantity and diversity of products available to the consumer. Mild preservation technology, particularly refrigeration and MAP, is increasingly being relied upon to ensure the safety and quality of RTU vegetables. Such technology has resulted in the increased significance of psychrotrophic and facultative anaerobic pathogens, including \textit{L. monocytogenes}, \textit{A. hydrophila}, and \textit{C. botulinum}. The emergence of such organisms, combined with continual product evolution, presents numerous questions with regard to the microbial safety of these products (Francis et al., 1999). The importance of strict temperature control from process to consumption is assumed by default. Refrigerated temperatures must be maintained during transportation, distribution, storage and handling in
supermarkets and by consumers. It is essential that contamination of produce be minimized through the use of good agricultural and strict hygiene practices, and that HACCP programs specific for the pathogen of concern be applied at all stages of production (Francis and O’Beirne, 2001).

### 6.3 Bacteriocins in packaging films

Examples of bacteriocin-coated surfaces include polyethylene films coated with nisin/methyl cellulose, nisin-coated poultry, and adsorption of nisin onto polyethylene, ethylene vinyl acetate, polypropylene, polyamide, polyester, acrylics and polyvinyl chloride (Appendini and Hotchkins, 2002). Bower et al. (1995) demonstrated that nisin adsorbed onto silanized silica surfaces inhibited the growth of *L. monocytogenes*. Nisin films were exposed to medium containing *L. monocytogenes*, and the contacting surfaces were evaluated at 4-hour intervals for 12 hours. Cells on surfaces that had been in contact with a high concentration of nisin (40000 IU/ml) exhibited no signs of growth, and many of them displayed evidence of cellular deterioration. Surfaces contacted with a 10-fold lower concentration of nisin (4000 IU/ml) had a smaller degree of inhibition. In contrast, surfaces contacted with films coated with heat-inactivated nisin allowed growth of *L. monocytogenes*.

### 7 Control of microorganisms by chemical antimicrobials

#### 7.1 Introduction

Food antimicrobial agents are ‘chemical compounds in foods that retard microbial growth or kill microorganisms, thereby resisting deterioration in safety and quality’. Most of the agents are only bacteriostatic or fungistatic, not bactericidal or fungicidal, and they will not preserve food indefinitely. Depending upon storage conditions, the food product eventually spoils or becomes hazardous. Thus, food antimicrobials are typically used in combination with other food preservation procedures, based on the hurdle concept discussed in section 4 (Davidson, 2001).

Food antimicrobials are sometimes called preservatives. However, this term often includes antioxidants and antibrowning agents in addition to antimicrobials. *Antimicrobials* is therefore a more specific term (Davidson and Harrison, 2002). In this chapter, the term ‘preservative’ is restricted to chemical preservatives obtained by synthesis. Classification of antimicrobials is often arbitrary, and here we have differentiated between chemical and natural antimicrobials. The first group comprises those agents that are either inorganic or produced by synthetic means, whereas the second group includes compounds that occur naturally and can be extracted from natural products. Nevertheless, some natural antimicrobials are now produced commercially by chemical synthesis. Cleaners and sanitizers used to remove dirt and to eliminate pathogenic and spoiling organisms from food facilities and equipment are also discussed under chemical antimicrobials.
7.2 Chemical preservatives

7.2.1 Nitrites
Sodium nitrite (NaNO₂) and potassium nitrite (KNO₂) have a specialized use in cured meat products. In fact, nitrites have other functions in cured meat in addition to serving as antimicrobial agents. As nitric oxide, they react with the meat pigment myoglobin to form the characteristic cured meat color, nitrosomyoglobin. They also contribute to flavor and texture and serve as antioxidants (Davidson, 2001). At one time, sodium nitrate (NaNO₃) and potassium nitrate (KNO₃) were used extensively; later, it was discovered that nitrates are converted into nitrites and that the latter are the effective antimicrobial agents (Gould, 2000). Nevertheless, nitrates are added to fermented dry sausage batter to serve as a reservoir for nitrite formation by bacterial reduction during the lengthy curing process.

The primary use of nitrites is to inhibit *Clostridium botulinum* growth and toxin production in cured meats. Essentially, nitrites inhibit outgrowth of the germinated spore, as only very high, unusable nitrite concentrations would significantly inhibit spore germination itself (Duncan and Foster, 1968). Nitrites are more inhibitory under anaerobic conditions. Ascorbate and isoascorbate enhance the antibotulinial action, probably by acting as reducing agents (Roberts et al., 1991). In addition, these compounds are important as inhibitors of nitrosoamine formation. Nitrosoamines are carcinogenic agents resulting from the reaction of nitrites with secondary or tertiary amines (Tompkin, 1993).

Nitrites have effects on microorganisms other than *C. botulinum*. They have proven to have inhibitory effects against *C. perfringens*, *E. coli* O157:H7, *Listeria monocytogenes*, *Achromobacter*, *Enterobacter*, *Flavobacterium*, *Micrococcus* and *Pseudomonas* (Tarr, 1941; Gibson and Roberts, 1986a, 1986b; Pelroy et al., 1994; Tsai and Chou, 1996). Certain strains of *Salmonella*, *Bacillus* and *Clostridium* are resistant (Perigo and Roberts, 1968; Rice and Pierson, 1982).

The antimicrobial action of nitrites on *C. botulinum* was elucidated by Woods et al. (1981) and Woods and Wood (1982), who showed that nitrites cause a reduction in intracellular ATP and excretion of pyruvate, thus inhibiting oxidative phosphorylation. A number of enzymes have been identified as being inhibited by nitrites, namely ferredoxin, oxido-reductase and pyruvate decarboxylase (Carpenter et al., 1987; McMindes and Siedler, 1988; Tompkin, 1993). The mechanism of inhibition of non-spore-forming microorganisms may be different from that of spore-formers. Rowe et al. (1979) reported that nitrites are capable of blocking active transport, oxygen uptake and oxidative phosphorylation by oxidizing ferrous iron of the electron carrier, cytochrome oxidase.

7.2.2 Sulfites
Salts of sulfur dioxide include potassium sulfite and sodium sulfite (K₂SO₃ and Na₂SO₃), potassium bisulfite and sodium bisulfite (KH₂SO₃ and NaHSO₃), and potassium metabisulfite and sodium metabisulfite (K₂S₂O₅ and Na₂S₂O₅). As antimicrobials, sulfites are used primarily in fruit and vegetable products to control three groups of microorganisms: spoilage and fermentative yeasts and molds, acetic acid bacteria, and malolactic bacteria.
In addition, they act as antioxidants and clarifiers, and inhibit enzymatic and non-enzymatic browning of foods (Ough, 1993). In some countries, sulfites are used in fresh meats and meat products. For example, they are effective in delaying the growth of molds, yeasts and salmonellae in sausages during storage at refrigerated or room temperature (Banks and Board, 1982).

The most important factor influencing the antimicrobial activity of sulfites is pH. As pH decreases, the proportion of $\text{SO}_2\text{H}_2\text{O}$ increases and the bisulfite ($\text{HSO}_3^-$) ion concentration decreases; the latter has no antimicrobial activity (King et al., 1981; Gould and Russell, 1991). Because of their extreme reactivity, it is difficult to pinpoint the exact antimicrobial mechanism for sulfites.

### 7.2.3 Phosphates

Phosphates have many important uses in food processing, including pH stabilization, acidification, alkalinization, sequestration or precipitation of metals, formation of complexes with organic polyelectrolytes (e.g. protein, pectin and starch), defloculation, dispersion, peptization, emulsification, nutrient supplementation, anticaking, and leavening (Ellinger, 1972). Some phosphate salts, including sodium acid pyrophosphate (SAPP), tetrasodium pyrophosphate (TSPP), sodium tripolyphosphate (STPP), sodium hexametaphosphate (SHMP) and trisodium phosphate (TSP), have variable levels of antimicrobial activity in foods (Shelef and Seiter, 1993).

Gram-positive bacteria are generally more susceptible to phosphates than are Gram-negatives. Bacteria inhibited by phosphates include *L. monocytogenes*, *Staphylococcus aureus*, *Bacillus subtilis* and *Clostridium sporogenes* (Kelch and Bühlmann, 1958; Jen and Shelef, 1986; Zaika and Kim, 1993; Lee et al., 1994). Post et al. (1968) preserved cherries against the fungal growth of *Penicillium*, *Rhizopus* and *Botrytis* using STPP. TSP is used as a sanitizer in chill water for raw poultry, particularly against *Salmonella* spp. (Davidson, 2001).

Several mechanisms have been suggested for bacterial inhibition by polyphosphates. Their ability to chelate metal ions appears to play an important role in their antimicrobial activity (Sofos, 1986). Maier et al. (1999) demonstrated that inhibition of *Bacillus cereus* by sodium polyphosphates is related to the chelation of divalent cations (Mg$^{++}$ and Ca$^{++}$), which inhibits cell division by blocking cell septation. Knabel et al. (1991) reported that antimicrobial activity of polyphosphates is reduced at lower pH, owing to protonation of the chelating sites. They concluded that polyphosphates inhibited Gram-positive bacteria and fungi by removal of essential cations from binding sites on their cell walls.

### 7.2.4 Parabens

Parabens are alkyl esters of *p*-hydroxybenzoic acid. Esterification of the carboxyl group of benzoic acid allows the molecule to remain undissociated up to pH 8.5, giving the parabens an effective range of pH from 3.0 to 8.0. In most countries, the methyl, propyl and heptyl parabens are allowed for direct addition to foods as antimicrobial agents, whereas the ethyl and butyl esters are also approved in some countries. Generally speaking, the antimicrobial activity of parabens is inversely proportional to the chain length of the alkyl component. As the alkyl chain length increases,
inhibitory activity generally increases. Increasing activity with decreasing polarity is more evident against Gram-positive than Gram-negative bacteria. Parabens are considered to be more active against yeasts and molds than against bacteria (Aalto et al., 1953; Davidson, 2001).

Parabens act primarily on the cytoplasmic membrane, causing structural damage that results in leakage of metabolites. This effect is proportional to the alkyl chain length. It has also been reported that parabens inhibit nutrient uptake through the membrane by blocking the membrane transport system (Judis, 1963; Furr and Russell, 1972; Freese et al., 1973).

7.2.5 Phenolic antioxidants

These compounds are used in foods primarily to delay oxidation of unsaturated lipids by interrupting the free radical chain mechanism of hydroperoxide formation during the autoxidation process (Davidson, 2001). However, they have also been shown to possess antimicrobial activity against a wide range of microorganisms – not only bacteria, yeasts and molds, but also viruses and protozoa. The most important members of this group are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and t-butylhydroxyquinoline (TBHQ) (Jay, 1996).

In general, phenolic antioxidants are more effective against Gram-positive than Gram-negative bacteria. TBHQ has been reported as an extremely effective inhibitor of *L. monocytogenes* and *S. aureus*. BHA is a more effective antifungal agent than BHT or TBHQ, acting against *Aspergillus flavus*, *Aspergillus parasiticus*, *Saccharomyces cerevisiae*, *Geotrichum* and *Penicillium* spp. Their mechanism of action is similar to that of parabens, as they are chemically related (Davidson and Doan, 1993).

7.3 Cleaners

7.3.1 Cleaning media

Water is the standard cleaning medium. Air is an alternative for the removal of packaging material, dust and other debris where water is not acceptable, and solvents may be used for the removal of lubricants and other similar petroleum-derived products. The major functions of water as a cleaning medium have been reviewed by Marriot (1999).

7.3.2 Classification of cleaning compounds

The essential functions of a cleaning compound are to ‘lower the surface tension of water so that soils may be dislodged and loosened, and to suspend soil particles for subsequent flushing away’. To complete the cleaning process, a sanitizer is applied to destroy residual microorganisms that are exposed as a result of the cleaning process. Most cleaning compounds that are used in the food industry are blends – in other words, ingredients are combined to produce a single product with specific characteristics that performs a given function for one or more cleaning applications (Marriott, 1999).

7.3.2.1 Soaps

Soaps are created by the reaction of an alkali with a fatty acid, and include either alkaline salts or carboxylic acids (Marriott, 1999).
7.3.2.2 Alkaline compounds
Alkaline compounds are divided into three simple classes: strong, heavy-duty, and mild. Strong alkaline cleaners have strong dissolving power and are very corrosive. They are used to remove heavy soils, but have little effect on mineral deposits. Examples are sodium hydroxide (caustic soda) and silicates having a high N₂O:SiO₂ ratio. Heavy-duty alkaline cleaners have moderate dissolving power and are slightly corrosive. Examples include sodium metasilicate (a good buffering agent), sodium hexametaphosphate, sodium pyrophosphate, sodium carbonate and tri-sodium phosphate (a good soil emulsifier). They are excellent for removing fats, but have no value for mineral deposit control. Mild alkaline cleaners are used for manual cleaning of lightly soiled areas. Examples are sodium bicarbonate, sodium sesquicarbonate, tetrasodium pyrophosphate, phosphate water conditioners (sequesters) and alkyl aryl sulfonates (surfactants). They have good water-softening capabilities, but are of no value for mineral deposit control (Marriott, 1999).

7.3.2.3 Acid compounds
Acid compounds are used for removing encrusted surface materials and dissolving mineral scale deposits. Organic acids such as citric, tartaric and gluconic are also excellent water softeners, rinse easily, and are mostly harmless. Inorganic acids are less used because they are corrosive and/or irritating to the skin. Two classes of acid cleaning compounds are distinguished: strong and mild. Strong acid cleaners are corrosive to concrete, metals and fabrics. When heated, they produce toxic gases. Examples are hydrochloric, hydrofluoric, sulfamic, sulfuric and phosphoric acids. They are mostly used to remove the encrusted surface matter and mineral scale frequently found on steam-producing equipment, boilers and some processing equipment. Mild acid cleaners are mildly corrosive. Examples are levulinic, hydroxyacetic, acetic, and gluconic acids, used primarily as water softeners (Marriott, 1999).

7.3.2.4 Active-chlorine cleaners
Active-chlorine cleaners containing active chlorine, such as sodium or potassium hypochlorite, are effective in the removal of carbohydrate and/or proteinaceous soils because they aggressively attack such materials and chemically modify them. Thus, active-chlorine products are especially valuable for cleaning surfaces containing starch and/or protein. Hypochlorites should be applied soon after they are made up, as they lack stability during storage (Wyman, 1996).

7.3.2.5 Synthetic detergents
Synthetic detergents are as good emulsifiers as soaps. They lower surface tension, promote wetting of particles, and deflocculate and suspend soil particles. Wetting agents are divided into three major categories: cationic, anionic and non-ionic. Anionic compounds are the most commonly used wetting agents because of their compatibility with alkaline cleaning agents and their good wettabiliy properties. Examples are sulfated alcohols, olefins, oils, monoglycerides and amides, alkyl-aryl polyether sulfates, alkyl sulfonates and heterocyclic sulfonates. Because of their neutrality, non-ionic agents are effective under both acidic and alkaline conditions.
They are not affected by water hardness, but they tend to foam. Examples are thioethics, pluronic, amine fatty acid condensates, alkyl-aryl polyether alcohols and ethylene oxide fatty alcohol condensates. In general, synthetic agents are non-corrosive, non-irritating and easily rinsed from equipment and other surfaces (Anonymous, 1976).

7.3.2.6 Enzyme-based cleaners
Enzyme-based cleaners are proteases and work best under alkaline conditions. They are not as effective on all types of soils as are chlorine compounds (Marriott, 1999).

7.3.2.7 Cleaning auxiliaries
Auxiliary compounds protect sensitive surfaces or improve the cleaning properties of a compound. There are two main categories: sequestrants and surfactants. Sequestrants can chelate by complexing with magnesium and calcium ions. This action effectively reduces water hardness. Sequestrants consist of polyphosphates or organic amine derivatives, the latter being considered as generally more effective agents. Commercially, most organic agents are salts of ethylene-diamine-tetracetic acid (EDTA). Surfactants are surface-active agents that function to facilitate the transport of cleaning and sanitizing compounds over the surface to be cleaned. They are classified according to their net charge – that is, anionic (e.g. linear alkyl-benzene sulfonates), cationic (e.g. quaternary ammonium salts) and non-ionic (ethylene oxide derivatives, alkanol-amides and amine oxides). Important physicochemical characteristics of surfactants were summarized by Marriott (1999).

7.4 Sanitizers
Sanitizers, as defined by the US Environmental Protection Agency, are ‘pesticide products that are intended to disinfect or sanitize, reducing or mitigating growth or development of microbiological organisms including bacteria, fungi, or viruses on inanimate surfaces in the household, institutional, and/or commercial environments’ (CFR, 2001). Soil that remains on food-processing equipment after use is typically contaminated with microorganisms nourished by the nutrients of the soil deposits. A sanitary environment is obtained by thoroughly removing soil deposits with an appropriate cleaning solution and subsequently applying a sanitizer to destroy residual microorganisms.

7.4.1 Thermal sanitation
The two major sources for thermal sterilization are steam and hot water, but in general the process is rather inefficient because of high energy costs (Marriott, 1999).

7.4.2 Radiation sanitation
Ultraviolet (UV) light or high-energy cathode or gamma rays will destroy microorganisms. However, the methods of sanitizing have been restricted to certain commodities and are not really useful in food plants and food service operations because of limited total effectiveness, equipment cost and operator safety issues. In the case of UV, light rays must actually strike the microorganisms, and the latter can
be protected by soil itself. In addition, light can be absorbed by dust, thin films of grease and opaque or turbid solutions, thus reducing killing power (Marriott, 1999). UV systems have potential, but require cleaned surfaces for effective use.

7.4.3 Chemical sanitation
The ideal sanitizer possesses important properties: broad-spectrum microbial destruction; resistance to factors such as soil load, detergent and soap residues, and water hardness; non-toxic and non-irritating properties; water solubility; acceptable odor or no odor; stability in solutions; ease of use; ready availability; low cost; and ease of measurement for preparation of solutions. The efficacy of chemical sanitizers depends on a number of physicochemical factors, as discussed by LeChevallier et al. (1988) and Marriott (1999).

7.4.4 Classification of sanitizers
7.4.4.1 Chlorine compounds
The main chemicals in this category are liquid chlorine, hypochlorites, inorganic chloramines and organic chloramines. When liquid chlorine (Cl₂) and hypochlorites are mixed with water, they hydrolyze to form hypochlorous acid (HOCl). This compound dissociates to form H⁺ and OCl⁻. Chlorine compounds are more effective antimicrobial agents at a low pH; the hypochlorite ion, which is not as effective as a bactericide, predominates at higher pH values. Another chlorine compound, chlorine dioxide (ClO₂), does not hydrolyze in aqueous solutions. ClO₂ is particularly suitable for sewage treatment because it is hardly affected by pH or organic matter (Marriott, 1999).

The mode of action of chlorine as an antimicrobial agent has not been fully determined. It appears to kill through inhibition of the glucose oxidation metabolic pathway and by oxidation of sulfhydryl groups of certain enzymes. However, other modes of action have also been proposed, including the disruption of protein synthesis, deleterious reactions with nucleic acids, inhibition of oxygen uptake, and oxidative phosphorylation coupled with leakage of some macromolecules, and promotion of chromosomal aberrations. Chlorine impairs membrane function, especially transport of extracellular nutrients. Chlorine is also known for stimulating spore germination and subsequently inactivating the germinated spore. Vegetative cells are more easily destroyed than are Clostridium spores, which in turn are killed more easily than Bacillus spores. Chlorine concentrations ≤ 50 ppm lack antimicrobial activity against L. monocytogenes, but higher concentrations effectively destroy this pathogen. This lethal effect is enhanced by increasing temperature; up to 52 °C, the reaction rate doubles for each 10 °C increase (Kulikkoosky et al., 1975; Camper and McFetters, 1979; Marriott, 1999; Meinhold, 1991).

7.4.4.2 Bromine and iodine compounds
Bromines have been used primarily for water treatment, either alone or in combination with other compounds. There is a synergistic effect when bromine and chlorine compounds are combined (Marriott, 1999).

The major iodine compounds used for sanitizing are iodophors (formed by complexing elemental iodine with surfactants such as nonyl phenolethylene oxide),
alcohol–iodine solutions, and aqueous iodine solutions. Iodophors are the most important agents: they are used in water treatment, disinfection of equipment and surfaces, and as a skin antiseptic (Marriott, 1999). The mode of antibacterial action of iodine compounds is not fully understood. It appears that diatomic iodine (I₂) is the major agent, able to disrupt bonds that hold cell proteins together and to inhibit protein synthesis, but free elemental iodine and hypoiodous acid (HOI) have also been identified as possessing antimicrobial properties. The iodophor complex releases an intermediate triciodide ion that rapidly converts to diatomic iodine and hypoiodous acid at low pH (Anonymous, 1996).

Iodine-type sanitizers are somewhat more stable in the presence of organic matter than are the chlorine compounds. They are as active in the deactivation of vegetative cells as chlorine agents, but not as effective in spore inactivation. In a concentrated form, their shelf-life is long. However, in solution, iodine may be lost due to sublimation – especially at temperatures above 50 °C. Because they are acidic, iodine compounds are not affected by hard water and will prevent accumulation of minerals if used regularly. Iodine compounds cost more than chlorines and may cause off-flavors in some products. In addition, they tend to be very sensitive to pH changes (Marriott, 1999).

### 7.4.4.3 Quaternary ammonium compounds

These sanitizers, commonly called the ‘quats’, are ammonium compounds in which four organic groups are linked to a positively charged nitrogen atom. Typically, the organic radical is the cation, and either chloride or bromide the anion. Examples are alkyl-dimethyl-benzyl ammonium chloride and methyl-dodecylbenzyl-trimethyl ammonium chloride. Their mechanism of germicidal action is not fully understood, but it may be that the surface-active quat surrounds and covers the cell outer membrane, causing enzyme inhibition and leakage of internal constituents. Quats are essentially bacteriostatic, not bactericidal. They are used most frequently on floors, walls, furnishings and equipment. Being good penetrants, they have value for porous surfaces (Frank and Chmielewski, 1997; Marriott, 1999). The major advantages of quaternary ammonium compounds have been summarized (Anonymous, 1997).

### 7.4.4.4 Acid sanitizers

Acid sanitizers are frequently associated with cleaners. The acid neutralizes excess alkalinity that remains from the cleaning compound, prevents formation of alkaline deposits, and sanitizes. They act by penetrating and disrupting cell membranes and acidifying the cytoplasm. These compounds are especially effective on stainless steel surfaces or where contact time may be extended, and have a high antimicrobial activity against psychrotrophic organisms, yeasts and viruses (Marriott, 1999).

Organic acids, such as acetic, peroxyacetic, lactic, propionic and formic, are most frequently used. A pH value ≤ 3.0 is ideal for their performance. They can be applied by clean-in-place (CIP) methods, by spray or foaming. All cleaning compounds need to be thoroughly rinsed away before these sanitizers are applied, as they can lose all of their effectiveness in the presence of cationic surfactants (Marriott, 1999).

Carboxylic acids as sanitizers are assorted mixtures of free fatty acids, sulfonated fatty acids and other organic acids. They have a broad range of bactericidal activity,
and are non-corrosive, cost-effective, and stable in dilutions both in the presence of organic matter and at high temperatures. They are negatively affected by cationic surfactants, so rinsing of previously used detergents is essential (Anonymous, 1997).

Increased interest in peroxyacetic acid has developed for CIP sanitizing systems. This sanitizer, which provides a rapid, broad-spectrum kill, works on the oxidation principle through reaction with the components of cell membranes. Because it is effective against yeasts (e.g., *Candida* and *Saccharomyces*) and molds (e.g., *Penicillium, Aspergillus, Mucor* and *Geotrichum*) it has gained acceptance in the soft drink and brewery industries. Its efficacy against *Listeria* and *Salmonella* justifies its application in dairy plants. It reduces pitting of equipment surfaces by being less corrosive than halogen compounds; it is also biodegradable (Anonymous, 1997).

Acid anionic sanitizers are formulated by combining an anionic surfactant with an acid sanitizer. Thus, an acidified rinse is combined with the sanitizing step. The advantages of these combinations have been discussed (Anonymous, 1997).

### 7.4.4.5 Miscellaneous

Hydrogen peroxide (H₂O₂) in 3–6% aqueous solutions has been found to be effective against biofilms. As a sanitizer, H₂O₂ may be used on all types of surfaces: equipment, floors and drains, walls, steel-mesh gloves, belts, and other areas where contamination exists. It is effective against *L. monocytogenes* when applied to latex gloves (McCarthy, 1996). Ozone (O₃) has been evaluated as a chlorine substitute. Like ClO₂, O₃ is unstable and should be generated as needed at the site of application. Because it oxidizes rapidly, it has low environmental impact (Marriott, 1999). Glutaraldehyde has been used to control the growth of common Gram-negative and Gram-positive bacteria, as well as species of yeast and filamentous fungi found in conveyer lubricants used in the food industry (Marriott, 1999).

### 8 Control of microorganisms by natural antimicrobials

Consumer perception that use of synthesized food antimicrobials may be associated with toxicological problems has generated interest in the food industry regarding the use of naturally occurring compounds. Organic acids that are routinely produced in large quantities through chemical synthesis are also found naturally in many food products. The extraction of these and other antimicrobials from natural sources, however, can be complex, inefficient and expensive. Yet, synthetic agents may be considered less desirable than naturally occurring antimicrobials by a segment of consumers. Thus, interest in and the incentive for the development and use of naturally occurring antimicrobials in foods have increased because of the growing interest in so-called natural foods.

Numerous naturally occurring antimicrobial agents are present in animal and plant tissues, where they probably evolved as part of their hosts’ defense mechanisms against invasion by microorganisms. Natural antimicrobials can be derived from
barks, stems, leaves, flowers and fruits of plants; various animal tissues; and even from other microorganisms. Noted sources of natural antimicrobials are herbs, spices, fruits, milk, eggs, and lactic acid bacteria used in food fermentation. However, the selection, manufacture and commercial application of a proper antimicrobial are challenging due to the complexity of food, the variety of factors influencing preservation, and the complex chemical and sensory properties of natural antimicrobials themselves. Food preservation can be enhanced by interactions among multiple antimicrobial factors, which can yield additive or synergistic effects. These combined factors may include natural product composition, microbial flora, pH, water activity (a_w), added chemicals, packaging, and processing and storage temperatures (Sofos et al., 1998).

For the successful application of a naturally occurring antimicrobial to a food, there is a need to determine its efficacy and the antimicrobial spectrum of the compound. The antimicrobial selected should not contribute to the development of resistant strains, nor alter the food in such a way that growth of another pathogen is possible. To be useful as a natural antimicrobial, a compound must show functionality in the targeted food system. Many antimicrobials act together and therefore might be most appropriately evaluated in combination. Success of application testing may be determined by increased shelf-life and reduced pathogen viability (Sofos et al., 1998).

The exact mechanisms through which antimicrobials affect microbial growth are complex and difficult to elucidate fully, but knowledge of the antimicrobial mechanism of a compound will allow selection of combinations of antimicrobials with different mechanisms that could be optimally utilized against microorganisms in a food product (Sofos et al., 1998).

An important aspect of any compound selected for use as a food preservative is its toxicological properties. A naturally occurring antimicrobial to be used in food must not be toxic either by animal testing or by its continuous consumption in a food over a long period. In addition to the absence of toxicity, the antimicrobial must be non-allergenic and be able to be metabolized and excreted so as not to lead to residue build-up. It should not react either to make important nutrients unavailable to humans or to destroy those nutrients, and should not interfere with the proliferation of desirable microorganisms, such as lactic acid bacteria.

Chemical and physical properties of the antimicrobial agent should be compatible with the composition and properties of the food to be preserved. Important properties to be considered include chemical reactivity, solubility, dissociation constant (pKa) and influence on product quality. The potential impact on the sensory characteristics is of significance. Many naturally occurring antimicrobials must be used at high concentrations to achieve antimicrobial activity. Obviously, compounds that negatively affect flavor or odor are unacceptable. For example, some spice extracts show antimicrobial activity, but only at concentrations that would cause the food to be rejected by most consumers. It is also unacceptable for a food antimicrobial to mask spoilage. Ultimately, the greatest roadblock to the use of naturally occurring antimicrobials is economics (Sofos et al., 1998). Antimicrobials can be applied to food in various ways; the method used is dictated by what the existing processing and packaging procedures are (Sofos et al., 1998).
8.1 Traditional antimicrobials

Antimicrobials that may be classified as traditional with long or frequent use in processed foods include sugars, common salt and wood smoke (Sofos and Busta, 1992).

8.1.1 Natural sugars

Natural sugars such as sucrose, fructose, glucose, syrups, and various corn and other products (which generally are useful in foods as sweeteners, flavorings and fermentable materials) can also exert antimicrobial activity through decrease of $a_w$. Sugars also have an indirect activity by serving as substrates in food fermentations leading to the formation of acids, alcohols and other antimicrobial agents (Foegeding and Busta, 1991).

Direct microbial inhibition requires sugar concentrations exceeding 40–50%; for example, a 50% sucrose concentration decreases $a_w$ to 0.935, enough to inhibit growth of *Clostridium botulinum* (Sofos and Busta, 1992). Foods preserved with high sugar concentrations include jams, jellies, preserves, syrups, fruit juice concentrates, sweetened condensed milk and candies. Some yeasts and molds have the ability to grow in the presence of high sugar concentrations. Small concentrations of sugar act as substrates and support the growth of various microorganisms, including spoilage and pathogenic agents, as well as those useful in the production of fermented foods (Sofos *et al.*, 1998).

8.1.2 Common salt

Common salt (sodium chloride) has been used as a flavoring or a preservative in foods since ancient times. Foods treated with salt include meat, fish, cheese, butter, margarine and brined vegetables. Curing of meat with impure salt led to the discovery of nitrates as additives for the curing meat products (Sofos and Busta, 1980). In recent years, because of the potential link between sodium consumption and the development of hypertension, there has been a trend to decrease salt concentrations in processed foods. Potential partial replacers are potassium chloride and phosphates. However, if certain levels of potassium chloride are exceeded, bitter flavors may result. Phosphates also may contribute to undesirable textural and flavor effects. In any case, complete elimination of salt from certain foods may be impossible because of its important contribution to taste and to technological properties such as protein extraction and texture development (Sofos, 1986).

The amount of salt necessary to decrease $a_w$ to 0.935, which inhibits growth of *C. botulinum*, is 10% in the water phase of a product. However, even lower levels are important because they act synergistically with other antimicrobials such as acids, nitrite, sorbate and benzoate (Sofos, 1984). *Listeria monocytogenes* and *Staphylococcus aureus* can survive and even proliferate in environments exceeding 5% salt concentration (Sofos, 1993).

8.1.3 Smoking

Smoking of foods is an ancient practice that is still in use. Wood smoke contributes to flavor but also incorporates antimicrobial components in a product, including
phenolics, formaldehyde, acetic acid and creosote. However, their antimicrobial activity in today’s mildly smoked foods is probably weak. The use of application of natural wood smoke to foods has not only decreased, but is also being replaced by liquid smoke flavorings isolated from natural wood-smoke condensates. These preparations are actually preferred because they are easy to apply uniformly, the concentration used can be controlled, pollution from crude tar products can be decreased, and polycyclic aromatic hydrocarbons can be removed (Sofos et al., 1988).

8.2 Organic acids

Most bacteria prefer pH values near neutrality, whereas yeasts and molds are more tolerant of lower pH values. Increasing the acidity (i.e. lowering the pH) is an effective way of limiting microbial growth. This can be achieved through acidulant addition or natural fermentation resulting in production of acids by desirable microorganisms (Sofos and Busta, 1992).

Acids inhibit microbial growth by lowering pH or through the antimicrobial activity of undissociated molecules or anions. As pH decreases, the antimicrobial activity of short-chain organic acids increases more than that of long-chain acids. As pH decreases and approaches the pKa of a short-chain organic acid, the undissociated shorter molecule is able to enter the microbial cell, where it dissociates, acidifies the cytoplasm and interferes with chemical transport across the cell membrane and/or with enzymatic activity (Banwart, 1989). As the cell tries to maintain cytoplasmic homeostasis, protons are pumped out, disrupting the proton motive force and consequently interfering with oxidative phosphorylation and nutrient transport systems (Dillon and Cook, 1994).

The antimicrobial activity of organic acids is enhanced when they occur in mixtures; their spectrum of activity is also increased. Shorter-chain organic acids inhibit or inactivate both Gram-positive and Gram-negative bacteria, whereas longer-chain organic acids are effective primarily against Gram-positive bacteria because they cannot penetrate the outer membrane of Gram-negative bacteria. In addition, mono-unsaturated fatty acids are generally less inhibitory to microorganisms than are saturated fatty acids (Kabara, 1978). Acidic environments not only limit microbial growth but also enhance the destruction of microorganisms by heat; thus, decreased pasteurization or sterilization times are possible (Doores, 1993). Increased acidity also enhances the antimicrobial activity of other hurdles. For example, the antimicrobial activity of preservatives such as nitrites and sorbates increases as product pH decreases (Sofos and Busta, 1980).

8.2.1 Acetic acid

Acetic acid is present in vinegar, and is one of the oldest preservatives in use. A natural process for the production of acetic acid may involve an alcoholic fermentation of sugars naturally present in grapes, malts, grains and other plant materials, followed by aerobic oxidation of ethanol to yield acetic acid. Thus it is found in fermenting plant materials and some dairy products (Sofos and Busta, 1992). Acetic acid can be used as a general preservative because it is soluble in water, readily available, of low
cost and low toxicity, and inhibits a wide spectrum of bacteria, including *Salmonella*, *S. aureus*, *L. monocytogenes* and the spore-formers *Bacillus* and *Clostridium* (Banwart, 1989; Doores, 1993). In the form of 1.5–2.5 % solutions, it can be applied in the decontamination of food animal carcasses (Dickens and Whittemore, 1994), and has also been tested as a vapor at low concentrations for control of fruit decay by post-harvest fungi (Sholberg and Gaunce, 1995).

Acetic acid derivatives such as sodium acetate, calcium diacetate and dehydroacetic acid are also used as antimicrobial agents. All these compounds are generally regarded as safe (GRAS). Sodium acetate has exhibited inhibitory activity against *L. monocytogenes* in catfish fillets and sausages (Chang *et al.*, 1995; Wederquist *et al.*, 1995; Rong-Yu *et al.*, 1996).

### 8.2.2 Benzoic acid

Benzoic acid is a natural constituent of cranberries, prunes, plums, apples, strawberries, cinnamon and ripe olives. In addition, it may be found in some yogurts as a by-product of microbial growth. Although pure benzoic acid is available commercially, sodium benzoate is more useful because of its higher water solubility (Chipley, 1993). As with other organic acids, the antimicrobial activity is due mostly to the undissociated form, even though the dissociated form has also shown activity (Eklund, 1988).

Benzoic acid is more effective against yeasts than against bacteria or molds, but the activity is highly dependent on food, pH, and $a_w$. In general, antimicrobial activity increases as the pH value of the food decreases near to its pKa of 4.19, and maximal antimicrobial activity occurs at pH values of 2.5–4.0 (Sofos, 1994). Pathogenic bacteria inhibited by benzoate include *Vibrio parahaemolyticus*, *S. aureus*, *L. monocytogenes*, *Bacillus cereus*, *Escherichia coli*, *Micrococcus*, *Pseudomonas* and *Streptococcus*. Susceptible yeasts include species in the genera *Candida*, *Rhodotorula*, *Debaryomyces* and *Saccharomyces*. Among the fungi affected are *Alternaria*, *Aspergillus*, *Mucor*, *Penicillium* and *Rhizopus*. Concentrations effective in inhibiting microorganisms are in the range of 0.05–0.1 % for yeasts and molds, and 0.01–0.2 % for bacteria (Chipley, 1993; Nassar *et al.*, 1995). Benzoate can be metabolized by some bacteria such as *Enterobacter*, *Pseudomonas*, *Corynebacterium glutamicum* and certain thermophilic bacilli. Osmotolerant species of yeasts such as *Zygosaccharomyces bailii*, as well as bacteria including *E. coli* and *Gluconobacter oxydans*, can acquire resistance to benzoic acid (Chipley, 1993).

Because of its low cost, among other advantages, benzoate is probably the most commonly used antimicrobial in commercial food preservation. When there are concerns regarding flavor defects, benzoate may be used at lower levels in combination with other preservatives such as sorbate or parabens. In general, benzoates are used extensively in the preservation of foods with pH < 4.5, including fruit products, beverage and bakery products, fruit juices and drinks, salads and salad dressings, pickles, sauerkraut, preserves, jams and jellies, and margarine. Typical usage levels range from 0.05 % to 0.1 % (Chipley, 1993; Sofos, 1994). Benzoic acid and its derivatives are GRAS substances and well tolerated by humans (Sofos and Busta, 1992; Chipley, 1993; Sofos, 1994).
8.2.3 Lactic acid

Lactic acid is formed by bacteria including Lactobacillus, Lactococcus, Streptococcus, Pediococcus, Carnobacterium and Leuconostoc, as well as certain molds. Heterofermentative organisms, e.g. Leuconostoc, also produce other end-products such as acetic acid, ethanol, carbon dioxide and diacetyl, as well as bacteriocins. Homo- and heterofermentative fermentations occur in cheese, sauerkraut, pickles and fermented meat. The lactic acid formed in these products through microbial degradation of sugars decreases the pH to levels unfavorable for growth of spoilage or pathogenic bacteria (Sofos and Busta, 1992).

Microorganisms inhibited by lactic acid and lactates include C. botulinum, C. perfringens, C. sporogenes, E. coli, L. monocytogenes, Salmonella, Serratia liquefaciens, S. aureus, Yersinia enterocolitica, Aeromonas hydrophila and Enterobacter cloacae. It is not effective against yeasts and molds (Maas et al., 1989; Harmayani et al., 1991; Shelef and Yang, 1991; Doores, 1993; Houtsma et al., 1994; Meng and Genigeorgis, 1994; Miller and Acuff, 1994; Pelroy et al., 1994). Overall, the antimicrobial activity of lactic acid ranges from good to poor depending on the substrate (Banwart, 1989). Used at a 3 % level in combination with potassium sorbate at 5 %, it extended the shelf-life of fresh, vacuum-packed poultry meat stored under refrigeration (Kolsarici and Candogan, 1995). The sodium and potassium salts, i.e. lactates, have been used relatively extensively in recent years as sensory potentiatiors, flavorings and antimicrobial agents in foods such as processed meats and poultry products (Shelef, 1994; Wederquist et al., 1994, 1995). Lactic acid and its derivatives are highly soluble in water and classified as GRAS (Sofos et al., 1998).

8.2.4 Propionic acid

Propionic acid is an oily liquid with a strong, pungent, rancid odor. It is produced by bacteria belonging to the genus Propionibacterium, as used in the ripening of Swiss-type cheeses. This natural acid acts as a flavoring agent and mold inhibitor in Swiss cheeses. In addition, propionic acid is formed by bacteria in the gastrointestinal tract of ruminant animals. In the pure form, it is miscible in water, alcohol, ether and chloroform, and it is somewhat corrosive. Its sodium and calcium salts, available commercially as white, free-flowing powders, are used as food preservatives (Sofos and Busta, 1992; Doores, 1993; Sofos, 1994). Propionic acid and its salts are classified as GRAS. Because they are metabolized as a fatty acid, propionates in concentrations as high as 1 % (as in Swiss-type cheeses) have no adverse health effects. Levels used in food preservation are in the range of 0.1–0.4 % (Sofos et al., 1998).

The most common use of propionates is as mold and rope (e.g. Bacillus subtilis) inhibitors in bread and other bakery products. They are inexpensive, and do not interfere with the yeasts involved in leavening. Propionates are better mold inhibitors than benzoates, and they are used as such in cheeses, cheese products, fruits, vegetables, jams, jellies, preserves, malt extracts and tobacco (Robach, 1980; Sofos and Busta, 1992; Sofos, 1994). Microorganisms inhibited by propionates also include E. coli, Salmonella, S. aureus, L. monocytogenes, Proteus vulgaris, Lactobacillus plantarum, Pseudomonas spp., Sarcina lutea, Aspergillus spp., Torula spp and Saccharomyces ellipsoideus (Doores, 1993).
8.2.5 Sorbic acid
Sorbic acid is another naturally occurring antimicrobial substance available commercially as a synthetic compound. Sorbates (potassium, sodium and calcium salts) are agents effective against a variety of yeasts, molds and bacteria at concentrations of 0.1–0.3 % (Sofos, 1989). Sorbic acid and its salts are GRAS. The antimicrobial effect of sorbates increases at lower pH values.

Sorbates inhibit yeasts in fermented vegetables, fruit juices, wines, dried fruits, cheeses, fish and meats. Specific products in which sorbates are applied for inhibition of yeasts are carbonated beverages, salad dressings, syrups, tomato products, jams, candies, jellies and chocolate syrups. Sorbates are also effective inhibitors of many molds. Mold inhibition by sorbates is important in cheeses as well as in butter, fruits and fruit juices, grains, breads, cakes, smoked fish and dry sausages. In addition, they inhibit mycotoxin formation. Species of bacteria inhibited by sorbates include Bacillus, Campylobacter, Clostridium, Enterobacter, Escherichia, Lactobacillus, Listeria, Mycobacterium, Salmonella, Staphylococcus, Vibrio and Yersinia (Sofos and Busta, 1993).

Strains of molds belonging to the genera Penicillium, Aspergillus and Mucor are able to decompose sorbates in cheese and fruit products; lactic acid bacteria have also been reported to degrade sorbates in wine and fermented vegetables (Liewen and Marth, 1985; Sofos and Busta, 1993).

8.3 Lipid antimicrobials
Fatty acids and their soaps have been used since antiquity for cleansing and disinfecting. Fatty acids and their polyhydric alcohol esters are considered to be of low toxicity and have been used as emulsifiers in foods since the early 1900s (Kabara, 1993). In general, fats and oils from animals and vegetables are known for their ability to inhibit microorganisms by their fatty acids or oxidation products, mainly peroxides, and through associated antioxidative phenolic compounds formed by plants (Dallyn, 1994).

8.3.1 Fatty acids
Fatty acids, as components of natural fats, are primarily even-numbered straight-chain molecules, often with one or more double or triple bonds. The fatty acid compositions of natural fats differ considerably depending on origin. Plant oils often contain an abundance of medium-chain fatty acids, whereas oils from marine animals and plants are more abundant in unsaturated fatty acids; animal fats, on the other hand, are more saturated (Beuchat and Golden, 1989). Short-chain fatty acids show inhibitory activity at relatively high concentrations (1–3 %) against bacteria and fungi (Doores, 1993). Medium-chain saturated fatty acids (C₈–C₁₄) and their potassium and sodium salts are inhibitory mainly towards Gram-positive bacteria and yeasts. Inhibition generally occurs in media with concentrations from 0.0005 % to 0.005 %, but higher concentrations are usually required in foods. Lauric acid is the most inhibitory fatty acid against Gram-positive organisms, whereas capric acid is most active against yeasts (Kabara, 1993). The antimicrobial effect of saturated fatty acids is generally caused by the undissociated form of the molecule, and activity in
foods is therefore controlled by pH. Activity is usually highest in acidic (pH ≤ 4.6) and mildly acidic (pH ≅ 5.0) foods (Tsuchido and Takano, 1988).

Branched-chain and hydroxylated fatty acids possess slightly less antimicrobial activity than their straight-chain counterparts. Fatty acids with chain lengths greater than C₁₄ are not sufficiently soluble in the suspending solution for adequate cell contact, so their activity is lower. Similarly, introduction of hydrophobic groups, such as phenyl rings, decreases inhibitory activity due to low solubility, whereas increasing polarity with a hydroxyl or an amine group restores this activity. The presence of unsaturated linkages can markedly increase the antimicrobial activity of fatty acids. The magnitude of inhibition is influenced by degree and position of unsaturation. Thus the most active monounsaturated fatty acid is palmitoleic, whereas linoleic is the most active polyunsaturated fatty acid. The cis forms of fatty acids are active whereas the trans isomers are inactive, probably because steric hindrance of straight-chain acids prevents contact with cell membranes (Kabara, 1993).

All the above facts support the notion that the plasma membrane is the likely site of action. In addition to direct interaction of the undissociated molecule with the cell membrane, unsaturated fatty acids may exert some antimicrobial activity by autoxidation, which results in the formation of peroxides and other active oxygen metabolites. This oxygen-dependent activity would be expected to be relatively pH-independent under physiological conditions, and to be most effective against anaerobes and other organisms lacking enzymatic defenses against active oxygen metabolites (Sofos et al., 1998).

Unsaturated fatty acids are well known to exert antimicrobial activity against Gram-positive bacteria and yeasts, but most Gram-negative bacteria are resistant. Oleic, linoleic and linolenic acids are inhibitory at 0.005–0.02 % against Gram-positive cocci, lactobacilli, corynebacteria, and spore-forming Bacillus and Clostridium (Nieman, 1954).

### 8.3.2 Fatty acid esters

Fatty acid esters, derived from the esterification of fatty acids with polyhydric alcohols or sugars, have shown considerable antimicrobial and emulsifying activities in foods (Shibasaki, 1982). In general, monoacylglycerols – that is, monoglycerides formed through the reaction of medium-chain fatty acids with glycerol – have more potent antimicrobial properties and show a wider spectrum of activity than do free fatty acids. Antimicrobial activity has been demonstrated for esters formed between fatty acids and a variety of polyhydric alcohols or compounds possessing hydroxyl groups, e.g. sugars and peptides. The requirement for antimicrobial activity seems to be that a hydrophilic group be attached to the lipid component (Conley and Kabara, 1973).

Monoacylglycerols are especially active against Gram-positive bacteria and certain fungi, and have little activity against Gram-negative bacteria. Several studies have indicated that many Gram-positive bacteria, including B. subtilis, B. cereus, S. aureus, S. epidermidis, streptococci groups A and D, Micrococcus spp., Pneumococcus spp and Corynebacterium spp. are sensitive to low concentrations of monolaurylglycerol in microbiological culture media (Conley and Kabara, 1973). Monolaurylglycerol prevents or delays growth and toxin formation by S. aureus and Streptococcus spp.
(Schlievert et al., 1992). Antimycotic activity of this compound was demonstrated against *A. niger*, *Penicillium citrinum*, *Saccharomyces cerevisiae*, *Candida utilis*, *C. albicans*, *Cladosporium* spp and *Alternaria* spp. (Kato and Shibashaki, 1975; Kabara et al., 1977; Marshall and Bullerman, 1986). Monoacylglycerols have also been demonstrated to inhibit *L. monocytogenes* in foods (Wang and Johnson, 1997).

### 8.3.3 Sucrose esters

Sucrose esters are heat-stable and, because they can withstand autoclaving, are useful in stabilizing canned or other heat-treated foods, especially because they seem active against spore-formers, including *Bacillus stearothermophilus*, *B. coagulans*, *Desulfotomaculum nigrificans* and several *Clostridium* spp. (Sofos et al., 1998). They are also active against molds (Marshall and Bullerman, 1986).

### 8.3.4 Lipopeptides

Lipopeptides, which are derived from natural components by means of the condensation of peptides or amino acids and fatty acids, would be expected to have excellent antimicrobial properties. Sorboyl-tryptophan, sorboyl-D-alanine, myristoyl-D-aspartic acid and glycy1-D-alanine strongly inhibited *C. botulinum* when combined with 0.006 % sodium nitrite (Paquet and Rayman, 1987). Several lipopeptides, including polymyxin and lipopeptide antibiotics, have potent antimicrobial activity and could be effective food preservatives.

Polymyxins consist of a fatty acid moiety covalently linked to a cyclic peptide. In contrast to many other lipophilic antimicrobials, polymyxins have a strong effect on Gram-negative bacteria, causing direct membrane damage through a detergent-like action but at much lower concentrations than ordinary detergents. Membrane damage can be recognized by leakage of solutes, including nucleotides and inorganic ions, or by penetration of normally excluded molecules into the cell. Thus, polymyxins are unique among related lytic agents in being bactericidal in the absence of cell growth (Davis, 1990). They are useful in the control of *Salmonella* infections in poultry (Goodnough and Johnson, 1991). However, even though many of the lipopeptides are synthesized by food-related bacteria such as *Bacillus* spp., they are classified as antibiotics and it is unlikely that they will be used in food (Davis, 1990).

### 8.4 Plant substances

Compounds exhibiting various levels of antimicrobial activity are present naturally in plant stems, leaves, barks, flowers and fruits. Information on the antimicrobial activity of plant substances and extracts has been available since the nineteenth century, but interest in naturally occurring antimicrobials declined during the first half of the twentieth century, possibly because of the development of highly effective synthetic antimicrobials (Delaquis and Mazza, 1995). Compounds responsible for some of the flavors and aromas of foods are also inhibitory to microorganisms. In many instances, however, the concentrations of spices and herbs necessary for inhibiting microorganisms exceed those resulting from normal usage levels in foods (Beuchat, 1994).
Plants have developed mechanisms for defense against invasion by bacterial, fungal or insect and animal predators. Compounds involved in plant defense mechanisms may be classified as pre-infectional or post-infectional (Walker, 1994).

8.4.1 Prohibitins
Prohibitins include phenolic compounds, flavonols, glucosides, glycosides, alkaloids, dienes, lactones, polyacetylenes and protein-like compounds.

8.4.2 Inhibitins
Inhibitins are mostly phenolic or flavonoid in nature. The latter’s activity is usually associated with the action of the enzyme diphenol oxidase. Many plants contain phenolic compounds in the form of hydrolyzable tannins that can denature proteins. These compounds cause an astringent taste and antimicrobial activity in plant extracts (Walker, 1994).

8.4.3 Post-inhibitins
Post-inhibitins are stored as inactive precursors and activated when needed to fight invasion. Activation is catalyzed by hydrolases or oxidases released by the host plant or the invading agent. Examples are the sulfoxides of onion and garlic. In garlic, the precursor alliin is degraded by the enzyme alliinase to yield allicin. Many plants contain cyanogenic glycosides hydrolyzed by specific $\alpha$-glycosidases to release HCN, a microbial inhibitor in plants such as sorghum and lima beans, which is also toxic to animals (Walker, 1994).

Isothiocyanates are an important group of post-inhibitins derived from the glycosides glucosinolates, stored in cell vacuoles of plants in the family Cruciferae (cabbage, brussels sprouts, cauliflower, broccoli, rutabagas, mustard and rapeseed). When plants are injured, glucosinolates are hydrolyzed rapidly by the enzyme myrosinase to produce thiocyanates, isothiocyanates, nitriles, and glucose. Isothiocyanates are believed to exert inhibitory activity against molds, yeasts, and bacteria (Delaquis and Mazza, 1995).

8.4.4 Phytoalexins
Phytoalexins are primarily formed due to the activity of the diphenol oxidase enzymes (e.g. catecholases) acting on phenolic compounds to yield products of increased antimicrobial activity. These enzymes are present in almost all plants, where they oxidize dihydroxyphenols to form quinines, which are quite reactive and toxic. When the plant tissue and its membranes are damaged the enzyme is released and, as it comes in contact with the substrate, it forms the quinone inhibitors. Quinines can react with themselves or with proteins and amino acids of the plant or of the invading agent to form dark, highly oxidized melanoidins that are inhibitory to microorganisms. Common substrates for phenol oxidases include chlorogenic acid, catechin, epicatechin and DOPA (dihydroxyphenylalanine). In addition, plant defense mechanisms are enhanced by the action of hydrolytic enzymes on phenolic compounds resulting in the formation of diphenol oxidase substrates – that is, aglycones. The mechanism of action of phytoalexins, which are broad-spectrum antimicrobial
agents, is not understood fully, although evidence suggests that they alter the properties of microbial plasma membranes (Walker, 1994).

Pisatin from peas was the first recognized phytoalexin, but the total number of compounds isolated exceeds 200 from more than 20 botanical families. Shredded carrots and carrot juice have a lethal effect on *L. monocytogenes* (Beuchat and Brackett, 1990). Extracts of carrot roots inhibit differentiation and aflatoxin formation by *Aspergillus parasiticus* (Batt et al., 1980).

8.4.5 Garlic and onion

Plants in the *Allium* genus, namely garlic, onion and leek, are probably the most widely consumed foods with substantial antimicrobial activity, mainly related to the compound allicin. As aforementioned, intact tissues of *Allium* species do not contain allicin but do contain the precursor alliin. When bulb tissue is disrupted, alliin undergoes hydrolysis to yield allicin, pyruvate and ammonia by the action of the phosphopridoxal enzyme alliinase. The mechanism of antimicrobial activity is inhibition of sulfhydryl-based enzyme activity, including alkaline phosphatase, invertase, urease and papain (Wills, 1956).

The spectrum of activity of *Allium* extracts is broad, including important pathogens such as *S. aureus*, *Bacillus* spp., *C. botulinum* type A (but not types B and E), *E. coli*, *Salmonella* spp and *Shigella* spp. Also, a large number of yeasts and molds are susceptible (Sofos et al., 1998). *Allium* extracts are also able to inhibit mycotoxin formation (Mabrouk and El-Shayeb, 1981).

8.4.6 Spices and herbs

In addition to contributing to the sensory quality of foods, many spices and herbs also exhibit antimicrobial activity, mostly due to phenolic compounds. Although in many instances concentrations of these compounds necessary for inhibiting growth or various metabolic activities in microorganisms exceed those normally used in foods, the preservative effects of such seasoning agents should not be discounted. Among the spices with the greatest antimicrobial activity are cinnamon, cloves and allspice. Their active principles are often present in the essential oil or in the extracted, isolated and concentrated natural oil. The most important compounds are cinnamic aldehyde (present in cinnamon), eugenol (a major constituent in clove oil and present in considerable amounts in the essential oil of allspice), and thymol (present in the essential oils of thyme, oregano, savory, sage and several other herbs) (Sofos et al., 1998).

These compounds exhibit a wide antimicrobial spectrum, including important pathogens such as *S. Typhimurium*, *S. aureus*, *E. coli* and *V. parahaemolyticus* (Deans and Richie, 1987), and inhibit *Aspergillus* growth and mycotoxin production (Bullerman et al., 1977). The mode of action of phenolic compounds in spices and herbs against microorganisms has not been defined (Sofos et al., 1998).

8.4.7 Plant pigments

Compounds responsible for the color of plant tissues have, in many instances, antimicrobial properties. Anthocyanins, present in almost all higher plants and predominantly
in flowers and fruits, consist of an aglycone portion (i.e. anthocyanidin) esterified with one or more sugars. Although these pigments are better known for their food-coloring capabilities, they also inhibit bacteria, including *E. coli* and *S. aureus* (Powers et al., 1960) and yeasts (Marwan and Nagel, 1986). The mechanism of antimicrobial activity is not fully understood, although it is generally accepted that their chelating ability may explain in part a proven anti-enzymatic action (Somaatmadja et al., 1964).

The term ‘annatto’ refers to a series of preparations containing the carotenoid-type pigments *cis*-bixin and nor-bixin. Essentially non-toxic, annatto is commonly classified as a natural colorant and is used to impart distinctive flavor and color to foods. Annatto preparations are, in fact, the primary colorant for dairy foods such as cheese and butter. Annatto plant extracts show antimicrobial activity against Gram-positive bacteria, such as *B. cereus*, *C. perfringens*, *S. aureus* and *L. monocytogenes*, but have no activity against Gram-negative bacteria or yeasts (Galindo-Cuspinera et al., 2003).

### 8.4.8 Other phenolic compounds

Several phenolic compounds, including oleuropein and its aglycone, are found in olives and olive oil. The hydrolysis products of oleuropein are complex alcohols, elenolic acid and the oleuropein aglycones. Oleuropein itself is not antimicrobial, but the elenolic acid and the aglycones are. Microorganisms inhibited by these compounds include *Lactobacillus* spp., *Leuconostoc mesenteroides*, *S. aureus* and fungi (Fleming et al., 1973).

Hydroxy-cinnamic and cinnamic acids are phenolic compounds present in plant parts used as spices (Beuchat and Golden, 1989). Compounds of this chemical family with antimicrobial activity include caffeic, chlorogenic, *p*-coumaric, ferulic and quinic acids. Depending on the botanical species, hydroxy-cinnamic acids may be present at concentrations sufficient to retard microbial invasion and delay rotting of fruits and vegetables. Gram-positive and Gram-negative bacteria, molds and yeasts are sensitive to these compounds (Davidson and Branen, 1981). Caffeic, ferulic and *p*-coumaric acids, for example, inhibit *E. coli*, *S. aureus* and *B. cereus* (Herald and Davidson, 1983), and *S. cerevisiae* (Baranowski et al., 1980). Caffeic and coumaric acids inhibited aflatoxin production (Paster et al., 1988).

Tannins and tannic acid are present in the barks, rinds and other structural tissues of plants, and are known to possess antimicrobial activity against *S. Enteritidis*, *L. monocytogenes*, *E. coli*, *S. aureus*, *A. hydrophila* and *S. faecalis* (Chung and Murdock, 1991). The antimicrobial effect of red and white wines is proportional to the amount of flavonoid tannin present (Singleton and Esau, 1969).

### 8.4.9 Hops

Flowers of the hop vine are used to impart bitter flavors and other desirable properties to beer. Resins, commonly termed α-acids (represented by humulone and its derivatives), and β-acids (represented by lupulone and its derivatives) are the major compounds responsible for this flavor. Most of them also inhibit microbial growth. Gram-positive bacteria and some fungi are most sensitive (Mizobuchi and Sato, 1985). Although hops have antimicrobial activity, they contribute little to the microbial stability of beer (Richards and Macrae, 1964).
8.4.10 Coffee, tea, kola and cocoa
Caffeine is present in coffee and cocoa beans, tea and kola nuts. It is antimycotic as well as antibacterial. Inhibition of growth of several mycotoxigenic Aspergillus and Penicillium spp. at concentrations of caffeine as low as 0.0001 % has been documented, although the mechanisms by which caffeine inhibits polyketide mycotoxin synthesis is unknown (Buchanan et al., 1983). Bacteria affected by caffeine include S. aureus, Salmonella spp., E. coli, S. faecalis, B. cereus and L. monocytogenes (Vanos and Bindschedler, 1985; Pearson and Marth, 1990). Tea and cocoa contain theophylline and theobromine, also regarded as antimicrobial agents. Growth of S. Typhimurium, E. coli, S. aureus and B. cereus was found to be inhibited in a 2 % total solids instant tea infusion, whereas Lactobacillus plantarum was inhibited at 10 % total solids (Vanos et al., 1987).

8.4.11 Carbohydrates
Perhaps the most widely occurring and abundant group of indirect natural antimicrobial compounds is the simple sugars in foods of plant origin. Sucrose, fructose and other sugars decrease the aw of foods and in sufficient concentration inhibit microbial spoilage. Carbohydrates also serve as substrates for microorganisms involved in fermentations, resulting in generation of antimicrobial components. Other carbohydrates are converted to toxic metabolites, thereby killing or inhibiting their producers (Scott, 1988).

8.5 Antimicrobial polypeptides
Among the natural substances considered to be safe alternatives to synthetic chemical preservatives in food processing are various enzymes and other peptides. Some polypeptides are being used, while others seem promising for food preservation. Polypeptides present in animal or plant tissue that are important in defense against pathogenic microorganisms are often classified as functioning by oxygen-dependent or -independent mechanisms (Sofos et al., 1998).

The oxygen-dependent defenses involve enzymes and metabolic processes generating toxic metabolites of oxygen, such as hydrogen peroxide, superoxide ions, hydroxyl radicals and halides. These enzymes include peroxidases and other oxygen-metabolizing enzymes. Another group of enzymes related to oxygen is the oxidases (e.g. glucose oxidase and catalase), which can deplete oxygen and therefore inhibit aerobic microorganisms. Oxygen-independent means of inhibiting or killing microorganisms involve peptides and proteins that react with the cell surface and disrupt the structural surface layers or membranes of microorganisms. This group of polypeptides includes cationic peptides and proteins, bacteriocins, lysozyme and other lytic enzymes, and hydrolases including lipases and proteases. These polypeptides often function in combination with each other or with other groups of antimicrobials (Sofos et al., 1998).

8.5.1 Lytic enzymes
Lysozymes act by cleaving glycoside bonds in the structural peptidoglycan of bacterial cell walls; by their activity lysozymes leave a punctured cell wall, which may lead to
cell lysis in hypotonic media (Tranter, 1994). Lysozymes have desirable properties as food preservatives; it is economically feasible to obtain them from sources such as egg white in good yield and purity by ion-exchange resins. They have very low or no toxicity when consumed orally at levels exceeding 0.2 %, even after extended periods of consumption. Because they are odorless with a slightly sweet taste and, even more importantly, because the quantities used in foods are very low (0.002–0.04 %), they do not interfere with the sensory quality of products (Johnson, 1994).

Egg-white lysozyme remains stable in a number of food-processing operations. It can withstand boiling for 1–2 minutes at acidic pH values in solution, but it is denatured upon boiling at high pH values. It can be frozen, and is stable to spray drying (Tranter, 1994). This enzyme is active primarily against specific groups of Gram-positive bacteria, mostly non-pathogenic Clostridium, Bacillus, Bifidobacterium, Corynebacterium and Streptococcus, and the pathogenic L. monocytogenes and C. botulinum. Because of its specificity, it does not interfere with food and beverage fermentations carried out by beneficial lactic acid bacteria and yeasts (Carini et al., 1985; Hughey and Johnson, 1989).

Lysozyme is used to prevent gas formation or ‘late blowing’ by Clostridium tyrobutyricum and other gas-producing Clostridium spp. during ripening of certain cheeses, such as Provolone, Grana, Padano, Emmental and Gouda (Carini et al., 1985). Japanese investigators have studied lysozyme as a preservative in foods such as fresh vegetables, tofu, sausages, fish cakes and seafood (Tranter, 1994). Applications include coating fresh fruits, vegetables, meat and fish surfaces, as well as addition to wine and sake. Lysozyme extends the shelf-life of sake and mirin (Japanese wines) and prevents the malolactic fermentation in Western wines (Pitotti et al., 1991). Using the Maillard reaction, lysozyme–dextran conjugates have been prepared. They show improved emulsifying properties and are able to inhibit both Gram-negative (V. parahaemolyticus, E. coli, A. hydrophila and Klebsiella pneumoniae) and Gram-positive bacteria (B. cereus and S. aureus) (Nakamura et al., 1991).

In addition to lysozyme, a number of other enzymes are able specifically to cleave carbohydrate or peptide linkages in the cell walls of bacteria and fungi. These enzymes could be useful in the control of bacteria on foods but not as therapeutic agents, due to their interference with immune reactions (Leive and Davis, 1980).

### 8.5.2 Oxidases and peroxidases

Oxidases are enzymes oxidizing organic substrates, with molecular oxygen, generating hydrogen peroxide. Xanthine oxidase is an example of a naturally occurring enzyme in milk that generates hydrogen peroxide (Sofos et al., 1998).

Glucose oxidase catalyzes a reaction between glucose and oxygen, and yields glucono-δ-lactone and hydrogen peroxide. This enzyme has been approved to remove glucose from food and as an antioxidant. For example, it is used to remove glucose from egg white, to remove oxygen from beverages and headspace of packages, and to prevent Maillard browning reactions (Frank, 1992). It is effective against S. infantis, S. aureus, C. perfringens and B. cereus, although C. jejuni, L. monocytogenes and Y. enterocolitica have shown resistance (Tiina and Sandholm, 1989).
Peroxidases are widespread in nature and oxidize molecules at the expense of hydrogen peroxide. There are many peroxidases with different redox potentials and substrate specificities. Often, the rate-limiting substrate for peroxidase activity in foods is the availability of hydrogen peroxide, which is reactive and must be provided continuously (Ekstrand, 1994).

Lactoperoxidase, a product of the mammary glands and the most abundant enzyme in bovine milk, is also produced in salivary glands and is present in the saliva of mammals. It oxidizes thiocyanate or halogens, thereby producing toxic metabolites. Its combination with hydrogen peroxide, thiocyanate and/or iodide leads to a potent antibacterial system known as the lactoperoxidase system, which is based upon the inactivation of bacterial metabolic enzymes due to their oxidation by hypothiocyanate or hypoiode. The enzyme alone has been documented to be responsible for some of the natural antibacterial activity present in cow’s milk. The antimicrobial activity of the lactoperoxidase system is both species- and strain-specific. The system is bactericidal to Gram-negative bacteria (e.g. spoilage psychrotrophs) but is usually only bacteriostatic or temporarily inhibitory to Gram-positive organisms (e.g. L. monocytogenes, B. cereus and Streptococcus uberis) (Zajak et al., 1981; Reiter and Harnulv, 1984; Marshall et al., 1986; Siragusa and Johnson, 1989).

The lactoperoxidase/thiocyanate/hydrogen peroxide system has been utilized industrially, and has been suggested as a preservative in several systems, including the temporary preservation of raw milk in developing countries where refrigeration is unavailable. This system can inactivate E. coli (including the pathogenic O157:H7), S. Typhimurium, Y. enterocolitica, and Pseudomonas aeruginosa in milk and infant milk formula (Ekstrand, 1994). Lactoperoxidase has also been used to prevent spoilage in other dairy-based products, such as soft ice cream and pastry cream. Its activity against Gram-negative pathogens suggests that it could be beneficial for the preservation of other foods, particularly those of animal origin that may contain enteric pathogens (Johnson, 1994). An interesting potential application of lactoperoxidase is as a probiotic. Calves fed milk supplemented with the lactoperoxidase system components showed better weight gain than calves receiving unsupplemented milk (Ekstrand, 1994).

8.5.3 Transferrins

Growth and survival of many bacterial and fungal pathogens, namely Staphylococcus, Clostridium, Listeria, Mycobacterium, Salmonella, Escherichia, Pseudomonas, Yersinia, Vibrio and Aeromonas, depend on the availability of iron ions (Weinberg, 1978). In contrast, lactic acid bacteria have a metabolism based on manganese ions instead of iron. It is therefore possible, by means of iron sequestration, to favor the growth of beneficial lactic acid bacteria over that of either pathogens or spoilage microorganisms in foods. This can be accomplished through chelation by iron-binding polypeptides, especially the transferrins and related proteins (Bruyneel et al., 1990). Unlike antimicrobial enzymes such as lysozyme and lactoperoxidase that act catalytically, transferrins must be present in stoichiometric excess of the quantity of iron ions available; therefore, they are useful only in foods such as milk or egg albumen that have low iron content (Sofos et al., 1998).
About 13% of egg-white protein is the iron binding ovotransferrin, also known as conalbumin. This compound is believed to be the key factor responsible for microbial inhibition in egg albumen. Its activity is enhanced in albumen’s alkaline pH (8.5–9.5), but microbial growth resumes when the albumen is saturated with iron ions (Tranter, 1994).

Lactoferrin is a transferrin present in the milk of mammals as well as in tears, saliva and mucosal secretions (Masson et al., 1969). The physiological functions of lactoferrin may include protection of young animals from enteropathogenic bacteria, protection of the non-lactating mammary gland against mastitis, the physiological transport and supply of iron ions, and immunoregulation (Ekstrand, 1994). In addition to metal chelation, lactoferrin damages the outer membrane of Gram-negative bacteria and sensitizes them to lysozyme, with which lactoferrin forms a complex (Ellison and Giehl, 1991). Microorganisms sensitive to this agent include Vibrio cholerae, Streptococcus mutans, E. coli, P. aeruginosa and the yeast Candida albicans (Arnold et al., 1980), whereas L. monocytogenes, S. Typhimurium, S. aureus, Pseudomonas fluorescens and Shigella sonnei show resistance (Payne et al., 1990).

Digestion of bovine lactoferrin with gastric pepsin yields a hydrolysate with antibacterial activity greater than that of native lactoferrin. This peptide, referred to as lactoferricin, is able to inactivate a broad range of Gram-positive and Gram-negative bacteria, including E. coli, L. monocytogenes, S. aureus, C. perfringens, C. jejuni, Y. enterocolitica, Corynebacterium diphtheriae, P. aeruginosa, Proteus vulgaris, K. pneumoniae, S. Enteritidis and S. mutans. Resistant organisms include P. fluorescens, Bifidobacterium bifidum and Enterococcus faecalis. Lactoferricin is lethal at concentrations ranging from 0.00003% to 0.0015%, depending on the microorganism (Wakabayashi et al., 1992; Hoek et al., 1997).

8.5.4 Bacteriocins
Bacteriocins are small peptides, produced by bacteria, that possess antibiotic properties. Bacteriocins are normally not termed antibiotics in order to avoid confusion with and concerns about therapeutic antibiotics (Cleveland et al., 2001). They differ from most therapeutic antibiotics in being proteinaceous and generally possessing a narrow specificity of action against strains of the same or closely related species (Tagg et al., 1976). Bacteriocins are rapidly hydrolyzed by proteases in the human digestive tract (Joerger et al., 2000). The effectiveness of bacteriocins as antimicrobial agents in foods can become limited for various reasons, and cost remains an issue impeding their broader use as food additives. Hence, not only do searches continue for new and more effective bacteriocins, but efforts are also being made to improve existing bacteriocins to address both biological and economic concerns (Chen and Hoover, 2003).

Most of the bacteriocins from lactic acid bacteria are cationic, hydrophobic or amphiphilic molecules composed of 20–60 amino acid residues (Nes and Holo, 2000). They are commonly classified into three groups (classes) that also include bacteriocins from other Gram-positive bacteria (Nes et al., 1996).

Class I includes small peptides containing the unusual amino acids lanthionine, α-methyllanthionine, dehydroalanine and dehydrobutyryl. They are also called
lantibiotics (from lanthionine-containing antibiotics). This class is further subdivided into type A and type B lantibiotics, according to chemical structures and antimicrobial activities (Guder et al., 2000). Type A lantibiotics are elongated peptides with a net positive charge that exert their activity through the formation of pores in bacterial membranes. Type B are smaller globular peptides and have a negative or no net charge; antimicrobial activity is related to the inhibition of specific enzymes.

Class II includes small, heat-stable, non-lanthionine-containing peptides. These peptides are divided into three subgroups. Class IIa includes pediocin-like peptides with remarkable anti-Listeria activity (Ennahar et al., 2000); class IIb are dipeptides, and class IIc contains the remaining peptides of the class.

Class III is not well characterized, and it includes large, heat-labile proteins that are of lesser interest to food scientists.

Nisin remains the commercially most important bacteriocin. It has led in popularity because of its relatively long history of safe use and its documented effectiveness against important Gram-positive foodborne pathogens and spoilage agents. In fact, it is the only purified bacteriocin approved for food use in the US, and has been successfully used for several decades as a food preservative in more than 50 countries (Chen and Hoover, 2003). Produced by Lactococcus lactis, nisin is a 34-amino acid peptide, categorized as a class I bacteriocin and a type A lantibiotic. At least six different forms have been discovered and characterized (designated as A–E and Z), with nisin A being the most active type (van Kraijj et al., 1999).

Nisin usually has no effect on Gram-negative bacteria, yeasts and molds, although Gram-negative bacteria can be sensitized to nisin by permeabilization of the outer membrane layer through sublethal heating, freezing and chelating agents (Delves-Broughton et al., 1996). Normally only Gram-positive bacteria are affected; and these types include lactic acid bacteria, Listeria, Staphylococcus and Mycobacterium, and the spore-forming Bacillus and Clostridium. The spores of bacilli and clostridia are actually more sensitive to nisin than are vegetative cells, although the antagonism is sporostatic, not sporicidal, thus requiring the continued presence of nisin to inhibit outgrowth of the spores. Heat damage of spores substantially increases their sensitivity to nisin, so that nisin is effective against spores in low-acid, heat-processed foods, resulting in its use as a processing aid in canned vegetables. The mechanism of its sporostatic action is distinct from its bactericidal effect on the cytoplasmic membrane of vegetative cells (Morris et al., 1984).

Purified nisin has been evaluated for toxicological effects and found to be harmless or at least to have very low toxicity using rat and guinea pig models (Shtenberg and Ignatev, 1970). Examples of marketed food products that can legally be amended with nisin are canned soups (Australia), ice for storing fish (Bulgaria), baked goods and mayonnaise (Czech Republic), and milk shakes (Spain). The majority of products approved for its use are dairy products (especially cheeses) and canned goods. In the US, use of nisin-producing starter cultures has never been regulated as lactococci are considered GRAS (Chikindas and Montville, 2002).

Nisin (100 IU/ml) may control L. monocytogenes in ricotta-type cheeses for 8 weeks or more, according to the cheese type (Davies et al., 1997). In a study using vacuum-packed cold-smoked rainbow trout, the inhibition of the above pathogen by
nisin or sodium lactate, or their combination, was determined. Both antimicrobial agents were capable of inhibiting the pathogen growth, but the combination of the two compounds was even more effective (Nykänen et al., 2000).

8.6 Miscellaneous antimicrobial agents

8.6.1 Ethanol

The value of ethanol as a naturally occurring antimicrobial has been recognized since the first alcoholic fermentations of fruits to produce wines, which occurred several thousand years ago. For ethanol to disinfect, water must be present. At 95 % ethanol most vegetative cells are resistant, whereas at 60–75 % ethanol most microorganisms are destroyed in less than a minute. Low concentrations are rarely biocidal alone, but may exert inhibitory effects. At 8–11 % (v/v), ethanol prevents growth of most molds and bacteria; a 15–18 % concentration is required to prevent growth of most yeasts. Gram-negative bacteria are more susceptible to ethanol than are Gram-positive bacteria, whereas bacterial spores are generally resistant. Various environmental factors influence the activity of ethanol as an antimicrobial. Increased sugar concentration, decreased temperature and decreased aw increase its effectiveness; but the presence of organic matter decreases activity (Shelef and Seiter, 1993).

Because ethanol is amphiphilic, the primary site of activity is thought to be the cytoplasmic membrane. It is also thought that ethanol may have a direct effect on membranes or membrane-bound enzymes, or an indirect effect due to impairment of membrane biosynthesis. Dissolution of ethanol in the cell membrane increases fluidity of the lipid and decreases the gel-to-liquid crystalline phase transition temperature of lipids. This results in disruption of membrane organization, leakage of ions, leakage of low molecular weight solutes, and even leakage of macromolecules (Seiler and Russell, 1991).

Ethanol has GRAS status as a food additive. It is primarily used as a solvent for flavor and color compounds. Evaluation of its antimicrobial activity is actually quite recent. Most studies have focused on the antymycotic activity of the compound. Increased shelf-life of bakery products such as bread or pizza crust was obtained by the addition of ethanol and sterile water as a dip or a spray. This research first demonstrated that ethanol is a vapor phase inhibitor; thus techniques such as vacuum packaging with ethanol, adding sachets or strips impregnated with ethanol, or encapsulation techniques are possible methods for incorporating ethanol as a mold inhibitor (Seiler and Russell, 1991).

8.6.2 Natamycin

Natamycin was first isolated in 1955 from a culture of *Streptomyces natalensis*, a microorganism found in soil from Natal, South Africa. It is a polyene macrolide antibiotic (Brik, 1981). Natamycin is active against nearly all molds and yeasts, but has no effect on bacteria or viruses. Natamycin blocks mycotoxin production in genera such as *Aspergillus* and *Penicillium* (Gourama and Bullerman, 1988). The mode of action of polyene macrolides involves binding of ergosterol and other sterol groups in fungal cell membranes.
8.6.3 Reuterin
A number of low molecular weight compounds with antimicrobial activity have been isolated and identified from culture filtrates of lactic acid bacteria. The most studied to date is reuterin (β-hydroxy-propionaldehyde), produced by the heterofermenter Lactobacillus reuteri. Reuterin exists in three forms – the aldehyde, its hydrate, and a cyclic dimer – and has very broad spectrum activity against bacteria, fungi, protozoa and viruses. The addition of reuterin to foods such as minced beef, milk and cottage cheese has been shown to control coliform growth and to inactivate L. monocytogenes and E. coli O157:H7 (El-Ziney and Debevere, 1998; Muthukumarasamy et al., 2003).

8.6.4 Diacetyl
Diacetyl is produced by the citrate-fermenting lactic acid bacteria Leuconostoc cremoris and Lactococcus lactis subsp. lactis var. diacetylactis. The compound produces a buttery flavor in fermented dairy products and in foods to which it is added for flavor. Acceptable sensory levels in dairy products range from 0.0001 % to 0.0007 %. The effective antimicrobial concentration is higher than its organoleptic detection threshold, limiting its use as a natural preservative. Its volatility also limits its usefulness.

8.6.5 Hydrogen peroxide
Hydrogen peroxide itself does not have antimicrobial activity. Rather, it produces powerful reaction products, such as singlet or superoxide oxygen, that are highly toxic to living organisms. Another possible mechanism is through the oxidation of sulfhydryl groups and double bonds in proteins and lipids. The compound is active against bacteria, molds, yeasts and viruses, and is particularly effective against anaerobes and facultative anaerobes because many lack catalase. In general, Gram-negative bacteria are more susceptible than Gram-positive ones (Cords and Dychdala, 1993). Organisms affected by hydrogen peroxide include E. coli, E. aerogenes and L. monocytogenes. Others, such as Lactobacillus bulgaricus, L. lactis and Bacillus megaterium, showed resistance. The susceptibility of spore-forming organisms (i.e. Bacillus and Clostridium species) depends on concentration, aw and temperature (Domínguez et al., 1987).

In the US, hydrogen peroxide is allowed as a direct additive at 0.05 % to pasteurized milk for making certain types of cheese and as an antimicrobial in whey (0.04 %) and starch (0.15 %). Catalase is added to products to remove residual hydrogen peroxide. It is also allowed for sterilization of polymeric food-packaging surfaces, which are used for aseptically packaged foods and for sanitizing of food contact surfaces (Code of Federal Regulations, 1992).

9 Alternative innovative technologies
Newer physical food preservation methods can be divided into thermal and non-thermal procedures. Special attention in research and development has been directed toward the non-thermal technologies that have the ability to inactivate microorganisms at ambient or near-ambient temperatures. In fact, alternative technologies for inactivating microorganisms without relying on heat are not new concepts, but their use
as food preservation treatments has received considerable attention recently in response to consumer demands for more ‘fresh’ and ‘natural’ products (Ross et al., 2003).

9.1 Ohmic heating

Ohmic heating is a thermal method that minimizes energy input and thus reduces thermal damage to food. If an electric current passes through a conductive medium, in this case the food, the medium warms up as a result of ionic movement. Essentially, ohmic heating utilizes the effect of the electrical resistance within a conductive liquid or solid material, allowing a direct conversion of electric energy into heat. It is therefore evident that its applicability is limited to foods with sufficient conductivity. In processing plants, the product is continuously pumped through a column equipped with several electrodes (Butz and Tauscher, 2002).

Ohmic heating is presently used for pasteurization and sterilization of liquid and particulate foods, especially ready-to-serve meals, fruits, vegetables, meat, poultry and fish, and is an alternative to sterilization of foods by means of conventional heat exchangers or autoclaves. There are several other potential applications, including blanching, evaporation, dehydration, fermentation and extraction (Butz and Tauscher, 2002).

Lethality within food particles undergoing ohmic heating was investigated by Kim et al. (1996). Meatballs containing spores of *Bacillus stearothermophilus* and precursors of chemical markers were thermally processed in a starch solution with 30–40% solids content using a 5-kW ohmic system. Higher temperature and microbiological lethality were observed at the center of the meatballs rather than near the surface. The time–temperature history of the ohmically processed meatballs equivalent to 1.06 minutes at 133 °C corresponded to an F0-value of 16.8, which is at least five times greater than that needed for a 12-log reduction in *Clostridium botulinum*.

9.2 Microwaves

The primary advantage of using microwaves, when compared to conventional electric oven heating, is time-saving. Within the food industry there are some unique applications associated with the singular heating properties of microwaves, including tempering, drying pasta and cooking bacon. Microwave technology has the potential and flexibility to be adapted for vacuum and freeze drying, pasteurizing, sterilizing, baking, roasting and blanching. A number of factors exert a significant influence in achieving uniform heating with microwave energy, namely the moisture and ionic content of foods, the specific heat of various food constituents, and the product density, shape and volume (Heddleson and Doores, 1994).

Microwave energy is a form of non-ionizing radiation that falls several orders of magnitude short of the energy necessary to break the weakest of chemical bonds (i.e. $1.2 \times 10^{-5}$ eV for the quantum energy of microwaves versus 5.2 eV for the breakage of hydrogen bonds) (Rosen, 1972). Two major microwave constituents can be distinguished: a magnetic field and an electric field, oriented perpendicularly to one
another. The latter is primarily responsible for heating, as it promotes the rotation of polar molecules and consequently yields heat generated by molecular friction (Curnutte, 1980).

There are two main schools of thought concerning the means by which microwaves injure or kill bacteria. On one hand, a number of studies have concluded that microwaves can reduce bacterial numbers entirely by heat. That includes irreversible heat-denaturation of enzymes, proteins, nucleic acids or other cellular constituents, resulting in cellular death as well as leakage of metabolites and/or cofactors crucial to cellular function through membranes damaged by heat. On the other hand, there are studies that suggest a non-thermal mechanism of lethality, an effect exclusive to microwave technology, apparently related to RNA damage (Chipley, 1980; Khalil and Villota, 1989).

Numerous studies have examined how microwave heating affects numbers of microorganisms present in various foods, often comparing microwave heating to similar time–temperature treatments performed in conventional ovens. The majority of these studies have focused on important pathogens, such as *Salmonella* spp., *L. monocytogenes*, *E. coli*, *S. aureus*, *Streptococcus faecalis* and *Clostridium perfringens* (Heddleson and Doores, 1994).

Heddleson *et al.* (1991) performed the first study using microwave heating to relate the composition of liquid model food systems to temperatures achieved and amounts of destruction of *Salmonella* spp. It was found that of the various food components examined, only NaCl significantly influenced temperatures and inactivation rates. The presence of salt caused large temperature gradients within small volumes, and the non-uniform heating contributed to a greater survival of the pathogen. Later, Heddleson *et al.* (1994) examined processing variables influencing the destruction of *Salmonella* spp. Heating medium volume, container shape and covering containers did not significantly alter the rate of inactivation at 60 °C. Increased post-heating holding times of ≥ 2 minutes increased bacterial destruction. Microwave heating with ovens of low power (*ca.* 450 W) was less effective than heating with units of high power (*ca.* 700 W).

Schnepf and Barbeau (1989) conducted a study to compare the effectiveness of microwave, convection-microwave and conventional electric ovens in eliminating *S. Typhimurium* from roasting chickens. The microwave oven proved to be the least efficient in killing the pathogen, while convection-microwave and the conventional oven proved to be of nearly equal efficiency. Minimum internal temperatures recommended by the US Department of Agriculture (USDA) (71 °C), the Food and Drug Administration (FDA) (74 °C) and the American Home Economics Association (85 °C) were used as the bases for selecting temperatures for examination. The researchers found that even at 85 °C, microwave heating did not eliminate *Salmonella*. It appears that *Salmonella* are not effectively killed by this thermal treatment when inoculated on the chicken surface, and therefore it may not be wise to assume that minimum internal temperatures can be used to recommend safe cooking practices for microwave ovens. Since microwaves penetrate within the food matrix and essentially exert a steam-cooking effect, the coolest temperatures may be found on the surface of solid food masses due to evaporation. This is the opposite effect to the one taking
place in conventional electric ovens, in which conduction transfers heat from the surface to the inner mass, thus providing the rationale for current cooking recommendations based on minimum internal temperatures (Heddleson and Doores, 1994). In fact, previous researchers (Chen et al., 1973; Lindsay et al., 1986) reached the common conclusion that a minimum internal temperature is a poor criterion for determining safety standards in foods heated with microwave ovens.

In a later study, Reis-Tassinari and Landgraf (1997) evaluated the destruction of *S. Typhimurium* during reheating of foods in two different types of microwave ovens: a conventional 750-W and a 700-W unit with preset controls. Heating times in the conventional microwave oven were established at 50 seconds for baby food and 75 seconds for mashed potatoes and beef stroganoff samples, while for the preset oven time periods were determined by a built-in temperature sensor. The percentage of food samples positive for the pathogen after treatment in the conventional oven was 47.8%, whereas in the microwave with preset controls it was 93.3%. The results therefore suggested that reheating contaminated foods in microwave ovens might not be adequate to destroy *S. Typhimurium* and to assure food safety.

Galuska et al. (1988) conducted one of the first studies examining the destruction of *L. monocytogenes* by microwave heating. These authors examined the thermostolerance of *Listeria* suspended in non-fat dry milk heated by microwaves, and calculated *D*-values at five temperatures between 60 °C and 82.2 °C. They compared the results with those found by heating in a water bath. *D*-values were lower in the water bath-heated sample than in the microwave-heated sample. Microwave treatment accomplished a 4.5 log₁₀ cycle reduction in viable cell numbers within 15 seconds at 71.1 °C. At conventional pasteurization processing temperatures, microwaves were as effective as conventional heating in destroying *L. monocytogenes*.

The effect of different microwave power levels (240, 400, 560 and 800 W) on the survival of *L. monocytogenes* in inoculated shrimp was investigated by Gundavarapu et al. (1995). *D*-values were determined using constant-temperature water baths to establish heat resistance of the pathogen in shrimp. Shrimp were inoculated with ca. 5 × 10⁵ CFU/g of a five-strain mixture of *L. monocytogenes*. Shrimp samples were then cooked in the microwave oven at the different power levels using cooking times predicted by a mathematical model, as well as 20% longer times than those obtained from the model. No viable *L. monocytogenes* was detected in uninoculated shrimp after microwave cooking at the lowest power treatment, but at least one replication of inoculated shrimp tested positive for the presence of *Listeria*. No viable pathogens were detected in shrimp cooked at 120% of predicted times.

Dahl et al. (1981) studied the survival of *S. aureus* in model cook/chill foodservice systems similar to those used in hospitals for food preparation. *S. aureus* was surface-inoculated onto beef loaves, potatoes and canned green beans, which were heated to times and temperatures recommended by the HACCP plan of a specific facility. It was found that microwave heating to mean end temperatures of 74–77 °C did not eliminate *S. aureus* from food samples. Furthermore, the destruction kinetics of *S. aureus* could not be predicted consistently, and time and temperature were poor parameters upon which to base microwave thermal processes. *S. aureus* was inoculated into beef-soy loaves and assayed for recovery of viable cells during various
stages of food handling in a hospital chill food-service system by Bunch et al. (1977). Heating the loaves at 121 °C in a conventional oven to an internal temperature of 60 °C substantially reduced the inoculum level at the center of the loaf. Chilling the loaves at 5 °C for 24–72 hours, followed by microwave reheating to an internal temperature of 80 °C, resulted in the elimination of \textit{S. aureus} from the samples. However, it was noted that preformed toxin, if present, would not be inactivated by the microwave reheating treatment.

The microbiological quality of scrambled eggs and roast beef prepared in a hospital foodservice system and reheated in a microwave oven for later consumption was investigated by Cremer and Chipley (1980a, 1980b). The average time from cooking to microwave reheating was 26.3 hours for the scrambled eggs, which were then reheated to an average internal temperature of 67.1 °C. Nevertheless, the range of final temperatures was rather wide: 35–92 °C. At the time of microwave reheating the natural flora of the eggs reached a total plate count of 30 CFU/g of \textit{Bacillus} spp., \textit{Clostridium sporogenes}, \textit{Staphylococcus epidermidis}, \textit{E. coli} and \textit{Enterobacter aerogenes}. This low count was attributed to the use of pasteurized, \textit{Salmonella}-free frozen whole eggs as a starting material. Both coliforms and staphylococci were recovered after microwave heating, indicating inadequate thermal processing as revealed by the wide range of final internal temperatures. Roast beef was cooked in a conventional oven, stored for approximately 45 hours and then reheated by microwave energy, achieving a final average temperature of 68 °C, with a 43–93 °C range. Between the time of initial roasting and microwave reheating, bacterial numbers increased 3- to 11-fold from an initial level of \( \leq 200 \) CFU/g, because of the lengthy storage time at temperatures favoring growth (7.5 °C was the mean internal temperature). Organisms present in the cooked meat included \textit{C. sporogenes}, \textit{C. perfringens}, \textit{Bacillus} spp., \textit{S. aureus} and \textit{S. epidermidis}. Although microwave reheating lowered their numbers, it was unable to eliminate them. Expectedly, \textit{Clostridium} and \textit{Staphylococcus} were more heat resistant than coliforms. This study concluded that contamination from food handlers should be minimized, because the non-uniform temperatures found after microwave reheating were not sufficient to reduce the number of pathogens to acceptable levels.

Destruction of \textit{E. coli} was studied by Koutchma and Ramaswamy (2000) in a continuous-flow microwave heating system in combination with low concentrations of hydrogen peroxide. The antimicrobial was added separately to the cell suspension at selected concentrations and immediately subjected to microwave heating, or the cell suspension was treated with hydrogen peroxide for 10 minutes at room temperature before microwave heating. Synergistic effects of microwave heating and hydrogen peroxide treatment on \textit{E. coli} destruction were observed, and the interaction reached a maximum with exposure to hydrogen peroxide at 0.075 g/100 g, and microwave heating set at 60 °C.

In conclusion, microwave heating may lead to increased risk of foodborne illness due to poor uniformity of temperature within products and accelerated heating profiles. Factors that influence microwave technology include many of those that are significant in conventional processes, such as the mass and shape of foodstuffs, and their specific heat and thermal conductivity. There are, however, other factors unique to microwave heating due to the nature of the electric field involved in causing
molecular friction; primarily moisture and salt contents of foods. It is therefore imperative that thermal processes involving microwave ovens take into account these additional unique factors (Heddleson and Doores, 1994).

### 9.3 Superheated steam

Superheated steam is defined as steam heated to a temperature higher than the boiling point corresponding to its pressure. It cannot exist in contact with water, nor contain water, and resembles a perfect gas (Dictionary.com, 2003). Typically, superheated steam is obtained by drastically dropping the pressure of saturated steam without changing the temperature, after which energy is applied to heat the steam to the desired temperature (Tang and Cenkowski, 2001).

Compared with hot-air drying, superheated-steam drying provides a number of advantages (Tang and Cenkowski, 2000). Due to its combination of high temperature and low moisture, superheated steam can be utilized to inactivate spoilage and pathogenic microorganisms in foods sensitive to moisture, such as ground spices and flour. Superheated steam is particularly effective for inactivating bacterial spores. Holley et al. (2003) found that treating oat groats with superheated steam at 145 °C and 15 psi for 10 minutes reduced the population of *B. stearothermophilus* spores from 6.3 to 3.6 log$_{10}$.

### 9.4 High electric field pulses

High-intensity pulsed electric field (PEF) processing involves the application of pulses of high voltage (typically 20–80 kV/cm) to foods placed between two electrodes. PEF may be applied in the form of exponential decaying, square wave, bipolar or oscillatory pulses, and at ambient, sub-ambient or slightly above-ambient temperature for less than a second. Energy loss due to heating of foods is minimized, reducing the detrimental changes of the sensory and physical properties of foods (Barbosa-Cánovas et al., 1999).

Microbial inactivation by PEF has been explained by a number of theories, among which electrical breakdown and electroporation predominate. Electric high-voltage impulses generate a trans-membrane potential across the cell membrane. If the difference between outer and inner membrane potential rises above a critical value of about 1 V, polarization and ultimately breakdown of the membrane are induced. At sufficiently high field-strength (above 10 kV/cm) and duration of the pulses (usually between nano- and micro-seconds), vegetative microorganisms in liquid media are inactivated due to irreversible membrane destruction. PEF effectiveness depends on process factors (electric field intensity, pulse width, treatment time and temperature, and pulse wave shapes), microbial entity factors (type, concentration and growth stage), and media factors (pH, antimicrobials and ionic compounds, conductivity and medium ionic strength). PEF differs from traditional thermal treatments in that the latter greatly affect the cell organelles, whereas the former does not rupture them. This is evidently related to a better final product from a textural and sensory viewpoint (Grahl and Maerkl, 1996; Pothakamury et al., 1997; Butz and Tauscher, 2002).
Microbial inactivation by PEF is a function of growth phase and medium temperature. Logarithmic phase cells are more sensitive to electric fields than are stationary phase cells. Hülsheger et al. (1983) showed that a 4-hour culture of *E. coli* was more sensitive to PEF than was a 30-hour culture. Inactivation increases with an increase in temperature of the medium. With exponential decay pulses, the population of *E. coli* was reduced by 2 log cycles after 20 pulses at 40 °C and 50 pulses at 30 °C. On the other hand, when square waves were used, the population of *E. coli* was reduced by 2 log cycles after 10 pulses at 33 °C and 60 pulses at 7 °C (Pothakamury et al., 1997). Inactivation also increased with a decrease in the ionic strength of the medium. After 30 pulses at 40 kV/cm, the population of *E. coli* decreased by 2 log cycles with a decrease in the ionic strength and a decrease in pH (Vega et al., 1996). The presence of sodium or potassium in the treatment medium did not affect microbial inactivation, whereas calcium and magnesium induced a protective mechanism against PEF (Hülsheger et al., 1981).

PEF reductions of 6–9 log cycles were obtained in skim milk inoculated with *E. coli* (Qin et al., 1996). Furthermore, the *E. coli* O157:H7 population in apple juice was reduced by 5 logs using PEF for a maximum treatment time of 172 µs at temperatures lower than 35 °C (Evrendilek et al., 1999). Similar results (i.e. 4–5 log reduction cycles) were obtained for *Staphylococcus aureus*, *Lactobacillus delbrueckii* and *Bacillus subtilis* in skim and raw milk (Pothakamury et al., 1995; Qin et al., 1995, 1998); however in yogurt the reduction was lower (2 log cycles) for *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *S. cerevisiae* (Dunn and Pearlman, 1987). *Mycobacterium paratuberculosis*, *L. monocytogenes* and *S. Typhimurium* have also been inactivated using PEF (Simpson et al., 1999; Rowan et al., 2001; Liang et al., 2002), whereas *Corynebacterium* spp. and *Xanthomonas* spp. have shown resistance (Raso et al., 1999).

PEF alone appears to have very little effect on bacterial spores (Pagán et al., 1998), although some studies have reported successful destruction in saline solutions (Dantzer et al., 1999). PEF does not induce germination of spores, but if germination is initiated by other methods, the resulting vegetative cells will become sensitive to an electric field (Barbosa-Cánovas et al., 1998).

Pasteurization of milk was undertaken by Smith et al. (2002) using PEF alone and combined with nisin and lysozyme, added singly or together. A 7-log reduction was achieved through a combination of PEF treatment (80 kV/cm, 50 pulses), mild heat (52 °C) and the addition of both natural antimicrobials (38 IU/ml and 1638 IU/ml of nisin and lysozyme, respectively).

Similarly, using heat, acidity, antimicrobials (nisin or lysozyme) and PEF, Hodgins et al. (2002) pasteurized orange juice. Optimal conditions consisting of 20 pulses of an electric field of 80 kV/cm, pH 3.5 and a temperature of 44 °C with 100 IU/ml of nisin resulted in greater than a 6-log reduction in the microbial population. Following treatment, there was 97.5 % retention of vitamin C. Studying the inactivation of *S. Typhimurium*, Liang et al. (2002) also concluded that the bactericidal effects of nisin/lysozyme mixtures on PEF-treated cells were more pronounced than addition of either antimicrobial alone.

Application of PEF is restricted to food products that can withstand high electric fields – that is, that have low electrical conductivity and do not contain or form
bubbles. It is expected that PEF will be developed into a reliable technology for reducing the pasteurization temperature of liquid foods (e.g. juices, milk, liquid whole egg). However, as existing PEF systems and experimental conditions are diverse, much more research is needed to assess properly the effect of critical processing factors on pathogens of concern, kinetics of inactivation, absence of potential health risks and process impact on food components. The economic feasibility of PEF compared to traditional pasteurization systems is also an issue (Butz and Tauscher, 2002).

9.5 Oscillating magnetic fields

The region in which a magnetic body is capable of magnetizing the particles present is called the magnetic field. When the susceptibility to magnetization is equal in all dimensions, the particle possesses isotropic susceptibility. On the other hand, when the susceptibility to magnetization is unequal along each dimension, the particle possesses anisotropic susceptibility. Isolated carbon atoms exhibit isotropic susceptibility, whereas two carbon atoms bonded by single, double or triple bonds exhibit anisotropic susceptibility (Barbosa-Cánovas et al., 1998).

Magnetic fields are differentiated as static (SMF) or oscillating (OMF). An SMF exhibits constant field intensity with time, and the direction of the field remains the same. An OMF, applied in the form of pulses, reverses the charge for each pulse and the intensity of each pulse decreases with time to about 10% of the initial intensity. Magnetic fields can also be classified as homogeneous or heterogeneous. In a homogeneous magnetic field the field intensity is uniform in the area enclosed by the magnetic coil, whereas in a heterogeneous one the field intensity is not uniform as it decreases at greater distances from the center of the coil. In addition, heterogeneous fields exert an accelerating force on the particles—an effect that is not present in homogeneous fields. Magnetic fields are usually generated by supplying current to electric coils. Magnetic flux is measured in weber (Wb), so that 1 Wb = 10^8 magnetic lines. Magnetic density is flux per unit area (Wb/m^2). The inactivation of microorganisms requires magnetic flux densities of 5–50 T (tesla); 1 T = 1 Wb/m^2 (Barbosa-Cánovas et al., 1998).

The influence of static and/or oscillating magnetic fields on living organisms became evident in the early twentieth century with the observation of protoplasmic streaming in cells, which was accelerated or retarded according to the direction of applied magnetic fields. Homogeneous magnetic fields do not have any effect on the morphology, growth or reproduction of microorganisms; but heterogeneous ones do, being able to translocate free radicals such as OH● or O● and produce metabolic interruptions. A characteristic example is the inhibition of budding in yeast cells (Barbosa-Cánovas et al., 1998). Biological membranes exhibit strong orientation in a magnetic field because of their intrinsic anisotropic structure. Orientation of cell membranes parallel or perpendicular to the applied magnetic field depends on the overall anisotropy of the biomolecules, mainly proteins associated with the membrane. Resonating peptide bonds possess diamagnetic anisotropy and therefore tend to orientate parallel to an external magnetic field, as does the plane of carbon–carbon double bonds. In addition, cell division rates can be altered by changes in ion...
flux across the plasma membrane following the application of a magnetic field (Maret and Dransfield, 1985).

Several experiments have shown that a strong SMF or a moderate OMF has the potential to inactivate both vegetative and spore forms. A single pulse with a flux density of between 5 T and 50 T and a frequency of 5–500 kHz reduces the number of microorganisms by at least 2 log cycles. The technology of inactivating microorganisms by placing foodstuffs in magnetic fields has significant potential use, such as stopping a fermentation process at the right time, or the improvement of quality and shelf-life extension of pasteurized foods (Barbosa-Cánovas et al., 1998). Microorganisms inactivated by magnetic fields have included *S. thermophilus* in milk, *Saccharomyces* spp. in yogurt and orange juice, and bacterial spores in dough (Hofmann, 1985).

Generally speaking, preservation of foods with magnetic fields involves sealing the food in a plastic bag and subjecting it to 1–100 pulses in an OMF with a frequency of between 5 kHz and 500 kHz, at a temperature of 0–50 °C for a total exposure time of between 25 µs and 10 ms. Frequencies higher than 500 kHz are less effective, and also tend to increase the temperature significantly. Exposure time results from the product of the number of pulses and the duration of each pulse. The duration of each pulse includes 10 oscillations, after which the substantially decayed magnetic field has a negligible effect. No special preparation of food is required before treatment by OMF, although metal packages cannot be used. These treatments are carried out at atmospheric pressure with no detectable changes in quality (Barbosa-Cánovas et al., 1998).

The main technological advantages associated with magnetic fields are minimal thermal denaturation of nutritional and organoleptic properties, reduced energy requirements for adequate processing, and the potential for treatment of foods inside a flexible film package (which preclude post-process contamination). Yet there are some important issues regarding this technique that should be addressed before it becomes commercially feasible, including a thorough understanding of the mechanism of microbial inactivation, the precise correlation between OMF operational parameters and microbial inactivation, and the long-term health effects on magnetic-field machine operators (Barbosa-Cánovas et al., 1998).

### 9.6 Ultraviolet radiation

UV is a form of non-ionizing radiation having a wavelength of between 200 and 400 nm. It is usually divided into long-wave UV (UVA), with a 320–400 nm wavelength range; medium-wave UV (UVB), with a 280–320 nm range; and short-wave UV (UVC), with a 200–280 nm range. Low-pressure mercury gas (< 10 Torr) able to emit UV at 254 nm is commonly used for UV radiation, although there are also medium-pressure UV lamps (*ca.* 1000 Torr) that emit radiation between 185 and 1367 nm (Bintsis et al., 2000).

UVC is lethal to most microorganisms, including bacteria, viruses, protozoa, mycelial fungi, yeasts and algae. Lethality is mostly related to the alteration of microbial DNA by dimer formation. The main types of photoprodut in UV-irradiated DNA are pyrimidine dimers, pyrimidine adducts and DNA-protein cross-links.
Once the DNA has been damaged, microorganisms can no longer reproduce. Temperature has little (if any) influence on the microbicidal action of UV radiation, but moisture exerts a strong effect. When bacteria are suspended in air, an increase in relative humidity results in a greatly reduced death rate, especially beyond 50 % RH. Similarly, bacteria in a liquid medium are more resistant (Bintsis et al., 2000).

### 9.6.1 Disinfection of surfaces
Packaging materials can be sterilized by arranging appropriate UVC lamps over conveyors. It is key to the success of this process to have clean materials, as any dirt will absorb the radiation and protect any microorganisms present. As an example, during the manufacture of aseptically filled UHT dairy products, UVC sterilization has been applied to cartons and caps of high-density polyethylene bottles (Kuse, 1982; Nicholas, 1995).

UVC can also be employed to treat the actual surface of foods. Thus it has been used to control *B. stearothermophilus* in thin layers of sugar, or *Pseudomonas* spp. on meat surfaces. It is important to mention that sometimes UVC treatment leads to the development of off-flavors in meat and milk, due to the absorption of ozone and nitrogen oxides as well as from direct photochemical effects on the lipid fraction. Filtering UVC by covering the product with a thin layer of inert gas before irradiation can reduce this undesirable effect. Other applications include the inactivation of mold spores from the surface of baked goods (Sharma, 1999), reduction of total aerobic and mold numbers from the surface of eggs (Kuo et al., 1997), and the extension of the shelf-life of fresh fish by reduction of initial contamination (Huang and Toledo, 1982).

Wright et al. (1999) considered using UVC for reducing *E. coli* O157:H7 in unpasteurized cider. Cider containing a mixture of acid-resistant strains of the pathogen (6.3 log CFU/ml) was treated using a thin-film UVC disinfection unit with output at 254 nm. Dosages ranged from 9402 to 61 005 µWs/cm². Treatment significantly reduced *E. coli* O157:H7, with a mean reduction of 3.81 log CFU/ml. This reduction was affected by the level of background flora in cider, but ultimately the technique proved effective for reducing and possibly eliminating the pathogen.

### 9.6.2 Disinfection of air
UVC lamps are typically used in hospitals to create a curtain or barrier through which air must pass before reaching patients sensitive to infection (Bintsis et al., 2000). For the handling of sensitive foodstuffs, a system combining laminar flow of air to remove particles of size > 0.1 µm and UVC radiation to kill any remaining live microorganisms has been suggested as a feasible means to supply clean sterilized air, particularly in food cold-storage areas (Decupper, 1992; Shah et al., 1994). The microbiological quality of mechanically peeled fruits and vegetables is improved when UV-treated air is blown through the peeling unit, countercurrent to the flow of product (Dornow, 1992).

### 9.6.3 Disinfection of liquids
UVC is one of the simplest and most environmentally friendly ways of destroying a wide range of microorganisms in water (Gray, 1994). It has been used to disinfect sewage effluent, drinking water and water for swimming pools, and the combination
of UVC and ozone has a very powerful oxidizing effect (WHO, 1994). In most cases UVC can disinfect without any significant change in color, flavor, odor or pH, so that both microbiological safety and appropriate organoleptic quality of water are ensured. The standard is a 99.999 % reduction of microorganisms with a treatment time of less than 1 minute (Urakami et al., 1997). The situation is different in the food industry, where a simple reduction in the water supply microbial load may be sufficient. In this respect, the brewing industry has become a major UVC user (Egberts, 1990). The treatment of opaque liquids is, however, a problem, considering the poor penetration of UVC. Yet there have been some indications that the US Food and Drug Administration may allow pathogen elimination from fruit juices using UVC, as long as the flow of the juice is turbulent rather than laminar and the temperature is continuously kept below 5 °C (Bintsis et al., 2000).

The increased use of UVC radiation as a drinking-water treatment has instigated studies of the injury repair potential of microorganisms following treatment. Zimmer and Slawson (2002) challenged the repair potential of an optimally grown non-pathogenic laboratory strain of *E. coli* after UVC radiation from low- and medium-pressure lamps. Following irradiation, samples were incubated at 37 °C under photo-reactivating light or in the dark. Samples were analyzed for up to 4 hours following incubation, using a standard plate count. These researchers found that *E. coli* was capable of undergoing photo-repair following exposure to the low-pressure UVC source, but no repair was detected after exposure to the medium-pressure UVC source. Eventually, minimal injury repair was observed in the latter case at very low doses of 3 mJ/cm². This study clearly indicated differences in repair potential according to the UVC source used.

UV A is far less effective as a biocidal agent than UVC. For example, the incident energy required to bring about 50 % reduction in microbial viability is 5 J/m² using UVA, whereas UVC can achieve the same result with only $10^{-5}$ J/m² (Bintsis et al., 2000). The mode of action of UVA on microbial cells is significantly different from that of UVC (Moss and Smith, 1981).

### 9.7 Light pulses

Pulsed light is a method of food preservation that involves the use of intense, short-duration pulses of broad-spectrum light, ranging from ultraviolet to near infrared. The material to be sterilized is exposed to at least one pulse of light having an energy density in the range of 0.01–50 J/cm² at the surface, using a wavelength distribution such that at least 70 % of the electromagnetic energy is distributed in the 170–2600 nm range. The process inactivates a wide range of microorganisms, including bacterial and fungal spores. Filtering of the spectrum eliminates wavelengths that may adversely affect food flavor and quality. Duration of the pulses used ranges from 1 µs to 0.1 s, at a rate of 1–20 flashes per second. For most applications, a few flashes applied in a fraction of a second provide high levels of microbial inactivation. Thus, the process is very fast and suitable for high throughput (Barbosa-Cánovas et al., 1998).

This technology is mainly applicable in sterilizing or reducing the microbial population on the surfaces of packaging materials, packaging and processing...
equipment, foods and medical devices, as well as many other surfaces. As light pulses penetrate many transparent packaging materials, wrapped items can also be treated. Applications within the food industry include aseptic processing, liquid foods, solid foods (e.g. fish and meat products) and baked goods. With regard to aseptic processing, packaging materials are traditionally sterilized with hydrogen peroxide, but residues of this chemical may be undesirable. Light pulses have the potential to reduce or even eliminate chemical disinfectants and preservatives (Barbosa-Cánovas et al., 1998).

On a smooth, non-porous surface, light pulses can lower the vegetative and spore populations of microorganisms by about 9 and 7 log cycles, respectively. On porous and complex surfaces such as meat, approximately 1–3 log cycle reductions are obtained (Barbosa-Cánovas et al., 1998). In the case of meat products, thin slices will allow light penetration through the food material. Based on the same principle, white bread slices treated through the packaging material maintained a fresh appearance for more than 15 days, whereas untreated slices became moldy. Prepared and processed meat products, such as sausages and ground meat patties, can be treated to increase their shelf-life under refrigeration without the necessity for freezing. Similarly, vegetables such as tomatoes and potatoes, fruits such as apples and bananas, and prepared food products such as pastas and rice entrees can be treated to increase shelf-life, with minimal changes in nutritional quality (Rice, 1994).

A variety of microorganisms, including *E. coli*, *S. aureus*, *B. subtilis* and *S. cerevisiae*, have been inactivated by using between 1 and 35 pulses of light with intensities ranging from 1 to 12 J/cm². Greater inactivation can be obtained when full-spectrum light rather than glass-filtered light spectra are used. Thus, it appears that the UV component of light is essential to inactivate microorganisms using light pulses (Dunn et al., 1991). The antimicrobial effect is primarily related to light absorption by highly conjugated carbon-to-carbon double-bond systems in proteins and nucleic acids, and evidently a similar mechanism to that of conventional continuous UV sources is involved. However, it has also been suggested that pulsed light causes an instantaneous lethal heating of the cell, leading to lysing or rupture of the cell wall (Dunn et al., 1995; McDonald et al., 2000; Wekhof, 2000).

A comparison of the disinfection rates due to pulsed light with those under conventional UV exposure suggests that doses for sterilization by the former are at least one order of magnitude lower than those of the latter. *B. subtilis*, for example, was sterilized (99.999 % destruction) by about 42 600 µWs/cm² of UV, but the same level of inactivation required a dose of only 4500 µW.s/cm² under pulsed light. Clearly, pulsed light results in an apparent synergy of the pulsed energy quanta as compared to the relatively continuous stream of lower density conventional UV quanta (Wekhof, 1991; Dunn, 2000).

Pulsed light can be used to enhance product shelf-life and safety. Reductions of 2 log cycles were achieved with respect to *Salmonella* on chicken wings, *Listeria* on hot dogs, and both pathogens on primal and retail beef cuts (Dunn et al., 1995). Curds of cottage cheese were inoculated with *Pseudomonas* and subjected to light with an energy density of 16 J/cm² and pulse duration of 0.5 ms. After only two flashes, viability was reduced by 1.5 log cycles. The temperature at the surface of the
curd closest to the light source increased by 5 °C. Sensory evaluation using a trained panel showed no effect on cheese taste as a result of the light treatment (Dunn et al., 1991). Pulsed light was also very effective in eliminating microbial contamination (mainly S. Enteritidis) from the surface of shell eggs. As much as an 8 log-cycle reduction was obtained. In fact, the inactivation effect was not limited to the surface but also extended to a certain degree into the egg shell pores (Dunn, 1996).

In vegetables and fruits, pulsed light was able to inactivate polyphenol oxidase, the enzyme that causes browning (Dunn et al., 1991). Spores of Bacillus cereus and Aspergillus niger were inactivated by pulsed light from the surface of different packaging materials, whereas higher levels of inactivation of bacterial spores were achieved in water. In general, mold spores are more resistant to pulsed light than are bacterial spores (Dunn et al., 1991; Dunn, 1996). DNA damage, such as the formation of single strand breaks and pyrimidine dimers, was induced in yeast cells by Takeshita et al. (2003) after irradiation by pulsed light. The effect was essentially the same as that observed with continuous UV light.

The generation of the pulsed light requires a considerable amount of energy. Thus, the power consumption of a typical pulsed light system is about 1000 W while similar results can be achieved with a conventional UV system drawing only 10 W of total power. Applications are consequently limited to situations where the benefits of achieving rapid sterilization outweigh the cost of pulse generation (Dunn et al., 1997).

9.8 Ultrasound

Ultrasound refers to the result of an event generating pressure waves with a frequency of 20 kHz or more. In general, ultrasound equipment uses frequencies ranging from 20 kHz to 10 MHz. High-power ultrasound at lower frequencies (20–100 kHz) has the ability to create cavitation. This feature has value in inactivating microorganisms (Butz and Tauscher, 2002).

Investigation of ultrasound as a potential microbial inactivation method began in the 1960s (Earnshaw et al., 1995). The mechanism of microbial killing is mainly thinning of cell membranes, localized heating, and production of free radicals (Fellows, 2000). During the sonication process, longitudinal waves are generated when a sonic wave meets a liquid medium, thereby creating regions of alternating compression and expansion (Sala et al., 1995). These regions of pressure change cause cavitation, and gas bubbles are formed in the medium. These bubbles have a larger surface area during the expansion cycle, which increases the diffusion of gas, and the bubbles expand. When the ultrasonic energy is no longer sufficient to retain the vapor phase in the bubble, rapid condensation occurs. The condensed molecules collide violently, creating shock waves that form localized areas of very high temperature and pressure (up to 5500 °C and 50 000 kPa). The pressure changes yield the main bactericidal effect in ultrasound treatments (Piyasena et al., 2003). The cavitation threshold of a medium (i.e. the minimum oscillation of pressure that is required to produce cavitation) depends on the dissolved gas, hydrostatic pressure, specific heat of the liquid, the nature of gas in the bubble, and the tensile strength of the liquid. Another essential variable is temperature, which is inversely proportional to the cavitation threshold. In
addition, we now know that the ultrasonic frequency must be below 2.5 MHz, as cavitation will not occur above this value (Rahman, 1999).

Ultrasound is much more effective when combined with pressure (manosonication), heat (thermosonication), or both (manothermosonication). The enhanced mechanical disruption of cells is the explanation for enhanced killing (Sala et al., 1995). It has also been suggested that microbial inactivation by ultrasound is more effective when combined with other decontamination techniques such as heating, chlorination or extreme pH (McClements, 1995).

Pagan et al. (1999) studied the application of ultrasound for the inactivation of *L. monocytogenes*. Ultrasonic treatment alone (20 kHz and 117 µm) at ambient temperature was not very effective, with a *D*-value of 4.3 minutes. However, the combination with pressures of 200 and 400 kPa lowered the *D*-values to 1.5 and 1.0 minutes, respectively. An amplitude increase of 100 µm decreased the resistance of the pathogen to manosonication by a factor of 6. Temperatures up to 50 °C did not have any significant effect on inactivation; but once they exceeded this threshold, an enhanced effect was noted. These authors also found that growth temperature affected heat resistance of *L. monocytogenes*. Cultures grown at 37 °C were found to be twice as heat-resistant as those grown at 4 °C; however, the cell growth temperature did not change the effect of manosonication itself. In addition, lower pH values resulted in greater inactivation rates, whereas greater sucrose concentrations increased *D*-values.

*Salmonella* Enteritidis, *S. Typhimurium* and *S. Senftenberg* were investigated by Manas et al. (2000) for their resistance to heat treatment, manosonication and manothermosonication in liquid whole eggs and citrate phosphate buffer solution. With manosonication (117 µm, 200 kPa, 40 °C), *S. Enteritidis*, *S. Typhimurium* and *S. Senftenberg* had *D*-values of 0.76, 0.84 and 1.4 minutes in whole egg, and 0.73, 0.78 and 0.84 minutes in citrate phosphate buffer, respectively. In comparison, *D*-values at 60 °C were 0.068, 0.12 and 1.0 minutes for the buffer, and 0.12, 0.20 and 5.5 minutes for whole egg, respectively. A linear increase in ultrasonic wave amplitude resulted in an exponential increase in the inactivation rate of the manosonic treatment. When manothermosonication (117 µm, 200 kPa, 60 °C) was applied an additive effect resulted, with a reduction of 3 log cycles for the most resilient of the pathogens, *S. Senftenberg*.

The effects of ultrasound on *E. coli* in an aqueous medium, using a frequency of 24 kHz with varying intensities, were examined by Scherba et al. (1991). A significant reduction of the bacterial population was achieved, which increased with treatment time; however, intensity did not affect the killing rate. Utsonomiya and Kosaka (1979) noted that the initial temperature, medium, and pH influenced the survival of *E. coli* treated at 700 kHz. When the pathogen was suspended in saline at 32 °C, survival rates of 0.83 % and 0.2 % were obtained after 10 and 30 minutes of treatment, respectively; however, at an initial temperature of 17 °C, these values increased to 37.9 % and 8.1 %. No inactivation occurred in milk. When 10 % orange juice was added to milk, only 0.3 % survival was found at pH 2.6, whereas the survival was 100 % at pH 5.6.

The use of ultrasound to inactivate *E. coli* in biofilms could be beneficial to the food and water-bottling industries. For example, Johnson et al. (1998) reported that
the combination of 70 kHz with gentamicin sulfate, an antibiotic, reduced *E. coli* numbers in a biofilm by up to 97% in 2 hours. The main reason for the enhanced killing was increased diffusion of the antibiotic through the cell membrane, as the lipopolysaccharide layer of the outer cell membrane was believed to be destabilized by ultrasound. However, there are obvious concerns about utilizing a combination of ultrasound with an antibiotic in food processing. Rather, these types of combinations should be more suitable for the removal of biofilms from medical devices.

Raso *et al.* (1998a) carried out comparisons between manosonication and manothermosonication for the inactivation of spores of *B. subtilis*, and found that heat treatment provided by the latter made the inactivation process more effective.

Ultrasound, whether used alone, with thermal challenge or in combination with pressure treatment, could be an important element in food-processing technology because it is more effective and energy-efficient compared to conventional heat treatments.

### 9.9 High pressure

The technology of high-pressure processing (HPP) has been known for more than a century, but relatively recent scientific and technical progress has led to a renaissance regarding its utilization. A number of pressure-treated food products are already present in the Japanese, French, Spanish and American markets. HPP is a flexible technique that can subject liquid and solid foods, with or without packaging, to pressures between 100 MPa and 800 MPa, at temperatures that range from below 0 °C to beyond 100 °C, with exposure times that can range from a few seconds to more than 20 minutes. Food treated in this way keeps its original freshness, color, flavor and taste, as HPP acts instantaneously and uniformly throughout a mass of food, independently of size, shape and composition (Butz and Tauscher, 2002). Because pressure is uniform throughout the food, preservation is uniform, with no particle escaping treatment. In addition, and unlike thermal treatment, HPP is not time/mass dependent (Barbosa-Cánovas *et al.*, 1998).

Compression can increase the temperature of foods approximately by 3 °C per 100 MPa, and may also shift the pH of the food. Nevertheless, pressure pasteurization is also feasible at room temperature. Water activity and pH are critical process factors in the inactivation of microbes by HPP. Besides destruction of microorganisms, there are further influences of pressure on food materials, namely protein modification or denaturation, enzyme activation or inactivation, changes in enzyme-substrate interactions, and changes in the properties of polymerized carbohydrates and fats (Butz and Tauscher, 2002).

There are three main ways of generating high pressures (Deplace and Mertens, 1992):

- **Direct compression**, where the medium is pressurized with the small-diameter end of a piston and the large-diameter end is driven by a low-pressure pump. This method allows very fast compression, but the limitations of the high-pressure dynamic seal between the piston and the vessel internal surface restricts its use to small-diameter laboratory or pilot plant systems.
- **Indirect compression**, where a high-pressure intensifier pumps a pressure medium from a reservoir into a closed high-pressure vessel until the desired pressure is reached. This is the most widespread industrial procedure.
Pressure-medium heating, where high pressure is generated by expansion of the pressure medium through high temperature. Thus, heating of the pressure medium is used when high pressure is applied in combination with heat treatment of food; it requires accurate temperature control within the entire internal volume of the pressure vessel.

Microbial inactivation is probably due to a number of factors, including protein conformation changes and membrane perturbation, leading to cell leakage. Moderately high pressures decrease the rate of growth and reproduction, whereas high and very high pressures cause microbial inactivation. The threshold pressures for retardation of reproduction and/or inactivation are dependent on the microorganism and species. In general, Gram-positive organisms are more resistant to pressure than Gram-negative ones (Barbosa-Cánovas et al., 1998; Farkas, 2001). Those biochemical reactions in which reactants undergo either a decrease or increase in free volume are the ones most affected by HPP, because pressure causes either a decrease in the available molecular space or an increase in chain interactions. On the other hand, reactions involving formation of hydrogen bonds are favored by high pressure because bonding results in a decrease in volume. As a result of HPP, proteins are denatured (disruption of hydrophobic and ion-pair bonds), whereas nucleic acids are baroresistant. However, DNA transcription and replication are disrupted by high pressure due to the involvement of enzymes which can be inactivated. Different enzymes have considerable differences in barosensitivity. Membrane phospholipids suffer conformational changes, which disrupts membrane permeability (Hedén, 1964; Hoover et al., 1989; Farr, 1990; Knorr, 1993).

The mode of action of pressure on bacterial spores is still not fully understood. Bacterial spores are killed directly by pressures higher than 1000 MPa, and they are sensitive to pressures of between 50 and 300 MPa. It is generally agreed that at such pressures spores germinate, followed by death of the germinated spore. However, it is not known whether pressure induces spore activation similar to reversible heat activation or triggers germination irreversibly. Ultimately, temperature and pressure ranges for germination and inactivation depend on the spore species (Smelt, 1998).

The pH of a food material plays a very important role in determining the extent to which HPP affects microorganisms. Yeasts and molds are quite resistant to low pH, and a pH < 4.0 does little to sensitize these organisms to pressure. By comparison, vegetative bacteria are quite sensitive to a combination of pressure and low pH. Low aw protects cells against pressure, but microorganisms injured by pressure are generally more sensitive to low aw. The net effect of aw is not always easy to predict (Smelt, 1998; Tewari et al., 1999). Microorganisms are particularly sensitive to nisin during or after pressure treatment. Masschalk et al. (2001) have proposed a mechanism of pressure-promoted uptake of nisin to explain the sensitization.

HPP can be combined with other processing techniques to enhance microbial inactivation. Raso et al. (1998b) studied the inactivation of Y. enterocolitica by combining ultrasonication, pressure and heat. The lethal effect of ultrasonication (20 kHz, 150 µm) increased with rising pressure until maximum inactivation occurred at an optimum pressure of 400 kPa. Pagán et al. (1998) studied the possibility of germinating Bacillus spores using HPP, then inactivating the germinated cells with a PEF treatment.
They found that germination of more than 5 log cycles of spores was initiated by pressurization, and that while the germinated cells did become sensitive to a subsequent heat treatment, they were not sensitized to PEF application below 40 °C.

Shigehisa et al. (1991) reported complete destruction of S. Typhimurium in beef at 300 MPa after 10 minutes at 25 °C. Carlez et al. (1994) reported that HPP caused reduction of vegetative mesophilic and psychrotrophic contaminants, and destruction of coliforms and S. aureus. Murano et al. (1999) considered combining HPP and temperature to inactivate L. monocytogenes in pork patties. Samples were inoculated with $10^9$ CFU/ml of the pathogen, vacuum-packed, and treated with a number of combinations of high pressure and temperature. Lowest $D$-values, ranging from 0.37 to 0.63 minutes, depending on the Listeria strain, were obtained using 414 MPa for 6 minutes at 50 °C.

Erkmen and Karatas (1997) studied the effect of HPP on S. aureus in milk at pressures in the range of 50–350 MPa for up to 12 minutes at a constant temperature (20 ± 2 °C). No survival was found at 350 MPa for 6 minutes and 300 MPa for 8 minutes. Ponce et al. (1998) investigated the inactivation of L. innocua inoculated in liquid whole egg using HPP by subjecting the food to different combinations of pressure, temperature and time. Reductions of greater than 5 log cycles were obtained at 2 °C for 15 minutes using 450 MPa. Reduction values were greater than at room temperature – an effect that was explained as being due to the greater susceptibility of some proteins to denaturation at low temperatures. The effect of HPP on the survival of a pressure-resistant strain of E. coli O157:H7 in orange juice was investigated by Linton et al. (1999) over the pH range 3.4–5.0. The juice was inoculated with $10^8$ CFU/ml of the pathogen and subjected to pressure treatments of 400, 500 and 550 MPa at 20 °C and 30 °C. A pressure treatment of 550 MPa for 5 minutes at 20 °C produced a 6 log cycle inactivation at pH 3.4, 3.6, 3.9 and 4.5, but not at pH 5.0. Combining pressure with mild heat (30 °C) did result in a 6 log cycle reduction at pH 5.0. Thus, it was concluded that microbiological safety of orange juice is achievable through appropriate combinations of HPP, temperature and time.

HPP is a very promising non-thermal food preservation method, not only for its inherent ability to cause proper microbial inactivation, but also due to the fact that the rheological and functional properties of foods remain unaltered (Barbosa-Cánovas et al., 1998). Although much research has been done, there is still a great deal to be discovered regarding critical limits of the process and the extent to which this might ensure appropriate treatment of food materials. HPP commercialization will depend on its economic viability (Tewari et al., 1999).

### 9.10 Food irradiation

Food irradiation is a non-thermal technology that involves exposing prepackaged or bulk foodstuffs to gamma rays, X-rays or electrons. The most common method is gamma radiation from a radioisotope source, typically cobalt 60. If electrons or X-rays are utilized, they are generated by an electron accelerator. The cost of gamma radiation is competitive with that of other methods of food preservation (Barbosa-Cánovas et al., 1998).
The degree of chemical and physical change produced when food is exposed to high energy radiation is determined by the energy absorbed. In irradiation processing, it is described as the absorbed dose, measured in units of kilogray (kGy), where 1 gray (Gy) has an energy absorption equivalent of 1 J/kg. The following terms are used to describe the application of radiation in foods (Barbosa-Cánovas et al., 1998):

- **Radiciation** is the application of ionizing radiation sufficient to reduce the number of specified viable non-spore-forming pathogenic bacteria to such a level that none is detectable in the treated food when examined by recognized bacteriological testing methods: it is a treatment with relatively low doses (0.1–8 kGy).
- **Radurization** is the application of ionizing radiation sufficient to cause a substantial reduction in the numbers of specific viable spoilage microorganisms: it involves doses of about 0.4–10 kGy to improve the shelf-life of a product.
- **Radappertization** is the application of ionizing radiation sufficient to reduce the number and/or activity of viable microorganisms (with the exception of viruses) to such a level that very few, if any, are detectable by recognized bacteriological or mycological testing methods applied to the treated food. No spoilage or toxicity of microbial origin must be detectable no matter how long or under what conditions the food is stored after treatment with doses of about 10–50 kGy to bring about virtually complete sterilization.

In 1983, on the basis of international agreement, the Joint Food and Agriculture Organization/World Health Organization Codex Alimentarius Commission accepted food irradiation as a safe and effective technology for the treatment of food and adopted a Codex General Standard for Irradiated Foods with an associated Code of Practice. Although the use of irradiation continues to grow worldwide, negative reactions in various countries have restricted its expansion. Introduction of commercial applications is rather slow because many governments require extensive data to support the wholesomeness of irradiated food, which leads to lengthy regulatory and approval-granting processes (Barbosa-Cánovas et al., 1998). It is essential to emphasize that food irradiation is neither a miracle food preservation method nor a sinister technology. It has advantages and limitations like any other food preservation method (Castleman, 1993).

There are three main advantages associated with the use of irradiation in foods (Barbosa-Cánovas et al., 1998): the ability to replace chemical treatments that are increasingly coming under suspicion or are even being banned; the capacity to slow the rate of food deterioration, thus extending shelf-life; and flexibility in application to packages or bulk units, in the frozen state or at room temperature.

Ionizing radiation is lethal for bacteria. The critical target for inactivation is the DNA, resulting in the loss of ability to reproduce, although alteration of membrane properties has also been indicated as a significant mechanism of inactivation. The proportion of a bacterial population that survives a given dose of irradiation depends on the intrinsic sensitivity of the microorganism, the stage of its growth cycle, the amount of irradiation damage inflicted, and the microorganism’s potential for repair. Irradiation sensitivity differs with species and among strains, although the range of resistance among strains of a single species is usually small enough to be considered negligible (Ingram and Roberts, 1980; Moseley, 1990).
Bacterial spores are more resistant than their corresponding vegetative cells by a factor of 5–15 (Moseley, 1989). In general, the irradiation resistance of molds is equivalent to that of vegetative bacteria. Yeasts are more resistant than molds, and as resistant as bacterial spores. Viruses are even more irradiation-resistant than bacteria, so that irradiation treatments that destroy bacteria will not reliably inactivate viruses. Although there is no definitive explanation for microbial resistance to ionizing radiation, it is evident that the mechanism involves enzymatic activity able to repair radiation damage of nucleic acids (Ingram and Roberts, 1980; Jay, 1996).

Gram-negative bacteria involved in the spoilage of refrigerated fresh meats are more sensitive to irradiation than are lactic acid bacteria. For this reason, the use of low doses of irradiation that would inactivate those spoilage organisms, but not pediococci and/or lactobacilli, have potential application in fermented sausage production (Monk et al., 1995). Of the Gram-negative pathogens, Salmonella is considered the most resistant. Thus, irradiation processes designed to eliminate this pathogen will also eliminate Escherichia, Yersinia, Aeromonas and Campylobacter species (Radomyski et al., 1994). Irradiation is also very effective for eliminating S. aureus from meat products (Monk et al., 1995).

Irradiation inactivation of L. monocytogenes is possible, even at relatively high concentrations (10^5 CFU/g), without inducing noticeable modifications to the taste, odor or textural properties (Ennahar et al., 1994). As in heat processing, the 12D reduction concept is applied to the process of radappertization designed to kill C. botulinum spores (Monk et al., 1995).

Aziz and Moussa (2002) studied the effect of gamma irradiation on the production of mycotoxins in fruits. Irradiation at doses between 1.5 and 3.5 kGy significantly decreased the total fungal counts compared to un-irradiated controls. After 28 days of storage at refrigeration temperatures, the un-irradiated fruits contained high concentrations of mycotoxins as compared with samples irradiated with 3.5 kGy. Mycotoxin production decreased with increasing irradiation dose, and mycotoxins were not detected after doses ≥ 5.0 kGy.

Radiation treatments at doses of 2–7 kGy can effectively eliminate potentially pathogenic non-spore-forming bacteria, including Salmonella spp., E. coli O157:H7, S. aureus, Campylobacter spp. and L. monocytogenes, without affecting sensory, nutritional and textural qualities. Candidates for radiation decontamination include poultry and red meats, egg products, fish and seafoods, and fresh fruits and vegetables. A unique feature is that irradiation can be applied to food in the frozen state. With today’s demand for high-quality, convenient foods, irradiation in combination with other processes holds promise for enhancing the safety of many minimally processed foods. Radiation decontamination of dry ingredients, herbs and enzyme preparations with doses of 3–10 kGy has proven an alternative to fumigation with microbicidal gases. Radiation treatments at doses of 0.15–0.7 kGy appear to be feasible to control foodborne parasites (Farkas, 1998).

To promote worldwide introduction of food irradiation, it is necessary to develop national and international legislation and regulatory procedures to enhance confidence among trading nations that foods irradiated in one country and exported are irradiated under acceptable standards of wholesomeness, irradiation dose and
hygienic practice (Barbosa-Cánovas et al., 1998). Identifying irradiated food as such
(i.e. properly informing consumers) is essential to reassure the public that consumer
rights are protected. This is not trivial, because the effects produced by irradiation are
often small and may be similar to changes produced by other means of food preserva-
tion (Brynjolfsson, 1989; Johnston and Stevenson, 1990). With regard to labeling,
some countries require irradiated foods to be labeled with the characteristic green
radura symbol and appropriate descriptive words; other nations demand just the
radura symbol and no descriptive words, while others do not require any special iden-
tification. A universal labeling system is therefore imperative (Morehouse, 2002).

10 Plant hygiene and contamination during food processing

10.1 Introduction

Bacteria may be transferred to food by the production environment and personnel,
either directly or by cross-contamination through surfaces, equipment, utensils
and/or hands that have not been properly cleaned or disinfected. Cleaning and disin-
fection are two separate but closely related concepts. Cleaning is removing dirt and a
portion of the microorganisms present, whereas disinfection is treating the surfaces
in such a way that the remaining microorganisms are killed or reduced to an accept-
able level. Cleaning comes always first, otherwise the subsequent disinfection will be
less effective. Zoning – i.e dividing the production area into dry and wet and/or high-
medium- and low-care areas – is also useful in preventing product contamination.
Nowadays zoning has evolved into a complex set of measures including equipment
layout and design, air filtration, personnel hygiene, routes for personnel movement,
and appropriate cleaning and disinfection procedures. Considering the cost involved,
it is evident that zoning is only useful if applied logically (i.e. embedded in Good
Manufacturing Practices, GMP).

Adoption and use of GMP and control through HACCP, coupled with equipment
that is easier to keep clean as well as air-conditioned processing environments, have
changed the profile of problematic organisms faced by the industry, but have not elim-
inated them. For example, outbreaks of foodborne illness caused by Staphylococcus
aureus are significantly lower than 30 years ago; however, problems caused by
psychrotrophic organisms are likely to increase as we continue to demand longer
refrigerated shelf-life from perishable products. It is of interest to consider the fact that
a subset of psychrotrophs that are alkali-tolerant could gain a selective advantage in
food-processing environments where only alkaline cleaners and sanitizers are used.
These psychrotrophic alkalitrophs include the pathogens Listeria monocytogenes,
Yersinia enterocolitica and the potential pathogen Aeromonas hydrophila. Although
not psychrotrophs, Campylobacter spp. are alkalitrophic and may be afforded the
same selective advantage. Following rigorous cleaning, routine sequential exposure of
food-contact surfaces to both alkali- and acid-based cleaners/sanitizers is imperative
to control these organisms.
Introduction of potential pathogens via raw materials can lead to the establishment of foci or niches where these organisms may persist in the processing plant. Examples include hollow rollers on conveyors, cracked tubular equipment port rods, small spaces or gaps between close-fitting metal–metal or metal–plastic parts, valves and switches, or even saturated insulation. Failure to find and remove organisms from these niches may mean that these ‘house flora’ are periodically shed and organisms find their way into processed product. This type of event can lead to periodic foodborne illness outbreaks on an irregular basis over weeks or months that may involve large or small clusters of people scattered over the region served by the food plant. This pattern is typical of the type of problem caused by \( L. \text{monocytogenes} \) when it has established itself in a niche on equipment surfaces. Twelve such instances were reported in a variety of food plants (meat, dairy, fish) located in five European countries and the US between 1975 and 2000 (Tompkin, 2000).

Colonization of food-processing facilities and equipment by undesirable organisms that can withstand challenges generated by food processing is important. Listeriosis in Switzerland was traced to consumption of smear-ripened soft cheese. The same organism had survived on wooden shelves in 12 cheese-ripening cellars from which cheese was supplied. It was evident that the organism is able to adapt to different plant environments, survive for years, and contaminate products (Davidson and Harrison, 2002).

In a recent study, Autio et al. (2002) examined restriction endonuclease patterns or pulsotypes of \( L. \text{monocytogenes} \), isolated from a variety of foods produced in 41 different plants in 10 different European countries, using pulsed field gel electrophoresis. Some of the pulsotypes were repeatedly recovered from the same product made by the same producer, which suggested persistence of the strain in the processing plant. In contrast, other pulsotypes were repeatedly found in products from different producers, suggesting that persistent ‘house strains’ may not always be producer-specific but have a wide geographic distribution. It has become generally accepted over the last 25 years that the preferential mode of existence for bacteria is not as free-living planktonic (free-floating) cells but as complex cellular communities at environmental interfaces. Such communities are referred to as biofilms (Characklis and Marshall, 1990).

### 10.2 Biofilms

**10.2.1 Definition and implications for the food industry**

Formally, biofilms are defined as ‘collections of microorganisms and their associated extracellular products at an interface and generally attached to a biological or non-biological substratum’ (Palmer and White, 1997). There are two main concerns regarding biofilms in the food industry: the presence of biofilms interfering with food-processing operations, and the potential for biofilms to serve as a reservoir for contamination of food with organisms of spoilage or safety concerns (Gill, 1998).

On most of the occasions where biofilms are a nuisance, the terms ‘biofouling’ and ‘microbial-influenced corrosion’ are applied.
10.2.1.1 Biofouling
Biofouling refers to the undesirable formation of a layer of living microorganisms and their decomposition products as deposits on the surfaces in contact with liquid media. Biofilm growth in pipes can reduce fluid flow rate and carrying capacity, which ultimately results in clogging. Biofilm formation on heat exchange surfaces can rapidly reduce heat transfer rates. The efficiency of ultrafiltration and reverse osmosis membranes may also be reduced by biofilm growth resulting in pore clogging (Kumar and Anand, 1998).

10.2.1.2 Microbial-influenced corrosion
Microbial-influenced corrosion is a process whereby the corrosion of metal surfaces is increased by the presence of biofilms. Corrosion results from a potential difference being created between sites on the surface that are covered by biofilm and those exposed to the surrounding liquid environment. This phenomenon can also result in the production of acid or of sulfides by sulfate-reducing bacteria (Little et al., 1990).

10.2.1.3 Biofilm accumulation
Common places for biofilm accumulation are floors, wastewater pipes, vents in pipes, rubber and teflon seals, conveyor belts, stainless steel surfaces, etc. (Blackman and Franck, 1996). Herald and Zottola (1988) observed that L. monocytogenes was able to attach to stainless steel through attachment fibrils. The pathogen also attached to glass, polypropylene and rubber (Mafu et al., 1990), and produced a sanitizer-resistant biofilm on glass, stainless steel and rubber surfaces (Ronner and Wong, 1993). Numbers of bacteria recovered from these surfaces were high, and dependent on the length of exposure time. It was also found that hydrophobic and electrostatic interactions were responsible for the attachment of L. monocytogenes to these surfaces (Mafu et al., 1991). With regard to food surfaces, studies have shown the attachment of different microorganisms to poultry (Lilliard, 1988) and beef (Butler et al., 1979) surfaces. These organisms, mainly coliforms, have not only been associated with slaughtering processes but are also responsible for cross-contamination of carcasses (Anand et al., 1989).

10.2.2 Biofilm development
Biofilm formation and growth are complex, dynamic processes in which a number of steps can be identified; these are described below.

10.2.2.1 Conditioning of the surface
The formation of a biofilm occurs on virtually any submerged surface in any environment in which the bacteria are present. In food-processing environments, bacteria with organic and inorganic molecules (like proteins and minerals from milk and meat) get adsorbed to the surface, forming a conditioning film. These organic and inorganic substances, together with the microorganisms, are transported to the surface by diffusion or, eventually, by a turbulent flow of the liquid. The conditioning film leads to a higher concentration of nutrients compared to the fluid phase. Nutrient transfer rates are higher in a biofilm than in the aqueous phase.
Conditioning also alters the physicochemical properties of the surface, including surface free energy, hydrophobicity and electrostatic charges (Dickson and Koochmarae, 1989; Jeong and Frank, 1994; Hood and Zottola, 1997).

Nevertheless, there appears to be no evidence that microorganisms always attach to a conditioned surface. In fact, the microtopography of the food-contact surface is equally important to favor bacterial retention, particularly if the surface consists of deep channels and crevices to trap bacteria (Kumar and Anand, 1998). It is also established that adsorption of certain proteins to surfaces plays an important role in the microbial adhesion per se. For example, albumin has been found to be inhibitory for the adhesion of *L. monocytogenes* to silica surfaces (Al-Makhlafi *et al.*, 1995). Milk proteins such as casein and β-lactoglobulin are also able to inhibit the attachment of *L. monocytogenes* and *S. Typhimurium* (Helve *et al.*, 1993). On the other hand, whey proteins seem to favor the attachment of milk-associated microorganisms to stainless steel, rubber and glass surfaces (Speers and Gilmour, 1985).

### 10.2.2.2 Adhesion of cells

The attachment of microorganisms to the conditioned surface may be active or passive, depending on bacterial motility or transportation of the planktonic cells by gravity, diffusion or fluid dynamic forces from the surrounding fluid phase. Adhesion is also dependent on nutrient availability in the surrounding medium, and the growth stage of the bacterial cells themselves (Kumar and Anand, 1998).

Two stages can be identified in this process: a reversible adhesion followed by an irreversible one. Initial weak, long-range interactions developed between bacterial cells and substratum are called reversible adhesion, and involve van der Waals forces, electrostatic forces and hydrophobic interactions. During this stage, bacteria still show Brownian motion and can easily be removed by fluid shear forces (e.g. merely by rinsing). In irreversible adhesion, short-range forces are involved: dipole–dipole interactions, hydrogen, ionic and covalent bonds, and hydrophobic interactions. The contact between bacteria and surface takes place mainly through bacterial appendages such as flagella, fimbriae, pili and fibrils, and removal of cells requires much stronger forces such as scrubbing or scraping (Marshall *et al.*, 1971; Jones and Isaacson, 1983; Hancock, 1991; Kumar and Anand, 1998). Spores exhibit a greater rate of adhesion than vegetative cells due to their relatively high hydrophobicity and hair-like structures on the spore surface. After surface adhesion has taken place, spores may germinate and the resulting vegetative cells multiply and produce exopolysaccharides (EPS) (Rönner *et al.*, 1990; Husmark and Rönner, 1992).

The temperature and pH of the contact surface have an influence on the degree of adhesion of microorganisms. *Yersinia enterocolitica* adhered better to stainless steel surfaces at 21 °C than at 35 °C or 10 °C (Herald and Zottola, 1988), and *Pseudomonas fragi* showed maximum adhesion to stainless steel surfaces at the pH range 7–8, optimal for its cell metabolism (Stanley, 1983).

### 10.2.2.3 Formation of microcolonies

The irreversibly attached bacterial cells proliferate by using the nutrients present in the conditioning film and the surrounding fluid environment. This leads to the
formation of microcolonies that enlarge and coalesce to form a layer of cells covering the surface. The attached cells produce additional polymers (exopolysaccharides, EPS), which help in the anchorage of the cells to the surface and in stabilizing the colony from environmental fluctuations (Characklis and Marshall, 1990).

10.2.2.4 Formation of biofilm
Biofilm formation is a direct consequence of the continuous attachment of bacterial cells to the substratum and subsequent growth, along with associated EPS production. Biofilm composition is heterogeneous due to colonization by different microorganisms with different nutritional requirements. Further increase in the size of a biofilm takes place by the deposition or attachment of other organic and inorganic solutes and particulate matter from the surrounding liquid phase (Melo et al., 1992; Costerton et al., 1994).

10.2.2.5 Detachment and dispersal of biofilm
As the biofilm ages, the attached bacteria, in order to survive and colonize new niches, must be able to detach and disperse from the biofilm. The daughter cells can become individually detached or be sloughed off. Sloughing is a discrete process whereby periodic detachment of relatively large particles of biomass occurs. This can be due to various factors such as fluid dynamics, shear effects of the bulk fluid, presence of certain chemicals in the fluid environment, or altered surface properties of the bacteria or substratum. The released bacteria may be then transported to new locations and restart the biofilm process (Rittman, 1989; Applegate and Bryers, 1991; Marshall, 1992).

10.2.3 Biofilm structure and properties
The present model of biofilm structure is that of ‘matrix-enclosed microcolonies interspersed with less dense regions that include highly permeable water channels’. These water channels penetrate throughout the biofilm and provide direct access to oxygen and nutrients from the bulk fluid, as well as allowing the removal of metabolic waste (Costerton et al., 1994).

Studies found that the density of the bottom layers was 5–10 times that of the surface. This variation in density was paralleled by a decrease in porosity (84–93 % at the top vs 58–67 % at the base) and mean pore size (1.7–2.7 µm at the top layers vs 0.3–0.4 µm at the bottom layers). Living cells constituted a much greater proportion of the total biomass in the top layers (91 %) than in lower layers (31–39 %). These results were obtained from a mixed biofilm community under aerobic conditions provided with a non-restrictive nutrient source. Results of experiments conducted under more restrictive conditions suggest that more complex structures can be expected in response to restrictive growth conditions (Wolfaardt et al., 1994; Zhang and Bishop, 1994).

It has been recognized for some time that microbial communities are capable of unique metabolic activity that isolated cells cannot conduct (Gill, 1998). Examples of this phenomenon can be seen in the metabolism of xenobiotic compounds (Lappin et al., 1985) and the degradation of straw cellulose (Kudo et al., 1987), but from
a more general perspective, in the very synthesis of EPS and their biofilm functionality. EPS are a diverse group of polysaccharides that are secreted by many bacteria into the surrounding environment. Many organisms will convert a very significant proportion of available carbon into EPS, with rates of conversion of 50% or higher (Sutherland, 1983). EPS are of great importance in the formation and structure of biofilms, composing a high proportion of the total biomass and serving a fundamental role in substratum attachment and in constituting a matrix for further cell attachment (Gill, 1998).

EPS production is stimulated by a variety of environmental conditions, including specific ion concentrations, aeration and low nutrient availability. Terms such as glycocalyx, slime, capsule and sheath have been used to refer to the EPS associated with biofilms. EPS production not only plays a role in initial adhesion and anchorage, but also in protecting bacteria from dehydration, as EPS can retain water several times its own mass and only slowly get desiccated. In addition, EPS help in the process of trapping and retaining nutrients, and protect cells against antimicrobial agents (Characklis and Cooksey, 1983; Roberson and Firestone, 1992; Ophir and Gutnick, 1994; Rinker and Kelly, 1996).

Biofilms are complex communities of organisms in which a variety of interspecies interactions may take place. It has been suggested that these interactions could influence the final development of the mature biofilm by inhibiting the attachments of some species while recruiting others (James et al., 1995). Biofilms formed by mixed species are often thicker and more stable than monospecies biofilms. The biofilm formed between *L. monocytogenes* and *P. fragi* (the latter as the primary colonizing organism) was much more extensive than the respective individual biofilms (Sasahara and Zottola, 1993).

It is well established that bacterial biofilms exhibit greater resistance to antimicrobial agents than the individual cells in suspension. It is difficult to establish that any single mechanism causes the resistance; rather, the combined mechanisms create resistant populations (Mustafa and Liewen, 1989; Frank and Koffi, 1990; Krysinsky et al., 1992). Biofilm formation protects the innermost cells. Antimicrobial resistance is linked to the three-dimensional structure of the film, because the resistance is lost as the structure is disrupted (Hoyle et al., 1992; Boyd and Chakrabarty, 1995).

Antimicrobial agents are generally far more effective against actively growing cells, which means that good disinfectants for planktonic cells are not necessarily suitable for biofilm cells. Typically, cells within the film receive less oxygen and fewer nutrients than those at the biofilm surface. In this condition, microorganisms may present altered growth and physiological states, resulting in increased resistance to antimicrobial agents (Gilbert et al., 1990; Holah et al., 1990; McFeters et al., 1995). In mixed biofilms competition for nutrients results in nutrient deficiency, which also plays a major role in increased resistance of biofilms to disinfectants (Berg et al., 1982; Jones and Pickup, 1989). Some studies with foodborne bacteria have indicated that resistance is more substantial in older biofilms (more than 24 hours) than in younger ones (Anwar et al., 1990; Frank and Koffi, 1990; Wirtanen and Mattila-Sandholm, 1992).

Bacterial biofilms can develop increased resistance towards different antibiotics by production of antibiotic-degrading enzymes such as β-lactamases. In biofilms, many similar hydrolytic enzymes are produced and they become trapped and concentrated...
within the biofilm matrix, consequently exhibiting enhanced protective properties (Nickel et al., 1985; Widmer et al., 1990; Anwar et al., 1992; Vergeres and Blaser, 1992).

10.2.4 Control and removal of biofilms
An effective cleaning and sanitation program will prevent biofilm formation, but it is a difficult task, and a thorough cleaning procedure should be developed and included in the food operation from the very beginning (Kumar and Anand, 1998). Food-processing equipment design is essential to achieve better cleanability of the food-contact surfaces once bacterial adhesion has occurred. With new surfaces, there is not much difference in cleanability among glass, nylon, polyvinyl or stainless steel, but the latter exhibits better hygienic properties with time by resisting damage caused by the cleaning process itself. It is important to realize that the application of sanitizers can and does cause surface corrosion (Dunsmore et al., 1981; LeClercq-Perlat and Lalande, 1994). Proper choice of equipment, material and accessories, and correct construction, process layout and process automation are essential (Mattila-Sandholm and Wirtanen, 1992).

10.2.4.1 Chemical methods
It is asserted that before application of a disinfectant, it is essential to eliminate as much ‘soil’ and as many microorganisms as possible. Indeed, microorganisms become far more sensitive to disinfectants once they have been detached from the surface to which they were adhering. The mechanical or chemical breakage of the EPS matrix is therefore vitally important (Kumar and Anand, 1998).

Chelators such as EDTA have proven quite effective in destabilizing the outer membranes of bacterial cells by binding calcium and magnesium ions (Camper et al., 1985). Some detergents are bactericidal, and some disinfectants may even depolymerize EPS, thus enabling the detachment of biofilms from surfaces. Examples are peracetic acid, chlorine, iodine, and hydrogen peroxide (Kumar and Anand, 1998). For example, the detergent monolaurin (50 µg/ml) combined with heat treatment at 65 °C for 5 minutes completely destroyed the biofilm formed by L. monocytogenes. A synergistic interaction between monolaurin and organic acids like acetic acid also caused a pronounced reduction of this pathogen (Oh and Marshall, 1995, 1996). Cetylpyridinium chloride (CPC) was reported to be valuable in the poultry-processing industry to reduce attachment of Salmonella on poultry skin (Breen et al., 1997).

The impregnation of materials with biocides has been shown to play a major role in resisting bacterial colonization for as long as the antimicrobial agent is released from the surface. For example, antifoulant paints containing silver have been effective in controlling mixed biofilms in which Legionella pneumophila is present (Rogers et al., 1995). Food-packaging materials containing antimicrobial compounds have gained practical importance in recent years for the control of spoilage and pathogenic microorganisms on food surfaces. These compounds are able to migrate to the food surface and eliminate microbial contamination.

10.2.4.2 Physical methods
Control of biofilms includes physical methods such as super-high magnetic fields, ultrasound treatments, high-pulse electrical fields (on their own and in combination...
with organic acids), and low-energy electrical fields (on their own and as enhancers of biocides) (Kumar and Anand, 1998). The biocidal effect of these procedures has been attributed to iontophoresis – that is, the generation of ions from chlorine-containing components such as NaCl, CaCl₂, and NH₄Cl, which are able to kill both Gram-positive and Gram-negative cells (Davis et al., 1994). Another possible bioelectrical effect is that the electric current applied drives the charged molecules and antibiotics into the cells through the biofilm matrix, thus increasing mass transfer rates (Rajnicek et al., 1994).

10.2.4.3 Biological methods

Newer strategies for the control of biofilms include the adsorption of bioactive compounds like bacteriocins onto food-contact surfaces for the inhibition or adhesion of bacteria. Bacteriocins are proteinaceous bactericidal agents. Nisin, a well-known and frequently applied antimicrobial peptide, has proven to be an effective inhibitor of many food pathogens and spoilage bacteria, including spore-formers (Kumar and Anand, 1998). There are reports showing that food-contact surfaces where nisin was adsorbed had lowered incidence of surface contamination by L. monocytogenes (Bower et al., 1995). Similarly, the application of lactic acid cultures and their cell-free extracts has also been reported to selectively inhibit different spoilage and pathogenic microflora on the surface of poultry (Anand et al., 1995).

Enzymes are very effective for degrading EPS and thus removing biofilms. The effectiveness of specific enzymes varies according to the type of microflora making up the biofilm (Kumar, 1997). Endoglycosidases able to degrade polymers, such as glycoproteins, were developed and used effectively in buffer and detergent solution for removing S. aureus and E. coli from glass and cloth surfaces (Lad, 1992). The most effective way to deal with biofilms is cleaning and sanitation to prevent the formation of conditioning films and to remove attached cells before colony growth can occur (Gill, 1998). Potthof et al. (1997) described the development of an in-place cleaning system for the dairy industry using a combination of enzymes and surfactants.

10.2.5 Beneficial aspects of biofilms

In many natural environments, maintenance of water quality is brought about by the microbial metabolism in biofilms. Bacteria present in these biofilms biodegrade many of the toxic compounds and minimize the buildup of pollutants, thus acting as pollutant moderators (Fuchs et al., 1996). These systems that use mixed microbial consortia have found application in fluidized beds and trickling filters for sewage and wastewater management, as well as in water purification plants and waste gas treatment. The organic nutrient-trapping capability of biofilms helps in reducing the organic content of wastewaters (Kanekar and Sarniak, 1995; Pedersen et al., 1997; Raunkjaer et al., 1997). Biofilms have also received considerable attention from the viewpoint of bioremediation of industrial effluents (Nigam et al., 1996) and in the nitrification process for treatment of high-strength, nitrogen-fertilizer wastewater (Beg et al., 1995; Cecen and Orak, 1996).

It is evident that biofilms represent a natural form of cell immobilization. Immobilized microorganisms have been successfully employed in bioreactors to
improve the productivity and stability of fermentation processes, including acetic acid and ethanol manufacture (Macaskie et al., 1995; Pakula and Freeman, 1996). Even the lactic acid bacteria and bifidobacteria that colonize the gastrointestinal tract, protecting it from pathogenic bacteria, are assumed to exert their action through formation of biofilms (Vanbelle et al., 1989).

11 Predictive microbiology

11.1 Introduction

In traditional practice, foods were preserved for storage by reduction of the water content, addition of solutes and/or reduction of the pH sufficient to ensure the bacteriological stability of the product at all temperatures. The spreading availability of refrigerated storage led to a reduced usage of preservatives, and this has been reinforced by a growing distrust of preservatives among consumers. Consumer demands dictate not only the reduced use of preservatives, but also the replacement of frozen by chilled products and the minimization of any thermal processing (Leistner, 1992).

There is now realization that several preservative actions, none of which alone is sufficient to stabilize a product microbiologically, may in combination be adequately inhibitory because of synergistic effects (Grant and Patterson, 1995). However, alterations in an established formula and/or process for a preserved food, which reduce the microbiological stability of the product, have inevitably raised questions as to the possible growth of pathogenic bacteria in the product (Russell and Gould, 1991). The usual approach to establishing the microbiological safety of minimally preserved and processed foods is challenge testing, in which the food is inoculated with pathogenic bacteria that might contaminate the commercial product, followed by monitoring of the behavior of the inoculated organisms in product stored under abusive as well as recommended conditions (Notermans and in’t Veld, 1994).

Modeling the behavior of microorganisms began in about 1920, with the development of methods for calculating thermal death times. This revolutionized the canning industry. A resurgence in predictive modeling began in the 1980s, driven by a proliferation of refrigerated and limited-shelf-life foods, the development of hurdle technology, and the advent of personal computers. Modeling techniques have become standard tools for designing experimentation and describing results (Whiting and Buchanan, 2002).

11.2 Modeling

Modeling in food microbiology assumes that the growth or inactivation of microorganisms in a food or model system is predictable within the limits of normal biological variability, and that it can be described by mathematical equations. It commonly assumes that the measured behavior of an appropriate model system predicts the microorganism’s behavior in foods with corresponding levels of environmental factors. Models should be validated with a number of selected tests (Whiting and Buchanan, 2002).
Modeling in food microbiology has taken an empirical approach. Empirical though these models may be, they are based on established linear and non-linear regression techniques. Growth data and/or model parameters are fitted to equations by using interactive least-squares computer algorithms. Assumptions regarding randomness, normal distributions, interpolations with tested ranges rather than extrapolations outside the ranges, parsimony and stochastic specifications must be made in microbial modeling, as they are for any other statistical application of regression (Whiting and Buchanan, 2002).

The responses of bacteria to environmental conditions of particular interest (with respect to the behavior of pathogens in perishable foods) concerns, for growth-permitting conditions, the time for resolution of the lag phase and the rate of increase of the numbers of viable cells (Zwietering et al., 1990). For conditions that are non-permissive of growth, or are intended to be rapidly lethal, the behavior of interest is usually the rate of decrease of the number of viable cells (Cerf et al., 1996). For some organisms, the duration of the lag phase and rate of increase may, for considerations of product safety, properly refer to the concentration of a toxin rather than to cell numbers, as there may be no simple relationship between the two under all circumstances (Ikawa and Genigeorgis, 1987).

Data on which to base lag duration and growth rate models have generally been collected by monitoring the growth of bacteria during their batch cultivation in liquid media. A common procedure has been to grow the test organism to the stationary phase in a non-defined medium such as tryptone soy broth or brain heart infusion. The stationary phase culture is suitably diluted with fresh growth medium, and the diluted culture is used to inoculate flasks containing the growth medium modified as required by the addition of acidulants, humectants and/or other preservatives at appropriate concentrations. Each culture is then held at one of several selected temperatures under aerobic or anaerobic conditions, or under an atmosphere containing a specified fraction of CO₂ (McClure et al., 1994). When modeling of the combined effects of multiple environmental variables is intended, fractional or complete factorial designs may be employed in deciding the compositions, as well as different incubation temperatures and atmospheric conditions for the individual media, to define the behavior of the microorganism within the range chosen for each environmental variable (Whiting, 1997). Growth has usually been monitored by spread or pour plating, spiral plating, or measurement of the turbidity of each culture. However, other procedures, such as determination of the time required for the culture to attain a specified turbidity or conductivity, have been employed. Data obtained by different procedures can give somewhat different estimates of lag times or growth rates for the same culture (Dalgaard et al., 1994).

It has been the established practice to determine the growth rate by fitting a line to a plot of the log transformed data against time, with the assumption that the growth is exponential. The lag time is then usually taken as being the time (t) at which the backward-extrapolated growth curve is at the log data value for the culture at t = 0. However, alternative procedures for estimating the lag time from a log plot have also been employed (Buchanan and Cygnarowicz, 1990). It has become common practice to use equations, such as the Gompertz equation, to fit sigmoidal curves to
log-transformed growth data. The lag time is therefore taken as the time at which the extrapolated tangent is the log data value for the culture at $t = 0$, or at some other point otherwise estimable from curve parameters. (Gibson et al., 1988).

Models can be conceived as having three levels (Whiting and Buchanan, 1997):

- **The primary level**, which describes changes in microbial numbers with time. A typical example is a growth model that estimates the change in log CFU/ml with time. Another example is the model that describes the decreasing counts with time during thermal processing. Microbial numbers may be estimated by counting, turbidity, conductance, or biochemical assays. Formation of a microbial toxin or other metabolic product with time constitutes another type of primary-level model.

- **The secondary level**, which describes how the parameters of the primary model change with changes in environmental or other factors. These equations may be based on Arrhenius or square-root relationships, particularly if temperature is the primary factor of concern, as is often the case when specific groups of foods are being modeled. When more factors are included in the model (e.g., $u_w$, pH, concentrations of antimicrobials), polynomial regression equations are needed, although they tend to be more difficult to interpret.

- **The tertiary level**, which makes predictions or estimations. Environmental values of interest are entered into the secondary-level model to obtain specific parameter values for the primary model, which is then solved for increasing periods of time to obtain the growth or inactivation curve expected from that specific combination of environmental values. Tertiary systems are essentially represented by applications software, varying in complexity from an equation on a spreadsheet to expert systems or risk-assessment simulations.

Because of their empirical nature, models predict bacterial behavior only uncertainly when the conditions are marginal for growth. This is particularly unfortunate for product formulation purposes, as here the requirement is often that a product be formulated to assure that there will be no growth of organisms, with adverse effects on human health, that may contaminate the product. To deal with such requirements, it has been proposed that probabilistic models, rather than kinetic ones, are needed. A probabilistic model would give, for specific sets of conditions, the probability of growth occurring, but not the rate of growth. Such models should allow narrow definition of the boundaries of a range of safe formulation/storage conditions, and so permit confident minimizing of preservatives and preserving treatments (Ratkowsky and Ross, 1995). However, published models of this type seem confined to descriptions of growth and toxin production by *Clostridium botulinum* (Roberts and Jarvis, 1983; Lund et al., 1990).

The predictive power of any model will always be constrained by the complexities of food–microbe interactions. Often, models for an organism are based on the growth of only one strain, or at most a few strains, in a homogeneous broth. Various strains of the same organism may, however, behave very differently (for example, by exhibiting different rates of growth under identical conditions of cultivation) (Barbosa et al., 1994). In addition, many food systems do not present a homogeneous environment. For example, on the larger scale, the outside of a product such
as a hamburger patty will, if it is not vacuum-packed, present an aerobic environment for bacterial growth, while the environment within is anaerobic (Gill and Jones, 1994). On the smaller scale, and particularly if the patty is formed of coarsely ground meat of high fat content, contact between particles of fat will provide regions of high pH within an environment where the bulk pH is close to pH 5.5, which is normal for post-rigor muscle (Grau and Vanderlinde, 1993). Similarly, with any emulsified product, the environment for bacterial growth at an oil/water interface is likely to differ considerably from that of the bulk medium, not only with respect to pH but also with respect to the availability of nutrients or even oxygen (Robins et al., 1994).

Even if the food does present an essentially homogeneous environment, it may contain micronutrients that are not present in the general purpose broths commonly used for generating data for models (Gill et al., 1997). Then, some organisms may grow more rapidly and/or grow at lower temperatures on the food than in the broth. Also, the effects of factors such as pH and \(a_w\) may differ between a food and a broth because the chemical nature of acidulants and humectants as well as the purely physical factors can affect the growth of bacteria (Adams et al., 1991). An additional difficulty arises in modeling the duration of the lag phase, in that the lag duration may depend upon the conditions that induced the lag. Despite that, most models of the lag phase have been based on the cultivation of organisms in which growth has ceased apparently as a result of carbon limitation. It is then not surprising that lag phase models generally appear rather unreliable for predicting the initiation of bacterial growth in foods (McKellar et al., 1997).

Verification of model reliability has usually taken the form of comparing literature data for the growth of an organism in foods with the growth predicted by the model. A major difficulty with such an exercise is that parameters, such as pH and \(a_w\), have to be assumed for a food (Sutherland and Bayliss, 1994). Because a substantial quantity of the data available from food often does not fit well with model predictions, it has been frequently suggested that a model be regarded as satisfactory provided that it underestimates observed growth relatively rarely, although it may often grossly overestimate growth. Overestimation of growth is then referred to as a ‘safe failure’ of the model (Jones et al., 1994).

**Bibliography**


