Progress in Food Preservation

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Preface

Food preservation is a critical control point that influences and determines a whole range of outcomes, ranging from preservation of nutritional quality, food safety, the wholesome nature of food, texture, taste and organoleptic qualities, and consumer appeal, along with compliance to several points in the value chain that include long-term storage, long-distance transportation and marketing. In an era that is becoming increasingly global, the economics of food preservation, shipping and transportation determine not only the availability of food globally, but also the availability of food to the consumer at a reasonable price that can sustain the whole food value chain. This is especially critical in situations involving the shipping of fresh food over large distances. If the perishability is high, the food must reach the destination in a short time to enhance the market window.

Geopolitical and climatic turmoil in recent times have increased the cost of fuel, resulting in increased food prices across the globe. Food prices in Canada were anticipated to increase over 5% in 2011, which is a substantial increase in a country that has enjoyed relatively stable prices for food. The price of fresh vegetables in India rose by over 100% in 2011. Such events highlight the need for food preservation practices that will enable the buffering of worldwide fluctuations in food prices, while enhancing food safety and dealing with security issues across the world (see the UN Secretary General's High-Level Task Force on the Global Food Security Crisis, www.un.org/issues/food/taskforce/index.shtml).

There are several factors that influence the properties of preserved food, and these factors determine the nature and method of preservation techniques that are employed. Preservation of dry foods with low water activity is relatively easy. When it comes to highly perishable foods such as meat, seafoods, fruits and vegetables, this is a challenge. In animal products, the major stress is on the prevention of microbial growth, and preservation techniques are used to achieve this goal. The shelf life of fruits and vegetables is highly variable. Rapidly respiring commodities have a very short shelf life, and consequently methods involving low temperature and anaerobic conditions are favoured. This is especially true for fresh-cut fruits and vegetables. Sun drying or drying in general has been practised as a mode of food preservation for centuries. Thus, reducing water activity is an efficient method for food preservation. The application of concentrated osmotic solutions for dehydration is another way to achieve the same goal. In every method, there is an added element of food safety and killing harmful microorganisms is essential to ensure the preservation as well as the safety of food. Food preservation methods involving thermal and non-thermal techniques have been widely employed in the food industry. At present, there are several new methods concurrently used in conjunction with traditional methods, making use of such technologies as microwaves, electricity (pulsed field), high pressure and irradiation, to provide better preservation of food.

This book has been compiled to provide an in-depth evaluation of the recent advances in the science and technology dealing with food preservation. The chapters of the book are

organized into four parts. The first part contains five chapters discussing various aspects of modified-atmosphere packaging, active packaging and coating technology. The second part deals with novel decontamination techniques, describing several modern trends in this area. The third section comprises four chapters which evaluate aspects of theoretical modelling in relation to various aspects of food preservation. The fourth part deals with the use of natural preservatives or methods for food preservation. Altogether, the chapters are written by experts in their respective fields and provide a complete approach to food preservation technologies, as applied to various food systems.

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Part I Active and Atmospheric Packaging

1 Selected Techniques to Decontaminate Minimally Processed Vegetables

Vicente M. Gómez-López

Abstract: Production of minimally processed vegetables entails a big challenge. The product should be stable, fresh-looking and safe. Cutting and/or peeling accelerate physiological degradation and expose inner tissues to contamination, decreasing produce shelf life and increasing the risk of foodborne pathogen growth. Use of decontamination techniques to control microbial populations is limited because they must be mild enough to avoid impairing sensory characteristics of the product. As a consequence, novel decontamination techniques and decontaminating agents are being tested to cope with the challenge posed by this kind of product. This chapter addresses some novel developments and chemicals potentially useful to prolong the shelf life of minimally processed vegetables, namely continuous UV light, pulsed light, electrolysed oxidizing water, ozone and low-temperature blanching. It defines every technique or decontaminant, and then focuses on the effects on microbial population, produce physiology, sensory quality and nutritional consequences, if known.

Keywords: electrolysed oxidizing water; low-temperature blanching; minimal processing; novel decontamination agents; novel decontamination techniques; ozone; pulsed light; UV light

1.1 INTRODUCTION

During recent decades an intensive search for novel decontaminants and decontamination methods for minimally processed vegetables (MPV) has been pursued by researchers worldwide. The delicate nature of MPV and the absolute requirement of maintaining a fresh-looking product seriously limit the kind of techniques that can be used to obtain an innocuous and stable product.

A decontamination technique for MPV should reduce the risk of foodborne infections and intoxications, decrease microbial spoilage, preserve fresh attributes and nutritional quality, not leave hazardous residues or by-products (Gómez-López *et al.*, 2009) and be environmentally friendly.

This chapter presents selected decontamination techniques that vary greatly, and which have not been extensively reviewed in recent years. It includes two photonic methods: UV-C light and pulsed light; a chemical method: ozonation; and a thermal method: low-temperature blanching. It briefly defines each method and its microbial inactivation mechanism, and then its effect on foodborne pathogenic and spoilage microorganisms, MPV shelf life and the sensory and nutritional quality of the MPV.

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1.2 UV-C LIGHT

1.2.1 Definition

UV-C is the portion of the electromagnetic spectrum corresponding to the band between 200 and 280 nm. Inactivation of microorganisms with UV systems can be performed by means of mercury lamps, xenon pulsed lamps, or excimer lasers. This section deals only with disinfection using mercury lamps, called continuous-wave UV (CW UV). UV inactivation of microorganisms is frequently achieved by using low-pressure mercury lamps designed to emit light at 254 nm, called germicidal light (Bintsis *et al.*, 2000), or medium-pressure UV lamps that emit germicidal wavelengths from 200 to 300 nm (Bolton and Linden, 2003). Since UV light is a non-ionizing radiation, and irradiated products have had serious marketing problems, the term 'illumination' as proposed by Lagunas-Solar and Gómez-López (2007) will be used in place of 'UV radiation' or 'UV irradiated' to avoid misconception. UV-C illumination as a technique to preserve foods was discovered in the 1930s (Artés and Allende, 2005), and water disinfection by UV has been widely applied in Europe since the 1980s (Hijnen *et al.*, 2006). In food-related industries, applications include disinfection of water supplies, food contact surfaces, air in food-preparation areas (Bintsis *et al.*, 2000) and packaging materials (Mimouni, 2001).

UV illumination is characterized by being a relatively inexpensive and simple technique (although it is subject to certain safety precautions; Bintsis *et al.*, 2000), the lack of residual compounds (López-Rubira *et al.*, 2005) and the avoidance of chemicals that can cause ecological problems and/or are potentially harmful to humans (Allende and Artés, 2003b), although conventional mercury lamps generate ozone, which must be exhausted.

The technique is limited to decontaminating surfaces or transparent liquids due to the low penetrability of UV light. When illuminating a three-dimensional object, it is necessary to ensure that all surfaces receive adequate exposure to UV light, requiring equipment with radically new designs (Gardner and Shama, 2000).

The effect of UV light on microorganisms and foods depends on the energy incident on their surface, which is termed fluence (measured in Joules/meter^2, J/m^2).

1.2.2 Inactivation mechanism

The germicidal effect of UV-C light is primarily due to the formation of thymine dimers (cyclobutane dimers) which inhibit the formation of new DNA chains (Mitchell *et al.*, 1992; Giese and Darby, 2000). UV-C treatment of bacterial spores results mainly in formation of the 'spore photoproduct' 5-thyminyl-5,6-dihydrothymine (Slieman and Nicholson, 2000). Microbial cells can repair themselves from photochemical damage, mainly through photoreactivation, where the enzyme photolyase uses visible light energy to split cyclobutane dimers (Kao *et al.*, 2005). Photoreactivation has been studied in water bodies; however, as far as is known by this author, its effect on decontaminated produce has not been studied.

1.2.3 Effect on microbial populations

The efficacy of UV-C to inactivate a wide range of microorganisms has been demonstrated in viruses (Eischeid *et al.*, 2009), Gram-negative and Gram-positive bacteria (Chang *et al.*, 1985; Yaun *et al.*, 2003), bacterial spores (Mamane-Gravetz and Linden, 2004), conidia (Marquenie *et al.*, 2002) and parasites (Zimmer *et al.*, 2003; Hayes *et al.*, 2008) *in vitro* as well as when inoculated onto (Yaun *et al.*, 2004) and into vegetable matrixes (Hadjok *et al.*, 2008).

UV-C illumination is able to increase vegetable shelf life based on microbial spoilage counts by either inactivating microorganisms and/or decreasing their growth rate, and can even trigger a lag phase. This lag phase is more apparent in yeasts than in other microbial populations (Erkan *et al.*, 2001; Allende and Artés, 2003a, 2003b; Allende *et al.*, 2006) and can account for lower counts in illuminated compared with control samples at the end of the storage time. In contrast, UV-C may sometimes stimulate growth of lactic acid bacteria, possibly due to a higher resistance to UV-C and elimination of competing microflora (Allende and Artés, 2003a, 2003b).

Erkan *et al.* (2001) treated tissue slices of zucchini squash with UV-C up to 9.86 kJ/m^2, and Allende *et al.* (2006) did it to both sides of minimally processed (MP) Red Oak Leaf lettuce up to 7.11 kJ/m^2. In these cases, UV-C did not inactivate total bacterial and yeast populations, but slowed down their growth during storage. In the latter example, this meant a shelf-life extension of at least 2 days. On the other hand, illumination of MP Red Oak Leaf lettuce (Allende and Artés, 2003a) and MP Lollo Rosso lettuce (Allende and Artés, 2003b) both with UV-C up to 8.14 kJ/m^2 inactivated important microbial groups, bringing about a shelf-life prolongation of 2–3 days. In different cases, the published literature indicates that UV-C illumination of MPV can cause a shelf-life extension of at least 2 days from the microbial point of view when properly used.

1.2.4 Effect on sensory attributes

UV-C illumination has been reported to preserve or not affect the sensory quality of MPV. Erkan *et al.* (2001) stated that the decay of zucchini squash slices illuminated with UV-C at levels of 4.93 and 9.86 kJ/m^2 was significantly less than controls during storage at 5 and 10°C. However, after 12 days of storage at 10°C, a reddish-brown discoloration induced by UV-C was observed on the surface of illuminated tissues, which suggests the accumulation of phenolic compounds.

No effects were reported by Allende and Artés (2003a) on MP Red Oak Leaf lettuce treated with up to 8.14 kJ/m^2 UV-C on sensory quality evaluated in terms of overall visual quality, colour and browning, during storage in MAP at 5°C for 8 days. On the other hand, for MP Lollo Rosso lettuce stored at 5°C for 8 days, Allende and Artés (2003b) reported a beneficial effect of UV-C. No significant differences were found for aroma, texture, taste and colour, but samples treated with the highest fluences (2.44–8.14 kJ/m^2) had better-preserved overall visual quality and presented less browning than untreated samples or samples treated with the lowest fluences (0.407 and 0.814 kJ/m^2), and a shelf-life prolongation may be estimated based on sensory scores. Lettuce tissue became shinier when the highest fluence was applied, which was attributed to a possible induction of lignification-like processes started by the lettuce tissue to protect itself against the UV-C stress.

Allende *et al.* (2006) illuminated two sides of MP Red Oak Leaf lettuce and reported no effect on overall visual quality of UV-C illumination at fluences up to 2.37 kJ/m^2, but 7.11 kJ/m^2 induced tissue softening and browning after 7 days of storage at 5°C. Polyphenol oxidase is the enzyme responsible for enzymatic browning of many types of produce. Manzocco *et al.* (2009) achieved complete inactivation of mushroom polyphenol oxidase *in vitro* by UV-C illumination.

1.2.5 Effects on the nutritional and phytochemical composition of MPV

It is very well known by post-harvest experts that UV illumination acts as an elicitor of resistance mechanisms in fruit and vegetables, and thus leads to a rapid increase of

stress-response compounds such as phenols, flavonoids and phytoalexins, which have phytochemical properties. This change can be instantaneous or progressive and observed during produce storage. The biosynthesis of phenolic compounds is affected by UV illumination due to the increased activity of phenylalanine ammonia-lyase (Schreiner and Huyskens-Keil, 2006). Among the different controlled abiotic stresses useful to enhance the nutraceutical content of MPV, UV illumination is considered to be of high potential by the industry (Cisneros-Zevallos, 2003). There are no studies yet on the effect of UV-C on phytochemical composition of MPV. Nevertheless, increasing evidence of positive effects on whole produce and fresh-cut fruits suggests positive results, such as that reported by Cantos *et al.* (2001). Trans-resveratrol is a phytoalexin with health-promoting properties. Those authors demonstrated that UV-C can increase the concentration of phytoalexins in table grapes as much as 10-fold during storage. UV-C illuminated whole peppers (Vicente *et al.*, 2005) and broccoli heads (Costa *et al.*, 2006) showed increased antioxidant capacity with respect to controls during storage. Similarly, UV-C illuminated fresh-cut mango (González-Aguilar *et al.*, 2007) banana and guava, but not pineapple (Alothman *et al.*, 2009), showed increased antioxidant capacity but also vitamin C degradation.

1.3 PULSED LIGHT

1.3.1 Definition

Pulsed light (PL) is a new method intended for decontamination of food surfaces by killing microorganisms using short-term, high-frequency pulses of an intense broad-spectrum illumination rich in UV-C light (Gómez-López *et al.*, 2005a). It derives from CW UV, with the main difference that PL delivers much higher fluence rates and uses xenon flash lamps instead of mercury lamps, and special hardware to produce a high peak power. Peak power is defined as the pulse energy divided by the pulse duration (Rice and Ewell, 2001). Very comprehensive reviews on the subject have been published by Elmnasser *et al.* (2007) and Gómez-López *et al.* (2007a); this section is an update, with a focus on MPV. It is common in the literature to find reports comparing the efficiency of PL versus low-pressure or medium-pressure UV lamps, where the biggest difficulty is comparing light sources which differ not only in peak power but also in emission spectrum. Recently, Bohrerova *et al.* (2008) compared the disinfection efficiency of PL and CW UV from low- and medium-pressure mercury lamps over *Escherichia coli* cells and phages T3 and T4, which were all inactivated more efficiently by PL at equivalent fluence levels.

PL has similar advantages and disadvantages to CW UV-C, but also some specific pros and cons. Perhaps the most important advantage of PL is the fast microbial inactivation. Specific disadvantages are high cost and that this process can increase produce temperature. When studying the inactivation of *Aspergillus niger* spores on corn meal, Jun *et al.* (2003) found that some experimental factor settings resulted in sample temperatures of 120°C.

1.3.2 Inactivation mechanism

Xenon flash lamps have an emission spectrum ranging from ultraviolet to infrared light, wherein the UV-C component is the most important for microbial inactivation. Rowan *et al.* (1999) reported that using a flash with high UV content the inactivation of seven micro-organisms was 5–6 log colony-forming units (CFU)/plate, but only 1–2 log CFU/plate with a

low-UV flash. The germicidal efficiency of xenon flash lamps determined against *E. coli* was found to be maximum around 270 nm, with a major contribution to inactivation in the 220–290 nm range (Wang *et al.*, 2005). Other wavelengths also contribute to microbial inactivation. Bohrerova *et al.* (2008) reported that a significant fraction of the enhanced PL inactivation efficiency compared to low- and medium-pressure mercury lamps was due to wavelengths greater than 295 nm, and even due to visible light in the case of viruses.

Different kinds of damage have been reported on viruses and microbial cells due to PL action. Lethal events are photochemical and/or photothermal. The primary lethal effect of PL, as with CW UV light, is the formation of cyclobutane pyrimidine dimers, as observed in cells of *Saccharomyces cerevisiae* (Takeshita *et al.*, 2003) and *E. coli* (Bohrerova *et al.*, 2008). Interestingly, the higher number of thymine dimers observed in flashed *E. coli* cells in comparison with CW UV-treated cells was attributed to an effect of light higher than 295 nm. Single-strand DNA breaks and cell-membrane damage have been reported for yeasts by Takeshita *et al.* (2003).

Cell rupture at fluences exceeding 0.5 J/cm^2 due to momentary overheating caused by absorbing all UV light from a flash lamp was revealed by Wekhof *et al.* (2001). Micrographs of flashed *A. niger* spores were presented showing severe deformation and rupture attributed to escape of the overheated contents of the spore. Bohrerova *et al.* (2008) have suggested that besides DNA damage, phage capsids might be ruptured by the visible portion of PL. As in CW UV treatments, photoreactivation can also occur after PL treatment (Otaki *et al.*, 2003).

In vitro studies performed by Gómez-López *et al.* (2005b) showed that proteins and oils decreased the decontamination effect of PL, while carbohydrates and water showed variable effects. For this reason MPV seem to be a suitable matrix for efficient PL decontamination.

1.3.3 Effect on microbial populations

PL has been proved to be effective against viruses (Roberts and Hope, 2003), Gram-negative bacteria (Sharma and Demirci, 2003), Gram-positive bacteria (Krishnamurthy *et al.*, 2004), bacterial spores (McDonald *et al.*, 2002), yeasts (Takeshita *et al.*, 2003), conidia (Marquenie *et al.*, 2003), fungal spores (Jun *et al.*, 2003) and parasites (Huffman *et al.*, 2000).

Research on the inactivation of human pathogens on vegetable surfaces is still pending. Regarding microorganisms naturally present on vegetables, Hoornstra *et al.* (2002) were first in demonstrating the potential of PL. The authors treated white cabbage, leek, paprika, carrots and kale with two pulses of PL amounting to 0.30 J/cm^2. The reduction in aerobic count at the surface of the vegetables varied from 1.6 log CFU/cm^2 for carrots to more than 2.6 log CFU/cm^2 for paprika. Gómez-López *et al.* (2005b) reported a reduction in mesophilic aerobic counts between 0.56 and 2.04 log CFU/g for spinach, radicchio, iceberg lettuce, white cabbage, carrots, green bell pepper and soybean sprouts. Later on, Kaack and Lyager (2007) used PL to inactivate *S. cerevisiae* cells inoculated onto carrot pieces.

In order to prove whether microbial inactivation brings about shelf-life extension of MPV, Gómez-López *et al.* (2005b) flashed shredded iceberg lettuce and shredded white cabbage, and stored them under modified-atmosphere packaging at 7°C. For iceberg lettuce, even though a 0.46 log reduction was achieved in psychrotroph counts, control and flashed samples did not last 3 days of storage due to excessively high psychrotroph counts and bad sensory quality. For white cabbage, a 0.54 log decrease in psychrotroph counts was achieved, but after 2 days of storage control and flashed samples had similar psychrotroph counts. It is worth noting that this kind of results is common for MPV shelf-life studies, and might be avoided using storage temperatures not higher than 4°C (Gómez-López *et al.*, 2008a).

1.3.4 Effect on sensory attributes

Little is known on the effect of PL on MPV. Except for some discoloration of iceberg lettuce, no adverse effects of PL on sensorial quality were reported by Hoornstra *et al.* (2002) after 7 days of storage at 7 or 20°C of five illuminated vegetables. Heating was a concern for Gómez-López *et al.* (2005b), who were unable to efficiently treat grated carrots without avoiding its dehydration. The same authors reported the appearance of off-odour in MP cabbage immediately after treatment, which disappeared overnight. MP cabbage stored at 7°C was sensorially acceptable for up to 9 days, while untreated controls were rejected after 7 days, which means a shelf-life extension of 2 days from the sensorial point of view. For MP lettuce, however, no shelf-life extension was achieved by PL treatment.

1.3.5 Effects on the nutritional and phytochemical composition of MPV

There is a lack of studies about the nutritional and phytochemical consequences of the application of PL to fruit and vegetables, but encouraging results for phytochemical synthesis enhancement by CW UV makes PL a potential fast method for phytochemical content improvement. Additionally, PL did not affect levels of riboflavin and vitamin C in beef, chicken and fish (Dunn *et al.*, 1995).

1.4 ELECTROLYSED OXIDIZING WATER

1.4.1 Definition

Electrolysed oxidizing water (EOW) is created by electrolysis of diluted sodium chloride solutions in an electrolysis chamber, having free chlorine as the major disinfection factor. The most common type of electrolytic cell is a two-cell chamber. The generation of EOW using the two-cell chamber involves reactions in cells containing positively charged and negatively charged electrodes, respectively, separated by a membrane, and through which a much diluted salt-water solution passes. By subjecting the electrodes to direct current voltage, two types of water possessing different characteristics are generated: an acidic EOW (AcEW) and an alkaline EOW (AlEW). The first one has been the most studied due to its microbicidal properties. AcEW is produced from the anode side, and is characterized by a pH of less than 3, a high oxidation-reduction potential (ORP), about 1150 mV, and the presence of HOCl. AlEW is produced from the cathode side, having a pH of 11.4 and low ORP, about −795 mV (Kim *et al.*, 2000a).

A single stream of neutral EOW (NEW), also named mixed-oxidant solution (or MIOX), with enhanced levels of HOCl, is produced by the single-cell system, where no membrane is present. Therefore, three kinds of EOW can be produced: AcEW, AlEW and NEW (MIOX). Very comprehensive reviews on EOW has been published by Al-Haq *et al.* (2005) and Huang *et al.* (2008).

EOW can be prepared by the electrolysis of a diluted saline solution, without the use of any chemicals other than sodium chloride (Koseki *et al.*, 2004). It has therefore less adverse impact on the environment (Kim *et al.*, 2000a). Furthermore the raw materials, water and sodium chloride, are found virtually everywhere (Venczel *et al.*, 1997). EOW can be generated on site (Len *et al.*, 2000), and therefore transportation and storage of potentially hazardous chemicals are not needed (Nakagawara *et al.*, 1998). EOW is not only a

decontaminant but can also prevent enzymatic browning during storage of MPV (Koseki and Itoh, 2002).

AcEW could be more effective in inactivating microorganisms than chlorinated solutions, having the same concentration of available chlorine (Koseki *et al.*, 2001). Consequently, there should be lower formation of chloramines and trihalomethanes. NEW has also the advantage of its neutral pH, so it does not contribute as aggressively as AcEW to the corrosion of processing equipment or irritation of hands. It is also more stable as chlorine loss is significantly reduced at pH 6–9 (Deza *et al.*, 2003).

1.4.2 Inactivation mechanism

It has been observed that AcEW produces blebs and breaks in the outer membrane of *Pseudomonas aeruginosa*, inactivates nitrate reductase and degrades chromosomal DNA (Kiura *et al.*, 2002). There is no agreement about which is the primary factor contributing to the microbicidal activity of EOW. Factors involved are concentration of free chlorine, redox potential (ORP) and pH. Kim *et al.* (2000a, 2000b) concluded that ORP may be the primary factor affecting microbial inactivation of AcEW, while Nakagawara *et al.* (1998), Len *et al.* (2000), Koseki *et al.* (2001) and Kiura *et al.* (2002) have concluded that HOCl concentration is the main contributor.

Other antimicrobial compounds besides HOCl are hypothetically present in EOW, which should explain the higher effectiveness of EOW over conventional HOCl solutions to inactivate microorganisms *in vitro* (Venczel *et al.*, 1997). But those compounds, such as hydroxyl radicals, have not been detected (Stan *et al.*, 2005), and disinfection experiments on lettuce under controlled conditions of chlorine concentration, pH and ORP do not agree with this hypothesis (Park *et al.*, 2001). Perhaps additional compounds become quickly degraded by organic matter, and are not relevant for decontamination of MPV.

Chlorine is certainly involved and pH determines the most important species: Cl_2 below pH 3 (typical of AcEW), and HOCl and ClO^- above pH 4 (typical of NEW) (Nakagawara *et al.*, 1998). The maximum *in vitro* antibacterial activity of AcEW occurs around pH 4 (Nakagawara *et al.*, 1998; Len *et al.*, 2000; Park *et al.*, 2004). However, results from *in vivo* tests do not confirm the applicability of *in vitro* results. Yang *et al.* (2003) dipped MP lettuce in EOW at different pHs, and found no pH effect for the inactivation of *Salmonella Typhimurium* and *Listeria monocytogenes*, and two inactivation peaks for *E. coli* O157: H7 at pH 4 and 8.

1.4.3 Effect on microbial populations

Different studies have proved the efficacy of EOW to inactivate human pathogens both *in vitro* (Venczel *et al.*, 1997; Venkitanarayanan *et al.*1999; Kim *et al.*, 2000a, 2000b; Nakajima *et al.*, 2004; Park *et al.*, 2004) and inoculated onto vegetable surfaces (see Table 1.1) (Deza *et al.*, 2003; Abadías *et al.*, 2008; Park *et al.*, 2008).

As for spoilage microorganisms, Izumi (1999) studied the effect of rinsing five MPV with NEW (20 ppm available chlorine, pH 6.8, 4 min) on mesophilic aerobic microorganisms, finding a 1.8 log reduction for trimmed spinach leaves to no significant effect for carrot slices, Japanese radish shreds or diced potatoes. The author concluded that the effect of NEW was influenced by the surface area, anatomy and microstructure of the tissues, which differ among vegetables, as well as the type of cut, which would affect the extent of contact of EOW with microorganisms. Koide *et al.* (2009) reported 1.5 and 1.3 log reductions in total aerobic

Table 1.1 Studies on the effect of electrolysed water on pathogenic microorganisms inoculated onto fresh or minimally processed vegetables.

Produce	Microorganism	Free chlorine concentration (mg/l)	Time (min)	pH	Log reduction	Reference
Tomato	*E. coli* O157:H7	89	1	8.1	4.92	Deza *et al.* (2003)
	Salmonella Enteriditis				4.30	
	L. monocytogenes				4.74	
Lettuce	*E. coli* O157:H7	89	3	8.6	1.2	Abadias *et al.* (2008)
	Salmonella				1.7	
Spinach	*E. coli* O157:H7	20	10	6.3–6.5	1.25	Guentzel *et al.* (2008)
	S. Typhimurium				2.14	
	L. monocytogenes				2.94	
Iceberg lettuce	*E. coli* O157:H7				0.14	
	S. Typhimurium				1.41	
	L. monocytogenes				2.99	
Green onion	*E. coli* O157:H7	37.5	1	2.1	4.45	Park *et al.* (2008b)
	S. Typhimurium				4.24	
	L. monocytogenes				4.82	
Tomato	*E. coli* O157:H7				>5.86	
	S. Typhimurium				4.27	
	L. monocytogenes				>5.91	
Iceberg lettuce	*E. coli* O157:H7	50	2	2.6	0.72	Keskinen *et al.* (2009)
Romaine lettuce					0.77	

bacteria, yeast and mould counts after dipping MP cabbage in NEW (20 mg/l, 10 min). As for shelf-life prolongation, Rico *et al.* (2008) achieved 1.4–1.6 log reduction in aerobic mesophilic counts on lettuce compared to water washing, a difference that persisted after 7 days of storage at 4°C.

Regarding AcEW, it reduced total aerobic plate count of MP cilantro leaves by 0.66 log compared to water washing (Wang *et al.*, 2004), by more than 1 log in whole chinjon, leafy cabbage, spinach, cucumber and snap beans, and just 0.9 log in green peppers. With AlEW alone, a no more than 0.5 log reduction was observed (Lin *et al.*, 2005). Monitoring the effect of AcEW during the storage of MP cilantro at 0°C, Wang *et al.* (2004) observed that the total aerobic plate count and total Enterobacteriaceae count exhibited a 4-day lag phase. AcEW (50 ppm free chlorine, pH 2.8) reduced *E. coli* O157:H7 counts by 0.72 and 0.77 logarithmic cycles in MP iceberg and Romaine lettuce respectively (Keskinen *et al.*, 2009).

AlEW lacks antimicrobial properties, but it is considered to act like a diluted sodium hydroxide solution. Thus, it would work like a surface-active agent on vegetable surfaces. Consequently, the microorganisms on the surface would be reached more easily by AcEW during a sequential process, which explains higher decontamination found in lettuce (Koseki *et al.*, 2001), whole cucumber (Koseki *et al.*, 2004) and MP lettuce and MP cabbage (Koseki and Itoh, 2001, 2002). The shelf life of the latter MPV was estimated to be extended

by at least 1 day in spite of bacteria growing faster in samples treated with AcEW than in water-washed samples.

1.4.4 Effect on sensory quality

EOW washing does not change sensory properties of vegetables immediately after treatment. It does not affect the surface colour of carrot slices, trimmed spinach leaves or cucumber slices (Izumi, 1999); the taste, appearance or smell of whole tomatoes (Deza *et al.*, 2003); or the appearance of whole cucumbers (Lin *et al.*, 2005), MP lettuce, carrot and endive (Abadías *et al.*, 2008). Panellists of triangle tests were unable to differentiate MP iceberg lettuce, white cabbage and carrots treated with NEW (40 mg/l free chlorine, 5 min) from water-washed samples (Gómez-López *et al.*, 2008b). A residual chlorine odour after washing vegetables with AcEW (50 ppm active chlorine) can be eliminated by soaking with AlEW (Lin *et al.*, 2005). Furthermore, Park *et al.* (2001) found no effects of AcEW on visual quality, stem discoloration, wilting and colour of whole lettuce leaves stored for 14 days at 4°C.

As for shelf-life prolongation, Gómez-López *et al.* (2007b) extended the shelf life of shredded cabbage stored at 4°C by 5 days due to treatment with NEW. Rico *et al.* (2008) also showed beneficial effects of increasing concentrations of free chlorine (12–120 ppm) in NEW to decrease browning potential of lettuce and polyphenol oxidase activity and improving sensory quality evaluated in terms of fresh appearance, photosynthetic and vascular browning, and general acceptability; although high free-chlorine content NEW produced changes associated to blanching and loss of crispness.

AcEW can have beneficial effects in the sensorial stability of some MPV during refrigerated storage, such as decreasing the progress of browning in MP lettuce and MP cabbage (Koseki and Itoh, 2002). The authors suggested the enzymes responsible for browning may have been oxidized and weakened by the strong ORP of AcEW. But AcEW can also have deleterious effects, such as loss of aroma of cilantro leaves during storage, possibly correlated, according to the authors, to a higher tissue electrolyte leakage caused by oxidation of the cilantro cell membrane by AcEW (Wang *et al.*, 2004).

1.4.5 Effects on the nutritional and phytochemical composition of MPV

Treating MP leek with NEW up to 30 mg/l free chlorine compared to water-wash treatment did not influence vitamin C, α-tocopherol and total phenol content, the antioxidant capacity measured by the ferric reducing antioxidant power (FRAP) method, and the concentration of the carotenoids all-*trans*-β-carotene and 9-*cis*-β-carotene; but decreased violaxanthin and lutein concentrations, as well as γ-tocopherol content (Vandekinderen *et al.*, 2009).

1.5 OZONE

1.5.1 Definition

Ozone (O_3) is a highly unstable triatomic molecule that is formed by addition of an oxygen atom to molecular diatomic oxygen. When a free oxygen atom encounters molecular oxygen, it combines to form the ozone molecule. Production of ozone is generally accomplished by

using the corona discharge method (Güzel-Seydim *et al.*, 2004). Ozone is a strong oxidant active against all types of microorganism. Excess ozone auto-decomposes rapidly to produce oxygen, and thus it leaves no residues in food. It can be applied as a gas, or in aqueous solution where the gas does not appreciably react with water; therefore it forms a true physical solution (Khadre *et al.*, 2001).

The use of this disinfectant has some limitations. Because of its instability, ozone must be generated at the usage site. Metal and other types of surfaces with which it comes into contact are subject to corrosion or other deterioration because of its strong oxidizing power (Beuchat, 1998). Several food components interfere with its microbicidal efficacy (Restaino *et al.*, 1995; Güzel-Seydim *et al.*, 2004). Decomposition of ozone is so rapid in the water phase of foods that its antimicrobial activity is restricted to surfaces; therefore microorganisms embedded in product surfaces are more resistant to ozone than those readily exposed to sanitizer (Mahapatra *et al.*, 2005). Ozone-detection and -destruction systems and respirators are needed for the safety of workers in food-processing facilities (Kim *et al.*, 1999b).

1.5.2 Inactivation mechanism

Ozone is a strong oxidant and disinfectant. It can react with contaminants directly as molecular ozone or indirectly as ozone-derived free radicals such as •OH and $\bullet H_2O$ (Koseki and Itoh, 2001). Ozone decomposes in solution in a stepwise fashion, producing in turn hydroperoxyl ($\bullet HO_2$), hydroxyl (•OH) and superoxide ($\bullet O_2^-$) radicals. The hydroxyl radical is an important transient species and chain-propagating radical. The reactivity of ozone is attributed to the great oxidizing power of these free radicals (Kim *et al.*, 1999b). Different targets have been proposed for its bactericidal action: double bonds of unsaturated lipids in the cell envelope, interference with the respiratory system and damage to the genetic material, and a general oxidation of protoplasm (Kim *et al.*, 1999b). Young and Setlow (2004) proposed that spore killing by ozone is due to some type of damage to the spore's inner membrane, although the identity of this damage was not clear.

1.5.3 Ozonated water

1.5.3.1 Effect on microbial populations

The effect of ozonated water on pathogen populations of vegetable surfaces is very variable. Singh *et al.* (2002) reported 1.42 and 1.80 log reductions respectively in *E. coli* O157:H7 after treating MP lettuce and baby carrots with ozonated water (16.5 mg/l, 10 min). In contrast, counts of *L. monocytogenes* and *E. coli* O157:H7 in shredded lettuce were reduced from 6.0 log CFU/g to less than 1.0 log CFU/g by washing with ozonated water (3 ppm, 5 min) (Rodgers *et al.*, 2004). Finally, counts of *Shigella sonnei* inoculated on shredded lettuce decreased 1.7 log units by ozonated water (5 ppm, 5 min) (Selma *et al.*, 2007); and counts of *L. monocytogenes* in green leaf lettuce were reduced by more than 2 log units (Ölmez and Akbas, 2009).

Ozonated water has also shown mixed effectiveness to reduce microorganisms naturally present in MPV. Kim *et al.* (1999a) observed that bubbling ozone while stirring at high velocity (300 rpm) was the best of different alternatives tested to decontaminate lettuce, with log reductions of 1.9 log CFU/g for both counts of mesophilic and psychrotrophic bacteria. Thereafter, a number of articles have reported from non-significant effects to almost 2-log reductions (Table 1.2).

Table 1.2 Studies on the effect of ozonated water on natural microflora of fresh or minimally processed vegetables.

Produce	Microbial group	Ozone concentration (mg/l)	Log reduction	Reference
Lettuce	Mesophilic aerobes	5	1.5	Koseki *et al.* (2001)
	Yeasts and moulds		1.0	
Lettuce	Mesophilic aerobes	2.5–7.5	0.6–0.8	Garcia *et al.* (2003)
	Psychrotrophs	7.5	0.5	
Cucumber	Mesophilic aerobes	5	0.7	Koseki *et al.* (2004).
Celery	Mesophilic aerobes	0.18	1.69	Zhang *et al.* (2005)
Potato strips	Mesophilic aerobes, psychrophiles, lactic acid bacteria, yeasts, coliforms, anaerobic microorganisms	20	NS	Beltrán *et al.* (2005a)
Lettuce	Mesophilic aerobes	20	1.6	Beltrán *et al.* (2005b)
Green pepper	Mesophilic aerobes	3.95	NS	Ketteringham *et al.* (2006)
Rocket leaves	Mesophilic aerobes	10	1	Martínez-Sánchez *et al.* (2006)
	Psycrophilics		>1	
	Coliforms, yeasts and moulds		NS	
Green leaf lettuce	Mesophilic aerobes	2	1.5	Ölmez and Akbas (2009)
	Psychrotrophs		1.1	
	Enterobacteriaceae		1.5	
Asparagus	Mesophilic aerobes	0.1	1.91	Sothornvit and Kiatchanapaibul (2009)
Carrot	Mesophilic aerobes	1	0.4	Alegria *et al.* (2009)
	Yeasts and moulds		0.7	

NS, non-significant effect.

MPV decontaminated by ozonated water and stored at 4°C have been shown to have longer shelf life than their untreated counterparts, from the microbiological point of view. Counts of mesophilic bacteria in ozonated MPV remained lower than in controls in the cases of shredded lettuce for up to 9 days (Rodgers *et al.*, 2004) and 13 days (Beltrán *et al.*, 2005b); and fresh-cut celery for up to 9 days (Zhang *et al.*, 2005). For ozonated MP potato, mesophilic bacteria counts were not different from controls during storage; however, no decontamination was observed due to treatment (Beltrán *et al.*, 2005a).

1.5.3.2 Effect on sensory attributes

It has been shown that ozonated water maintains the sensory quality of MPV, among which MP lettuce is the most studied. According to microscopy observations, lettuce surface is not affected by ozone (Koseki *et al.*, 2001). Ozonated water had positive effects on sensory quality of MP lettuce stored at 4°C for 9 days (Ölmez and Akbas, 2009), the shelf life of which can be 4 days longer than chlorinated samples (Garcia *et al.*, 2003). Samples of MP lettuce washed with ozonated water and stored in air maintained an excellent visual quality during

storage at 4°C up to 13 days without significant differences compared to the initial visual quality, and no browning was observed. In contrast, water-washed controls decreased in visual quality and browned sharply after 5 days. Moreover, ozone did not affect its texture (Beltrán *et al.*, 2005b).

Fresh-cut cilantro leaves washed with ozonated water and stored at 0°C for up to 14 days had the same colour as leaves washed with tap water. The typical cilantro aroma was maintained with a higher score by the ozone treatment at day 14. The ozonated samples exhibited better overall quality retention during storage. The leaves appeared to be near the fresh or initial conditions of the cilantro, with a green and fresh appearance, no yellowing or dehydration and no trace of off-odour (Wang *et al.*, 2004). Ozonated water (0.18 ppm) was beneficial in maintaining colour, visible structural integrity and general appearance of MP celery during storage at 4°C (Zhang *et al.*, 2005). As for MP rocket leaves, ozone (10 mg/l) affected neither colour nor visual quality (Martínez-Sánchez *et al.*, 2006).

Potato strips treated with ozonated water (20 mg/l) stored under vacuum at 4°C during 14 days showed no evidence of browning, and maintained the full typical aroma and a very firm and turgid texture (Beltrán *et al.*, 2005a). Treatment of MP green asparagus with 1 mg/l aqueous ozone for 30 min delays the synthesis of the structural cell-wall constituents that lead to asparagus-toughening lignin, cellulose and hemicelluse during storage at 3°C for 25 days (An *et al.*, 2007).

Shredded carrots were affected by immersion in ozonated water (1 ppm, 5 min), suffering from loss of colour due to leaching, softening, losses of perceived aroma and low general acceptance, although the highly exposed cut area could account for the observed losses (Alegria *et al.*, 2009).

1.5.4 Gaseous ozone

1.5.4.1 Factors affecting the efficacy of ozone gas

The factors affecting the decontaminant efficacy of ozone gas were studied by Han *et al.* (2002) in *E. coli* O157:H7 inoculated onto green bell pepper surface. The order of significance and from the most important to the least was: ozone concentration (2–8 mg/l), exposure time (10–40 min) and relative humidity (60–90%), in the ranges indicated. A synergism was found between gas concentration and relative humidity. Li and Wang (2003) also found that germicidal efficiencies over four microorganisms increased as relative humidity increased, which could be related to the higher number of radicals from the ozone reaction with more water vapour at higher relative humidity.

1.5.4.2 Effect on pathogenic microorganisms and sensory quality

There are few studies about the application of ozone in the gas phase to decontaminate MPV. Singh *et al.* (2002) reported 1.79 and 2.64 log reductions in *E. coli* O157:H7 populations after treating respectively MP lettuce and baby carrots with gaseous ozone (7.6 mg/l, 15 min). Decoloration of lettuce leaves was observed after treatment with 5.2 or 7.6 mg/l O_3 for 10 and 15 min. Han *et al.* (2002) reported 7.35 log reduction (log CFU/5 g) of *E. coli* O157:H7 inoculated onto green bell pepper surface after treatment with 8 mg/l O_3 at 90% relative humidity for 25 min. Klockow and Keener (2009) developed an ozone-generation system where bags of vegetables, spinach in this case, were subjected to an electric field to generate ozone, and then stored. *E. coli* O157:H7 populations decreased by 5.8 and more than 6.4 log CFU/leaf after 24 h of storage at 5 or 22°C

respectively, but leaf colour changed from green to white, especially at the stem and outer edges of the leaf.

1.5.5 Effects on the nutritional and phytochemical composition of MPV

As with other disinfectants, the effect of ozonated water has been primarily studied in MP iceberg lettuce, while the effect of gaseous ozone is still pending for tests. It seems that in general ozone has neutral nutritional consequences for MPV. Ozonated water at 5 ppm, 5 min (Koseki and Isobe, 2006) and at 2–4 ppm, 2 min (Akbas and Ölmez, 2007; Ölmez and Akbas, 2009) did not affect the ascorbic acid content of MP lettuce; although harder treatments (10 or 20 mg/l O_3) did reduce vitamin C content (Beltrán *et al.*, 2005b). In contrast, 0.03 and 0.08 ppm of ozone in washing water delayed the oxidation of vitamin C in MP celery during storage at 4°C up to 9 days (Zhang *et al.*, 2005). According to the authors, inhibition of polyphenol oxidase and delay of tissue metabolism by ozonated water could account for this effect.

Ozone (10 mg/l) had no effect on the total polyphenol content of MP lettuce (Beltrán *et al.*, 2005b) or rocket leaves (Martínez-Sánchez *et al.*, 2006), and ozonated water (2–4 ppm, 2 min) did not affect β-carotene content of MP lettuce (Akbas and Ölmez, 2007; Ölmez and Akbas, 2009).

1.6 LOW-TEMPERATURE BLANCHING

1.6.1 Definition

Low-temperature blanching consists of immersion of MPV in hot water for up to 50°C and 120 s, high enough to provoke beneficial physiological changes and microbial reduction without cooking. Most of the literature on low-temperature blanching has been published in articles dealing with the effect of warm chlorinated water. However, because of the concerns on the use of chlorine for decontamination of fruits and vegetables, only the results of blanching in pure water will be summarized in this section.

1.6.2 Effect on microbial populations

The first results on the effect of mild heat treatment on the stability of MPV were discouraging from the microbiological point of view. Compared to samples washed at 20°C, immersion of MP lettuce in warm water (50°C, 90 s) decreased psychrotrophic counts by 0.56 log, but did not significantly affect counts of mesophilic microorganisms, Enterobacteriaceae, and yeasts and moulds. After less than 4 days of storage at 5 or 15°C the effect on psychrotroph counts was lost (Li *et al.*, 2001). Experiments under identical conditions showed that mild heat treatment enhances growth of *L. monocytogenes* during subsequent storage (Li *et al.*, 2002). The enhanced growth of microorganisms after a decontamination process is also frequent for other decontamination techniques as revised by Gómez-López *et al.* (2008a).

Compared to washing at 20°C for respective times, washing lettuce with warm water (50°C, 1 or 5 min) reduced counts of *E. coli* O157:H7 by 1.68 and 1.98 logs respectively, and *Salmonella* counts by 1.51 and 2.03 logs (Koseki *et al.*, 2004). A more than 1.0 log reduction was reported for total bacteria, pseudomonades and Enterobacteriaceae after pre-washing of iceberg lettuce with warm water (50°C, 60 s) after trimming and coring but before shredding

in comparison with washing with cold water (4°C, 90 s) after shredding. The sanitizing effect persisted throughout storage (4°C, 9 days) (Baur *et al.*, 2005). Similarly results were reported by Klaiber *et al.* (2005) for aerobic mesophilic bacteria, lactic acid bacteria and enterobacteria of shredded carrots pre-washed in warm water (50°C, 120 s) after peeling and topping but before shredding, in comparison with non-washed samples.

1.6.3 Effects on sensory quality

The main beneficial effect of low-temperature blanching is suppression of lettuce browning. Immersion in warm water (50°C, 90 s) retarded browning for at least 7 days at 5°C, and 2 days at 15°C (Li *et al.*, 2001). Fukumoto *et al.* (2002) studied the effect of warm water (47°C, 3 min) compared with cold water (4°C) on the photosynthetic and vascular tissues of iceberg lettuce stored for 7 days at 5°C. Washing at 47°C strongly inhibited edge browning, phenylalanine ammonia-lyase activity and peroxidase activity. Baur *et al.* (2005) reported that pre-washing of MP iceberg lettuce with warm water (50°C, 60 s) decreased cut-edge vascular tissue browning and phenylalanine ammonia-lyase during storage (4°C, 9 days).

Low-temperature blanching has been also applied to other MPV. Colour and odour of low temperature blanched MP endives stored for 2 days at 4°C were negatively affected when blanching conditions were 50°C/10 min. When 52.5°C/10 min and 55°C/5 min conditions were used, appearance, taste and texture were additionally damaged (Mayer-Miebach *et al.*, 2003). Pre-washing of MP carrots with warm water (50°C, 120 s) did not affect overall visual quality, colour, odour, texture, sweetness and flavour during storage (4°C, 9 days) as determined by sensory panel; it also did not affect whiteness index, and contents of glucose, fructose, sucrose, as well as total sugar. However, longer blanching times (60°C, 40 min) decreased carrot hardness due to pectin demethoxylation (Lemmens *et al.*, 2009).

1.6.4 Effects on the nutritional and phytochemical composition of MPV

Low-temperature blanching of detached whole endive leaves at conditions 50°C/10 min, 52.5°C/10 min and 55°C/5 min reduced polyphenol contents and antioxidant capacity (Mayer-Miebach *et al.*, 2003). In contrast, blanching carrots at 60°C for 40 min did not modify β-carotene levels of carrots (Lemmens *et al.*, 2009).

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2 Active and Intelligent Packaging of Food

István Siró

Abstract: Active packaging materials are designed to actively maintain or improve the condition of the food either by eliminating unwanted components from the package headspace and/or from the food itself or by releasing active components into the food or its surroundings. In this way, unlike traditional packaging, active packaging plays a dynamic role in food preservation. The main applications have mostly focused on delaying oxidation and controlling moisture migration, microbial growth, respiration rates, volatile flavours and aromas. Intelligent or smart packaging materials are designed to monitor the condition of the food and to communicate. These smart devices may be incorporated into packaging materials or attached to the inside or outside of a package. Examples include time-temperature indicators, freshness indicators, biosensors and radio frequency identification. This chapter is devoted to providing a general overview of active and intelligent packaging. It covers the main concepts and the latest developments in those fields. It also reports the most relevant aspects related to advances and challenges in the use of such packaging. Related nanotechnology innovations such as detection of pathogens and toxins by nanosensors, controlled delivery of active compounds via nano-encapsulation or absorption by nanoparticles are also discussed briefly.

Keywords: antimicrobial packaging; antioxidants; controlled release; emitter; ethylene; nano-sensor; oxygen scavenger

2.1 INTRODUCTION

The increasing consumer demand for minimally processed and ready-to-eat 'fresh' food products, the globalization of food trade as well as the distribution from centralized processing have created major challenges for the food-packaging industry with respect to maintaining safety and quality. Traditional packaging systems seem to reach their limits with regards to further extension of shelf life of packaged food. To provide such extension and to improve the quality, safety and integrity of the packaged food, innovative active and intelligent (A&I) packaging concepts have been developed.

While traditional food packages are passive barriers designed to delay the adverse effects of the environment on the food product, active packaging (AP) allows packages to interact with food and the environment and to play a dynamic role in food preservation. AP has been defined as packaging which 'changes the condition of the packed food to extend shelf life or to improve safety or sensory properties, while maintaining the quality of packaged food' (FAIR-project CT-98-4170, ACTIPAK).

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Table 2.1 Potential applications of AP technologies.

Type of AP	Food products
Oxygen scavengers	Smoked and cured meats, fish, cheese, bakery products, fresh pasta, ground coffee, tea, nuts, chips, vegetable oils, spreads, milk powder, cakes, cookies, beer, wine and beverages
Ethylene scavengers	Fresh produce, fresh-cut products
Carbon dioxide scavengers	Ground coffee
Moisture regulators/absorbers	Dry products, meat, poultry, fish
Aroma scavengers/absorbers	Citrus juices
Antioxidative packaging	Cereals, milk powder
Antimicrobial packaging	Meat, poultry, cooked ham, fish, cakes, bakery products, fruits, cheese
Carbon dioxide releasers	Fish, meat, butter, poultry
Microwave susceptors	Ready-to-eat meals, french fries, popcorn

AP is a broad concept covering diverse packaging systems designed specifically for a certain niche of food product (Table 2.1). Due to such diversity distinct classifications may appear in the literature. APs are often divided into two main categories: (i) active scavengers/absorbers, i.e. packaging that absorbs unfavourable components from the package headspace and/or from the food itself, and (ii) active releasers/emitters, i.e. packaging that releases beneficial active compounds into the food or its surroundings. The former group consists of oxygen, carbon dioxide or ethylene scavengers, and moisture and aroma absorbers. Antimicrobial and antioxidative AP, as well as aroma and carbon dioxide releasers, may be cited as typical examples for the latter. AP systems with combined mechanisms (e.g. combined oxygen scavenger/carbon dioxide releaser) also exist. These are also referred as 'regulators' in some publications (Galdi *et al*., 2008). Some APs, however, cannot be put into any of these categories. Self-heating and self-cooling packaging concepts, for example, may also be considered as active systems. These APs utilize different chemicals to generate exothermic or endothermic reactions. Microwave susceptors consist of aluminum or stainless steel deposited on substrates such as polyester films or paperboard and serve to dry, crisp and ultimately brown microwave food. Recent increasing consumer demand for convenient, ready-to-eat food is likely to facilitate the spread of such packaging.

Alternative classifications of APs can be done on the basis of their form; i.e. 'sachet/pouch type' or film-based APs, where the active compound is incorporated into the packaging film. This latter type is more favoured from a safety point of view, as there is no risk of accidental ingestion by the consumer or undesirable leak of the active compound, which may happen in the case of sachet-type APs.

Intelligent packaging (IP; or smart packaging) systems monitor the condition of packaged foods to give information about the quality of the packaged food during transport and storage (Brody *et al*., 2008). Typical examples of IP systems include indicators of gas leaks, time-temperature history and microbial spoilage. Although they differ from the concept of AP, IP systems may be used to check the effectiveness and integrity of AP systems.

The following sections of this chapter are devoted to reviewing the major types of A&I packaging, with particular emphasis on the latest developments as reported in the literature. The advances and related challenges are also discussed.

2.2 ACTIVE SCAVENGERS

Certain types of AP technology are designed to remove undesirable substances from the headspace of a package through absorption, adsorption or scavenging. Thus, a physical or chemical absorbent or adsorbent is either incorporated in the packaging material or added to the package by means of a sachet. It should be noted that the terms absorber, adsorber and scavenger are often used inconsistently in the literature to describe any system that removes a substance from the headspace. Absorption, however, involves a substance being taken into the bulk of a phase while adsorption comprises a substance being taken onto a surface. Both, absorption and adsorption are physical phenomena while scavenging implies a chemical reaction. In the following, the terms presented by the original authors of a particular work are used.

2.2.1 Oxygen scavengers

High levels of oxygen present in food packages may facilitate aerobic microbial growth, development of off-flavours and off-odours, colour change and nutritional losses due to oxidation processes, thereby causing significant reductions in the shelf life. Oxygen also influences the respiration rate and ethylene production of respiring produces. Therefore, the control of oxygen levels in food packages is important to limit the rate of these deteriorative and spoilage reactions. Vacuum and modified-atmosphere packaging combined with high-barrier packaging films still have their limitations in terms of complete oxygen removal and prevention of oxygen ingress through the packaging film. Furthermore, vacuum packaging systems cannot be applied for the packaging of fragile (e.g. chips), sharp (e.g. nuts with shells, certain meat with bones) and liquid food products. Application of an oxygen scavenger may overcome these problems. In general, existing oxygen-scavenging technologies are based on the oxidation of an active substrate. Typical examples include iron powder, ascorbic acid, photo-sensitive dye, sulphites, catechol, some nylons and enzymes (e.g. glucose oxidase and alcohol oxidase). Scientific evidence of the effectiveness of different oxygen scavengers in improving the shelf life of numerous oxygen-sensitive food products have been published. Recent examples include olive oil (Cecchi *et al*., 2010), table wine (Mentana *et al*., 2009), ready-to-eat cheese spread (Gomes *et al*., 2009), cheesecake (Sanguinetti *et al*., 2009) and catfish steaks (Mohan *et al*., 2008). The potential of oxygen scavengers for the packaging of fresh or minimally processed fruits (i.e. respiring products) has also been evaluated (Galdi *et al*., 2008). Oxygen scavengers with dual functionality may provide even more shelf-life extension. The combination of oxygen scavengers with an ethylene absorber, or carbon dioxide-releasing or antimicrobial systems can be cited as promising examples.

Most commercial oxygen scavengers are available in the form of a sachet or labels containing iron powder which is to be introduced into the package. Some of these iron-based scavengers require moisture for their operation. Recently, models have been developed describing the effect of humidity on the oxygen absorption by the iron powder (Polyakov and Miltz, 2010). One of the main drawbacks of using sachet-type oxygen scavengers is the risk of accidental ingestion of the active compound (i.e. iron powder). Oxygen-scavenging sachets are also not appropriate for liquid foods, as direct contact of the liquid with the sachet usually causes the spillage of sachet contents.

Circumvention of these problems could be done by incorporating the active compound into the packaging material itself (i.e. into film or closures). Several suppliers, for example, offer oxygen scavengers for compounding into flexible and rigid packaging including PET

bottles for beverage packaging. Such scavenger material incorporated into PET bottles or films (also referred to as active barrier technology) has been evaluated recently (Di Felice *et al.*, 2008).

In principle, different kinds of organic, inorganic or polymeric scavenging compounds can be applied; however, the nature of the polymer matrix (i.e. the packaging film) and the properties of the food product should be considered upon selection. Iron powder, for example, may cause loss of mechanical properties and reduction of transparency. Other scavenger materials include aromatic polyamide or ethylenic unsaturated hydrocarbons, such as squalene, fatty acids or polybutadiene. Polybutadiene-based scavengers, in particular, are promising candidates due to their transparency, good mechanical properties and processing characteristics, which are similar to those of polyethylene. During the scavenging reaction, however, by-products (e.g. organic acids, aldehydes or ketones) can be generated which negatively affect the sensorial quality of the food and pose potential health risk (Galdi *et al.*, 2008). Application of functional barriers that limit the migration of undesirable oxidation products can be used to minimize such problems (López-Rubio *et al.*, 2004).

Enzymatic oxygen-scavenging systems are more expensive than iron-based systems. The enzymes used are often sensitive to temperature, pH and water activity; therefore the use of these enzyme-based systems is limited. Incorporating enzymes into the packaging materials by means of current industrial processes applying relatively high temperatures (i.e. extrusion, lamination) is also challenging. Recently, however, carboxylated styrene acrylate latex samples have been functionalized by the immobilization and entrapment of glucose oxidase to be used as an oxygen scavenger (Nestorson *et al.*, 2008).

A common problem related to the application of oxygen scavengers is ensuring that the active compounds do not react with oxygen before this function is needed. To prevent scavengers from acting prematurely, they need to be stored in an oxygen-free atmosphere or alternatively specialized mechanisms used to trigger the scavenging reaction. For example, photosensitive dyes irradiated with ultraviolet light can activate oxygen removal.

To summarize, oxygen scavengers may be useful in extending the shelf life of many food products; however, the proper system should be selected with great caution in order to obtain maximal shelf-life extension while avoiding potential safety risk (e.g. unwanted migration, anaerobic headspace condition, pathogenic proliferation, etc.).

More detailed information on the different types of oxygen scavengers can be obtained from other reviews (Vermeiren *et al.*, 2003; Müller *et al.*, 2010).

2.2.2 Ethylene scavengers

Ethylene (C_2H_4) is a natural growth-stimulating plant hormone that accelerates ripening and senescence by increasing the respiration rate of produce, thereby decreasing its shelf life. Thus, elimination of ethylene from the package headspace is of high importance. Removal of ethylene can be done by potassium permanganate ($KMnO_4$) imbedded in silica or other inert substrates (e.g. perlite, vermiculite, alumina) at a level of 5–6 wt%. Potassium permanganate, having a purple colour, oxidizes ethylene to form either ethylene glycol or acetic acid, which in the presence of excess permanganate could be further oxidized to carbon dioxide and water, while manganese dioxide, with a dark brown colour, is generated. Due to the toxicity of potassium permanganate, however, products based on potassium permanganate cannot be integrated into food-contact materials (i.e. packaging), but are only supplied in the form of sachets highly permeable to ethylene.

Ethylene can also be removed by physical adsorption and subsequent breakdown on active surfaces such as activated carbon dispersed in the packaging film. It has also been shown that activated carbon efficacy is higher when used in combination with catalysts (e.g. palladium) (Meyer and Terry, 2010).

Other potential adsorbers include zeolites, Japanese oya or clays finely dispersed in the packaging film. Zeolites, which are diverse type of volcanic aluminosilicate crystalline materials, have a three-dimensional structure with interconnected cages and channels. Due to their cation-exchange capacity, and molecular sieving and adsorption capacity, they are considered as promising ethylene adsorbers. Although such minerals may adsorb ethylene, they also alter the permeability of the film, resulting in decreased ethylene concentration in the package headspace independently of any ethylene adsorption. As a drawback, these films are often opaque due to the incorporation of such minerals (Vermeiren *et al.*, 2003). Addition of nanosize clays, however, may eliminate the problem of opacity of these films.

Different types of ethylene scavengers and adsorbers have been tested for packaged produces including papayas (Silva *et al.*, 2009), bananas (Smith *et al.*, 2009), avocados (Meyer and Terry, 2010) and tomatoes (Martínez-Romero *et al.*, 2009) to limit the citations to recent publications only. The effectiveness of such systems is rarely assessed by direct measuring of ethylene removal capacity, but by following particular quality attributes of the products. Furthermore, the use of ethylene scavengers and adsorbers is often combined with modified atmosphere in order to achieve maximal shelf-life extension, thus their ethylene removal efficacy is difficult to substantiate by these indirect methods. It also has to be emphasized that their effectiveness varies greatly with product type (e.g. cultivars) and storage conditions (e.g. temperature) as well as the permeability properties of the packaging film. Different approaches for the inhibition of ethylene action, including the use of different ethylene scavengers, has been comprehensively discussed elsewhere (Martínez-Romero *et al.*, 2007; Smith *et al.*, 2009).

2.2.3 Carbon dioxide scavengers

Carbon dioxide scavengers are used to avoid pressure build-up or volume expansion inside packaging by removing carbon dioxide produced by fermented, roasted or respiring products. Carbon dioxide can be removed either by physical fixation (e.g. by zeolites or active carbon) or via chemical reaction (e.g. $Ca(OH)_2$, Na_2CO_3, $Mg(OH)_2$). A typical application area for carbon dioxide scavengers is roasted coffee, which may contain up to 15 atm dissolved carbon dioxide as a result of the Strecker degradation reaction between sugars and amines. Unless carbon dioxide is removed by a scavenger, the package may explode.

Another carbon dioxide-producing food product is kimchi, a general term for fermented vegetables such as oriental cabbage, radish, green onion and leaf mustard mixed with salt and spices. Carbon dioxide absorbers, however, remove carbon dioxide not only from the package headspace, but also from the juices, thus causing the loss of the product's characteristic fresh carbonic taste. Shin *et al.* (2002) suggested the combined use of sodium carbonate and zeolite, which can alleviate pressure build-up and volume expansion of kimchi packages while maintaining a low stabilized carbon dioxide partial pressure since zeolites and other finely dispersed minerals can absorb/adsorb carbon dioxide reversibly.

2.2.4 Moisture regulators

Desiccants in combination with high-water-barrier packaging have been used to maintain as low levels of moisture as possible in the packaging of dried food products, such as chips, nuts, spices, biscuits, crackers, milk powder and instant coffee. Moisture may cause caking in powdered products, softening of crispy products and moistening of hygroscopic products. Although such desiccants are mainly available in the form of internal porous sachets, the high thermal stability of the active substances used (e.g. silica gel, molecular sieves, calcium oxide, natural clays) allows, in principle, their incorporation as finely dispersed fillings in extruded films to produce desiccant plastic structures (López-Rubio *et al.*, 2004).

Internal humidity controllers are used to remove excess humidity from the package of high moisture foods (e.g. meat, poultry, fish and fresh produce) in order to suppress microbial growth and to avoid low consumer appeal. The excess moisture development inside a food package usually occurs due to the respiration of fresh produce, temperature fluctuations in food packages with high equilibrium relative humidity, or the drip of tissue fluid from cut meats, poultry and produce. Drip-absorbent sheets for liquid water control basically consist of a super-absorbent polymer (e.g. polyacrylate salts and graft copolymers of starch) in between two layers of a microporous or non-woven polymer. The excess moisture can also be captured in the vapour phase by placing humectants between two layers of a plastic film with high permeable to water vapour (e.g. poly(vinyl alcohol), PVOH) or by using a moisture absorbent sachet.

2.2.5 Aroma scavengers/absorbers

Aroma scavengers/absorbers are designed to eliminate undesirable flavours, aromas and odours present in the package headspace. The formation of these off-flavours and off-odours in food products originates mainly from the oxidation of fats and oils, leading to the formation of aldehydes and ketones or the breakdown of proteins of fish muscle into amines. Although these malodours can, in principle, be removed from the package headspace by using active aroma scavengers, these systems may mask or absorb off-flavours and off-odours that are indicative of spoilage, and therefore their application may pose a health risk to consumers.

Another area where aroma scavengers may be applied is the packaging of citrus juices, in which naringin and limonin are the main components responsible for bitterness. In particular, limonin, which is formed from a non-bitter precursor present in the fresh fruit due to heat treatment during processing, as well as chemical action in the acidic juice medium, may cause unfavourable bitterness. Reduction in the naringin and limonin contents of grapefruit juice by PVOH hydrogel containing immobilized naringinase enzyme has been demonstrated (Busto *et al.*, 2007).

Packaging materials may also generate undesirable odours that must be minimized for consumer acceptance. Specifically, in plastic materials, some polyolefin components tend to degrade or oxidize into short-chain, odorous hydrocarbon compounds during plastic processing which can be removed by aroma scavengers. Materials with high specific area, such as natural clays, zeolites and active carbon, are often used as aroma absorbents. These fillers, however, can absorb components other than aromas from the package headspace, including moisture, ethylene, carbon dioxide and other gases (Vermeiren *et al.*, 2003).

2.3 ACTIVE RELEASERS/EMITTERS

Release-type AP systems are engineered to release beneficial substances into the food product or into the package headspace. As oxidation reactions and microbial activity are the main processes limiting shelf life, antioxidative and antimicrobial packaging systems are the most relevant representatives of such APs. Others with less significance are carbon dioxide emitters and aroma releasers. In any case, a long-lasting protection of food requires the controlled release of the active compound. The main types of active releasers/emitters and some issues of controlled release are discussed in the following.

2.3.1 Antimicrobial packaging

When antimicrobials are directly mixed into initial food formulations their protective activity generally ceases due to neutralization in reactions and/or interactions in the complex food system. In addition, an antimicrobial compound present in the bulk food cannot selectively target the food surface where spoilage reactions occur more intensively. In order to compensate such activity loss high concentrations of antimicrobials are often applied, which may induce resistance in microorganisms, as well as consumer rejection. The use of antimicrobial packaging can protect the food, while minimizing the use of additives.

Antimicrobial APs aim to extend the lag phase and suppress the growth rate of microorganisms in order to extend the shelf life and to maintain product quality and safety (Brody *et al.*, 2008). The antimicrobial functionality can be introduced to the packaging in several ways, such as: (i) incorporation of volatile and non-volatile antimicrobial agents directly into polymers; (ii) coating or adsorbing antimicrobials onto polymer surfaces; as well as (iii) immobilization of antimicrobials to polymers by ionic or covalent linkages. Besides, sachets containing antimicrobials can be added to the package; alternatively, polymer films that are inherently antimicrobial can also be used.

According to the antimicrobial mechanism two main groups can be distinguished: (i) in migrating mechanisms the active compound migrates partially or completely into the food or onto its surface and there exercises its preservative action; (ii) non-migrating mechanisms contain compounds (e.g. enzymes or other antimicrobial proteins) that have antimicrobial action when the target microorganism comes into contact with the antimicrobial surface. As both concepts require direct contact between the product and the packaging mainly vacuum-packaged products can benefit from such technique. When volatile antimicrobials are used, however, no direct contact is necessary; thus such mechanisms can be applied, for instance, for the packaging of fruits and vegetables.

A whole range of antimicrobial APs has been described and tested in the literature. Considering the high number of related publications and the high complexity of this area, only some arbitrarily selected topics are discussed here.

2.3.2 Antimicrobial substances

The number of potential active agents is virtually unlimited and among others includes organic acids and their salts, alcohols, metals, bacteriocins, enzymes, chelators, fungicides, plant extracts and essential oils, as well as polysaccharides. Examples of antimicrobial agents for potential use in active food packaging are listed in Table 2.2.

Possible antimicrobial substances are food preservatives, such as sorbates, benzoates, propionates and parabens. Incorporation of sodium benzoate (Ye *et al.*, 2008a, 2008b) and

Table 2.2 Some examples of potential antimicrobial AP systems.

Antimicrobials	Polymer/carrier	Food/test medium	Reference
Organic acids/salts			
Potassium sorbate	Chitosan	Aqueous solution	Yoshida *et al*. (2010)
Sodium benzoate	Chitosan		Ye *et al*. (2008a, 2008b)
Potassium lactate	PP/PA/PP	Cooked ham	Jofré *et al*. (2007)
Metals			
Silver nanoparticles	Absorbent pad (cellulose/PE)	Fresh-cut melon	Fernández *et al*. (2010)
	Porous chitosan	Culture medium	Vimala *et al*. (2010)
	Chitosan-starch blend	Culture medium	Yoksan *et al*. (2010)
	Chitosan-lactate film	Culture medium	Tankhiwale and Bajpai (2010)
Bacteriocins			
Nisin	HPMC/PE	Culture medium	Chollet *et al*. (2009)
	LDPE/EVA/VDC	Beef cuts	Ercolini *et al*. (2010)
	HPMC/EC	Culture medium	Guiga *et al*. (2010)
	WPI coatings on PP	Culture medium	Lee *et al*. (2008)
	BC film	Vacuum-packaged frankfurter sausages	Nguyen *et al*. (2008)
Pediocin	CA	Sliced ham	Santiago-Silva *et al*. (2009)
Enterocin	Alginate	Cooked ham	Marcos *et al*. (2008)
	PP/PA/PP	Cooked ham	Jofré *et al*. (2007)
Enzymes			
Lysozyme	PE, PVOH	Culture medium	Conte *et al*. (2007, 2008)
	CA	Culture medium	Gemili *et al*. (2009)
	Sodium caseinate	Culture medium	Mendes de Souza *et al*. (2010)
Polysaccharide			
Chitosonium acetate	EVOH	Culture medium	Fernandez-Saiz *et al*. (2009)
Chitosan/trimethylchitosan	HPC	Culture medium	Belalia *et al*. (2008)

Plant extracts/aromas			
Allyl isothiocyanate	Polymeric resin	Sliced mozzarella cheese	Pires *et al.* (2009)
	SPI/PEO and PLA electrospun fibres		Vega-Lugo and Lim (2009)
	Glass vial/HDPE sealing	Chicken breast	Shin *et al.* (2010)
2-Nonanone	Alumina	Strawberry	Almenar *et al.* (2009)
Cinnamon essential oil	Chitosan	Culture medium	Ojagh *et al.* (2010)
	Wax paraffin on paper	sliced bread	Rodríguez *et al.* (2008)
Thymol, carvacrol, cinnamaldehyde, hydrocinnamaldehyde, oregano essential oil	PP	Culture medium	Gutiérrez *et al.* (2009)
Thymol	Zein film	Culture medium	Del Nobile *et al.* (2008)
Oregano essential oil			
Antibiotics			
Natamycin	Cellulose polymeric base	Mozarella cheese	Pires *et al.* (2008)
Others			
Triclosan	LDPE	Cooked ham	Camilloto *et al.* (2009)
	PP	Culture medium	Pinto *et al.* (2008)

BC, bacterial cellulose; CA, cellulose actetate; EC, ethylcellulose; EVA, ethylene vinyl acetate; EVOH, ethylene vinyl alcohol; HDPE, high-density polyethylene; HPC, hydroxypropyl cellulose; HPMC, hydroxypropyl methylcellulose; LDPE, low-density polyethylene; PA, polyamide; PE, polyethylene; PEO, poly(ethylene oxide); PLA, poly(lactic acid); PP, polypropylene; PVOH, poly(vinyl alcohol); SPI, soy protein isolate; VDC, vinylidene chloride; WPI, whey protein isolate.

potassium sorbate (Yoshida *et al.*, 2010) into chitosan film has been reported. These antimicrobials, however, may be incompatible with more apolar matrices (e.g. low-density polyethylene, LDPE); therefore, respective acid anhydrides with lower polarity are often applied instead.

Ethanol-releasing sachets or films can be used to suppress mould growth, especially in the case of cheese and soft bakery products with moderate water activity (a_w). Difficulties exist, however, in applying ethanol for antimicrobial packaging due to its off-flavour, rapid volatilization and some consumer resistance. Ethanol is generally encapsulated in carrier materials (e.g. silica gel) and a vanilla aroma is often used to mask the off-odour.

Metal ions such as silver, copper and platinum have been known for their antimicrobial activity for a relatively long time. Silver ions, which inhibit a wide range of metabolic enzymes, have strong antimicrobial activity with a broad spectrum; however, some resistant strains that absorb silver have been found. Silver-substituted zeolites, in particular, are widely used as polymer additives for food applications in Japan.

Bacteriocins are produced by microorganisms and can also inhibit the growth of spoilage and pathogenic microorganisms. These fermentation products include nisin, lacticins, pediocin, diolococcin, enterocins and propionicins. Extensive research has been related, for instance, to nisin, which is a water-soluble polypeptide produced by lactic bacteria and effective against Gram-positive organisms. Nisin on the other hand, is normally ineffective against Gram-negative bacteria, since they possess an outer cell membrane that blocks the active site. This can be overcome by the combination of nisin with food-grade chelators such as ethylenediaminetetra-acetic acid (EDTA) and citric acid (Ko *et al.*, 2009).

Similarly, improved antimicrobial effectiveness of lysozyme, a single-peptide protein, is reported through combination with EDTA (Sinigaglia *et al.*, 2008). Another enzyme with antimicrobial properties is glucose oxidase, which produces hydrogen peroxide to destroy bacterial cells upon contact, although the lowering of pH by the production of D-gluconic acid may also influence the growth of some microorganisms (Vartiainen *et al.*, 2005a).

Imazalil is a fungal sterol biosynthesis inhibitor and is permitted for use on fresh fruits to prevent mould infection. It is thermally stable at high temperatures and has antimicrobial activity at low concentrations, which makes it as an ideal candidate for incorporation into packaging films (Cha and Chinnan, 2004).

Triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether) is a non-ionic, broad-spectrum antimicrobial agent with a relatively high thermal stability and favourable safety profile. Little work has been done, however, on the use of triclosan for food packaging (although see recent studies by Pinto *et al.* 2008; Camilloto *et al.* 2009).

The use of natural antimicrobials, like plant extracts or volatile aroma compounds, is a promising alternative to synthetic antimicrobial compounds due to the appeal of a natural product, consumer preference and less conflict with legislation. Some of these compounds also have antioxidant properties, which makes them suitable for designing packaging materials with greater stability for the contained food. Extracts from grapefruit seed, cinnamon, clove, thyme, rosemary, oregano, garlic, basil, mint, lemongrass, horseradish and mustard are among the antimicrobial substances that have been added to packaging systems to demonstrate effective antimicrobial activity against spoilage and pathogenic bacteria. Several of these active compounds are volatile; they therefore have the potential to protect food when full contact with the packaging material cannot be attained. In particular, allyl isothiocyanate (AITC), which is derived from members of the Brassicaceae and can be found in mustard, horseradish and Japanese wasabi, has proven to be an effective antimicrobial (Pires *et al.*, 2009; Vega-Lugo and Lim, 2009). The main

disadvantage of using these natural compounds is related to the presence of a strong aroma, which may negatively influence the organoleptical properties of the food (Peltzer *et al*., 2009). Besides, the high volatility results in an activity loss, as well as too quick release from the packaging. These obstacles may be overcome through microencapsulation (e.g. in cyclodextrins, CDs), which also makes the active substance easier to handle (del Toro-Sánchez *et al*., 2010).

Chitosan, which derives from chitin, has been extensively studied as an antimicrobial agent in food packaging due to its ability to form a suitable film (Fernandez-Saiz *et al*., 2009) or coating (Vartiainen *et al*., 2005b), or to serve as carrier of other additives (Ojagh *et al*., 2010; Yoshida *et al*., 2010). The mechanism of its antimicrobial action is believed to occur by the rupture of the external membrane of bacteria. The use of chitosan-based films in food applications has been recently reviewed elsewhere (Dutta *et al*., 2009; Aider, 2010).

2.3.3 Development of antimicrobial packaging

The direct incorporation of antimicrobials in packaging films appeals to be a convenient means by which antimicrobial activity can be achieved. Numerous synthetic and naturally occurring antimicrobials have been incorporated into thermoplastics and thermosets, and have been tested against a variety of microorganisms. Along with the recent trend, research has also been conducted on biopolymer-based films with antimicrobials incorporated into their structure (Cha and Chinnan, 2004). Polylactic acid (PLA) (Mascheroni *et al*., 2010), polycaprolactone (PCL) (Sanchez-Garcia *et al*., 2008), starch and its derivatives (Flores *et al*., 2010), cellulose or its derivatives (Nguyen *et al*., 2008; Gemili *et al*., 2009), and zein (Del Nobile *et al*., 2008; Mastromatteo *et al*., 2009) are just few examples of the biopolymers for antimicrobial packaging, which have been studied recently.

During the development of an antimicrobial packaging several issues should be taken into consideration. As with any antimicrobial agent, those to be incorporated into polymers have to be selected on the basis of their spectrum of activity, mode of action, chemical composition and the growth rate and physiological state of the targeted microorganisms. Further considerations in choice of antimicrobial packaging are the concentration of antimicrobials in the polymer film, the effect of film thickness on activity, and the physical and mechanical properties of the polymer. For example, if the antimicrobial is entrapped into the bulk of the material, the film thickness will play a role in the diffusion and consequently the concentration at the surface of the film. As an alternative to this approach, the active substance can be incorporated into the food-contact layer of a multilayer packaging material (Quintavalla and Vicini, 2002).

Polymer additives including fillers, antifog and antistatic agents, lubricants, stabilizers and plasticizers may negatively affect the activity of antimicrobial polymers. These additives may change polymer conformation, altering diffusion, or may interact directly with the antimicrobial (Appendini and Hotchkiss, 2002). The effect of the antimicrobial on polymer properties should also be taken into account. For example, incorporation of particles that carry antimicrobials into the polymer matrix may change the mechanical, barrier and optical properties of the film. Several antimicrobials are polar; they are therefore often incompatible with non-polar synthetic polymers. Besides, thermal polymer-processing methods such as extrusion and injection moulding can only be used for heat-stable antimicrobial agents. For heat-sensitive antimicrobials such as enzymes and volatile compounds, solvent compounding, electrospinning (Vega-Lugo and Lim, 2009) or coating (Han *et al*., 2007) seems more suitable.

Indeed, antimicrobials that cannot tolerate the temperatures used in polymer processing or incompatible with the polymer are often coated onto the material after forming or are added to cast films. Coating a package surface also allows a quick release of the antimicrobials and their activity may be independent of film thickness. Such coatings also can serve as a barrier to moisture and oxygen. Apparently, one of the most studied antimicrobials coated on films is nisin (Cannarsi *et al*., 2008; Nguyen *et al*., 2008; Chollet *et al*., 2009; Rossi-Márquez *et al*., 2009; Ercolini *et al*., 2010; Guiga *et al*., 2010).

Some antimicrobial packaging uses chemically immobilized antibiotics, fungicides or active moieties, such as amine groups. This type of immobilization requires the presence of functional groups on both the antimicrobial agent and the polymer. Hence, potential antimicrobials to be immobilized include peptides, enzymes, polyamines and organic acids. Possible reduction in antimicrobial activity due to immobilization should be considered. For proteins and peptides, for instance, changes in conformation and denaturization by solvents may result in decreased activity (Appendini and Hotchkiss, 2002). Antimicrobials adsorbed or immobilized onto polymer surfaces may alter heat-sealing strength, adhesion and printing properties of the plastics. While ionic bonding onto polymers may allow slow release of antimicrobials into the food, diffusion to the product is less of a concern when the antimicrobial is covalently bonded to the polymer. More information on ionic and covalent immobilization of antimicrobials onto polymers can be obtained elsewhere (Kenawy *et al*., 2007).

2.3.4 Antioxidative packaging

Antioxidants have been used historically both in food products to improve oxidation stability of lipids and to prolong their shelf life, as well as in plastic films for polymer stabilization. Although some amount of the antioxidant may migrate from the packaging film into the food product anyway, in the case of antioxidative APs antioxidants are intended to be released to suppress oxidation reactions. Depending on the nature of the antioxidant, the release mechanism may involve evaporation and/or diffusion. Antioxidants with relatively high volatility, for example butylated hydroxytoluene (BHT) or butylated hydroxyanyzol (BHA), can be transferred into the food product without having direct contact between the food and the packaging material, while for non-volatile antioxidants such contact is essential. BHT and BHA, which are common antioxidants used for plastics, have been shown to prolong the shelf life of, for example, fish (Torres-Arreola *et al*., 2007), milk powder (Granda-Restrepo *et al*., 2009a) and cheese (Soto-Cantú *et al*., 2008). There has been some concern, on the other hand, related to the physiological effects of BHT and BHA in humans; therefore, research has been oriented towards the potential application of natural antioxidants.

The use of tocopherol (Granda-Restrepo *et al*., 2009b; Jin *et al*., 2009; Graciano-Verdugo *et al*., 2010), ascorbic acid and its salts (Gemili *et al*., 2010; Yoksan *et al*., 2010), rosemary extracts (Nerín *et al*., 2008) and oregano extracts (Peltzer *et al*., 2009) has been studied and suggested as a favourable alternative of synthetic antioxidants in AP applications. To improve their thermal and oxidative stability, as well as to ensure a controlled release, these natural antioxidants may be incorporated into CDs (Koontz *et al*., 2009; Arana-Sánchez *et al*., 2010) or chitosan nanoparticles (Yoksan *et al*., 2010). Some of these antioxidants may also change certain properties of the polymer films (e.g. transparency, tensile properties), which should also be considered when defining optimal concentrations.

The number of commercial applications and related scientific reports dealing with antioxidant releasers is limited when compared with that of antimicrobial APs. A possible explanation is that such systems are only capable of decreasing the effect of oxidation

reactions and they do not suppress microbial growth. To address this challenge, applications with dual functions are expected to be developed in the near future.

2.3.5 Other releasers/emitters

For certain food products moderate to high carbon dioxide levels (10−80%) are desirable in order to inhibit microbial growth and thereby extend shelf life. Carbon dioxide emitters have been proved to be efficient in improving the shelf life of extruded oats, cheese, butter, fresh salmon, smoked salmon, fresh cod, ground beef and cooked poultry meat cuts (Eie *et al.*, 2007). Some microorganisms, such as lactic acid bacteria, on the other hand, can be stimulated by carbon dioxide. The inhibition of spoilage bacteria using carbon dioxide may also reduce bacterial competition and thus permit growth and toxin production by pathogenic bacteria, while the product appears safe to eat. Research into the safety risks associated with the use of carbon dioxide emitters in packaging systems is therefore necessary. This concern, however, is also relevant in the case of other APs or modified-atmosphere packaging.

Release of carbon dioxide can also be necessary to overcome the collapse of flexible packages due to the development a partial vacuum caused by the dissolution of carbon dioxide in the product or the depletion of oxygen when using oxygen scavengers. In order to avoid such phenomena, dual-function oxygen scavengers/carbon dioxide emitters have been commercialized in the form of sachets containing either ferrous carbonate or a mixture of ascorbic acid and sodium bicarbonate (Vermeiren *et al.*, 2003).

Flavour scalping by polymer films or aroma permeation through packaging material may result in loss of flavour and taste intensities and may alter the organoleptic profile of the product. The incorporation of desirable food aromas into the plastic material or into an internal sachet can be used to compensate for aroma scalping and even to enhance food aroma to attract consumers when the package is opened. As an example, the headspace of dry instant coffee is often filled with volatiles distilled from the dehydration process to deliver fresh coffee fragrance when the package is opened.

Heat stability of the aroma compounds, however, is a crucial parameter. Indeed, most aromas are highly volatile, so thus unless they are protected a significant loss is expected during the film manufacturing process. Certain materials, such as CDs, can protect the aromas from evaporation through molecular encapsulation, which therefore facilitates the manufacture of aroma emitters.

2.3.6 Controlled release of active compounds

Controlled release may be defined as a method by which one or more active agents or ingredients are made available at a desired site and time and at a specific rate (Madene *et al.*, 2006). The aim of controlled-release packaging is to maintain effective levels of active compounds over a period of time by continual replenishment of inhibitory substances. There have been some approaches to ensure longer protection by merely decreasing the release rate of active compounds from the packaging. One of the most diffuse techniques is represented by the use of multilayer films, which include an outer barrier layer, a matrix layer containing the active agent and a control layer (Buonocore *et al.*, 2005). This latter has a tailored thickness and diffusivity with respect to the characteristics of microbial spoilage of food product. As recent examples, nisin-containing multilayer films based on renewable cellulosic derivatives (i.e. hydroxypropyl methylcellulose (HPMC), and ethylcellulose (EC)) have been described by Guiga *et al.* (2010).

In other studies the release rate of the active compound has been controlled (i.e. decreased) by varying porosity, or the asymmetry of the polymer film (Gemili *et al.*, 2009, 2010). Films made of sodium caseinate containing lysozyme, for instance, were modified by chemical or biochemical cross-linkers to achieve a controlled release of the antimicrobial (Mendes de Souza *et al.*, 2010). Changes in glass transition, as a consequence of combined effect of temperature and relative humidity, could also explain some particular behaviours of release from a biopolymer matrix as noted by Chalier *et al.* (2009).

Lacoste *et al.* (2005) and more recently Jin *et al.* (2009) have proposed a new technique, referred as 'smart blending', for developing novel controlled-release packaging materials. These researchers claimed that this technique allows formation in an extrusion of a wide variety of polymer blend morphologies even with common thermoplastics and fixed compositions so that release rates or dissolution rates can be adjusted to meet performance requirements.

In a study by Sanchez-Garcia *et al.* (2008) the decrease of thymol diffusion coefficient in biodegradable PCL has been achieved by the addition of novel mica-based organoclays. Such decrease has been attributed to the larger tortuosity effect imposed to the diffusion of the active compound by the dispersed nanoclay. Natural fibres have also been used to tune the release rate of thymol from mono- and multilayer zein films (Mastromatteo *et al.*, 2009).

Different strategies have been explored for achieving more sophisticated controlled release, where the release of active compounds is triggered by a certain stimuli (e.g. temperature, relative humidity, pH). These include, for example, micro-/nanoencapsulation, or immobilization on inorganic lamellae (e.g. layered double hydroxide (LDH)) with ionic bonds.

In microcapsules the active compounds can be protected from adverse environmental conditions while the capsules serve as a delivery system to reduce the threshold concentrations of encapsulated agents necessary to achieve the desired effects. Starch, dextrins, alginates, protein and lipid materials can be employed as encapsulating materials. Numerous release mechanisms have been proposed for microcapsules, including diffusion, dissolution, swelling, melting and compressing. The different microencapsulation techniques are reviewed comprehensively elsewhere (Madene *et al.*, 2006). Some relevant examples for the microencapsulation of active compounds are AITC in gum acacia (Chacon *et al.*, 2006), ascorbyl palmitate in chitosan nanoparticles (Yoksan *et al.*, 2010) or thyme oil in PLA (Martins *et al.*, 2009).

Recently, there has been an increasing interest in the use of CDs as a tool for controlled release of active compounds due to their outstanding ability to form molecular complexes with hydrophobic guest molecules. The release of active compound is determined by the association/dissociation equilibrium of the complex and is facilitated by high relative humidity. Several active compounds have been complexed mainly with α- or β-CDs in order to achieve controlled release. These include α-tocopherol (Koontz *et al.*, 2009), AITC (Vega-Lugo and Lim, 2009), 2-nonanone (Almenar *et al.*, 2009), cinnamon leaf and garlic oils (Ayala-Zavala *et al.*, 2008), quercetin (Koontz *et al.*, 2009), oregano oils (Arana-Sánchez *et al.*, 2010) and thyme oil (del Toro-Sánchez *et al.*, 2010). CD-containing active compounds have been incorporated into polyvinyl chloride and polyethylene (Fenyvesi *et al.*, 2007), as well as into PLA-PCL copolymer (Plackett *et al.*, 2007).

Fillers with high surface area and active groups on their surfaces have also been proved to be appropriate to realize controlled release. Modified LDHs, for instance, can be used to immobilize active molecules on the inorganic lamellae with ionic bonds, which can be successively released by anion exchange or displacement reactions (Tammaro *et al.*, 2009; Bugatti *et al.*, 2010). This method is particularly attractive as a controlled release packaging with improved mechanical and barrier properties may be provided.

Due to their submicron to nanoscale diameter and very large surface area, electrospun fibres are hypothesized to be more responsive to changes in the surrounding atmosphere (e.g. relative humidity and temperature) than conventional films, thus they may be suitable to perform controlled release of active compounds. AITC release from electrospun soy protein isolate (SPI) and PLA fibres triggered by moisture, for instance, has been reported to be a promising antimicrobial AP (Vega-Lugo and Lim, 2009).

In summary, controlled release of drug delivery has been used for some time in the pharmaceutical industry. On the other hand, significant research on testing the concept of controlled release of active compounds from food packaging did not appear until the last decade. The latest advances in this area are recently reviewed by Mastromatteo *et al.* (2010).

2.4 INTELLIGENT PACKAGING

IP covers a variety of applications, where some element of intelligence is added to food (e.g. sensing, recording, tracing and communicating). IP systems can be classified as: (i) quality indicators (e.g. gas indicators, time-temperature indicators or freshness indicators); and (ii) packaging which provides more convenience, improved traceability or improved logistical handling (e.g. radio frequency identification, microwave doneness indicators or thermochromic inks).

Based on an alternative classification, IP systems can be divided into sensors and indicators; however, some overlap is unavoidable. Most sensors contain two basic functional units: a receptor and a transducer. In the receptor, physical or chemical information is transformed into a form of energy, which may be measured by the transducer. Unlike sensors, indicators do not comprise receptor and transducer components and communicate information through direct visual change (Kerry *et al.*, 2006).

The most typical IP concepts are presented briefly in the following with an emphasis on quality indicators.

2.4.1 Gas indicators and sensors

Gas indicators in the form of a package label or printed on packaging films can monitor changes in the gas composition, in particular for modified-atmosphere packaging. They can be used as a leakage indicator or to verify the efficiency of, for example, an oxygen scavenger. Systems which both indicate leakage and absorb residual oxygen also exist. Oxygen indicators are the most common gas indicators for food-packaging applications; however, carbon dioxide indicators are also available. Most oxygen indicators are based on colour change as a result of: (i) an oxygen-binding reaction; (ii) a redox reaction; or (iii) a light-activated redox reaction. A tutorial review on these oxygen indicators for food packaging is available elsewhere (Mills, 2005).

The concept of optical on-pack sensors for monitoring the gas composition (i.e. oxygen and carbon dioxide) of the modified-atmosphere package at different stages of the distribution process is a very attractive alternative to the conventional destructive gas monitoring techniques such as gas chromatography and gas chromatography/mass spectrometry. In such systems the intensity or lifetime of a luminescence dye is measured and usually decreases with increasing partial pressure of oxygen. These types of sensors have been used to monitor the oxygen levels in different packaged food products.

2.4.2 Time-temperature indicators

The basic idea behind temperature-related food-quality indicators is that generally the quality of food deteriorates more rapidly at higher temperature, and thus maintaining correct storage temperatures ensures food safety. Time-temperature indicators (TTIs) provide a visual summary of a product's accumulated chill-chain history, recording the effects temperature in time (Wanihsuksombat *et al.*, 2010).

TTIs may be classified as either partial history or full history indicators, depending on their response mechanism. Partial history indicators only respond when a temperature threshold has been exceeded, while full history TTIs give a continuous temperature-dependent response throughout a products history. The major mechanisms on which TTIs are based include enzymatic reaction, polymerization and chemical diffusion. Special TTI/radio frequency identification (RFID) tags also exist containing a microchip to sense and integrate temperature over time. These monitoring systems, however, are not applicable for consumer packages, where interruptions of the cold chain often appear.

As a recent example of TTI developments, Japanese authors suggested that certain oil/water emulsions or aqueous solutions of some amide compounds can be used as simple partial TTI, as a phase separation occurring at a defined temperature causes clear visual change (Kitsunai *et al.*, 2008; Mizoguch *et al.*, 2009). The development of a new enzyme-type TTI based on the reaction between amylase and starch has also been reported (Yan *et al.*, 2008). Kreyenschmidt *et al.* (2010) characterized a novel TTI based on a photochromic solid state reaction under specific temperature conditions and UV irradiation and found that such TTI as a reliable tool to monitor the cold chains of a broad range of food products. A TTI prototype based on the vapor diffusion of lactic acid has been developed by Wanihsuksombat *et al.* (2010).

A large number of commercial TTIs have also been developed. Their principles of operation and performance have been discussed extensively in the literature.

2.4.3 Freshness/spoilage indicators

Freshness or spoilage indicators provide direct product quality information resulting from microbial growth or chemical changes within a food product. The concepts described in the literature are generally based on the detection of some volatile metabolites produced during aging of foods, such as carbon dioxide, ethanol, diacetyl, amines, ammonia and hydrogen sulphide (H_2S). The formation of different potential indicator metabolites in food products generally depends on the product type, associated spoilage flora, storage conditions and packaging system. Some organic acids (e.g. lactic acid, acetic acid) are also regarded as indicator metabolites; colour-based pH indicators, therefore, also can be used as freshness/spoilage indicators (Vaikousi *et al.*, 2008).

The number of scientific publications related to package indicators for spoilage or freshness of food is relatively limited. Among the few examples Pacquit *et al.* (2007) used a colorimetric dye-based sensor and indicator for monitoring fish spoilage on the basis of the presence of total volatile basic nitrogen (TVB-N). More recently another colorimetric mixed-dye-based food spoilage indicator for real-time monitoring of intermediate-moisture dessert spoilage has been described (Nopwinyuwong *et al.*, 2010).

Freshness indicators based on broad-spectrum colour changes, however, suffer from some disadvantages which need to be solved for realizing widespread commercial uptake. A lack of specificity, for example, means that colour changes indicating contamination may occur in products free from any significant sensory or microbiological quality deterioration. Thus,

more exact correlations appear necessary between target metabolites, product type and organoleptical quality and safety (Kerry *et al*., 2006).

2.4.4 Biosensors/nanosensors

Biosensors are a compact analytical device that detects, records and transmits information pertaining to biological reactions. These devices consist of a bioreceptor specific to a target analyte and a transducer to convert biological signals to a quantifiable electrical response. Bioreceptors are organic materials such as enzymes, antigens, microbes, hormones and nucleic acids. Transducers may be electrochemical, optical, calorimetric, etc., and are system-dependent (Yam *et al*., 2005). Biosensors have been developed to detect, for instance, *Escherichia coli* (Cheng *et al*., 2009) and *Salmonella typhimurium* (Lakshmanan *et al*., 2007). An overview of foodborne pathogen detection by biosensors has been published recently (Velusamy *et al*., 2010). Particular types of biosensors are the nanosensors, which comprise nanoparticles to attach to pathogens or other contaminants, which are then selectively identified by fluorescence or magnetic devices (Kaittanis *et al*., 2010). The detection is most often based on conductance or bioluminescence; however, enzyme sensors and immune sensors also exist (Mahalik and Nambiar, 2010). Detected microorganisms and toxins include *E. coli* O157:H7, *Salmonella* spp., *Shigella flexneri*, *Listeria monocytogenes* and *Staphylococcus* enterotoxin. Although bio- and nanosensors have been mainly used in clinical, biochemical and environmental analyses, application of biosensors in intelligent packaging systems is also anticipated in the future.

2.4.5 Radio frequency identification

Radio frequency identification (RFID) systems use radio waves to track items wirelessly (Brody *et al*., 2008). They based on tags affixed to assets (cattle, containers, pallets, etc.) to transmit accurate, real-time information to a user's information system. RFID tags may be classified into two types, such as: (i) passive tags that have no battery and are powered by the energy supplied by the reader; and (ii) active tags that have their own battery for powering the microchip's circuitry and broadcasting signals to the reader (Yam *et al*., 2005). Other classifications distinguish between low-, intermediate- and high-frequency RFIDs.

Although RFID has been available for many years for tracking expensive items and livestock, its application in packaging has only begun in recent years. An RFID tag can be attached to a package, which thus becomes intelligent in a sense that the data provide valuable information that can be stored and read by appliances. Such technology is still at its early stages of implementation and currently the focus is on simple tasks such as product identification and tracking, and not on complicated matters that involve the application of scientific food principles. Furthermore, RFID has some weaknesses including signal loss due to metal shielding or absorption by water molecules, relatively high price and limited recycling ability which need to be addressed before a broad application can occur (Brody *et al*., 2008).

2.5 NANOTECHNOLOGY IN ACTIVE AND INTELLIGENT PACKAGING

The potential benefits of nanotechnology have already been recognized by many industries, such as microelectronics, aerospace and the pharmaceutical industry. Nanotechnology applications are also beginning to live up to their promise in the field of food packaging.

Introduction of different nanofillers into polymer films (e.g. nanoclays, metal nanoparticles and nanofibres), for example, may result in nanocomposites with improved mechanical and barrier properties, thermal stability and reduced weight. In particular, renewable and biodegradable polymers can benefit from nanofiller addition. Comprehensive reviews of such biobased nanocomposites for food packaging are available elsewhere (Azeredo, 2009; Arora and Padua, 2010).

Nanotechnology-related applications of A&I packaging, on the other hand, are rather scarce. The few examples include food packaging materials with antimicrobial properties and nanosensors which are integrated into food packaging and are able to detect bacteria, toxins, viruses and allergens on the surface of food.

The idea to insert active nanoparticles into polymer matrices could in principle bring the two-fold advantage of improving the performance of food packaging material and imparting an additional functionality (i.e. antimicrobial, antioxidant, scavenger), thus promoting the prolongation of the shelf life of the packaged product.

Silver nanoparticles, for example, have excellent antibacterial properties (Kasprowicz *et al*., 2010). Silver ions can adhere to the negatively charged bacteria cell wall causing a change in the cell wall permeability, which, eventually coupled with protein denaturation, induces lysis of the bacterial cell (Munro *et al*., 2009). Such nanoparticles interact well with other particles due to their high specific area, which increases their antibacterial efficiency (Xu *et al*., 2008). The development of films and pads with antimicrobial properties based on silver nanoparticles has been reported (Tankhiwale and Bajpai, 2009; Fernández *et al*., 2010; Vimala *et al*., 2010).

As other examples for antimicrobial AP based on nanomaterials, benzoic acid, benzoate and its derivatives have been bonded to either a magnesium–aluminium LDH (Tammaro *et al*., 2009), or to zinc–aluminium LDH (Bugatti *et al*., 2010). When these complexes have been blended with PCL a decreased release of the antimicrobial molecules could be obtained.

Carbon nanotubes are another type of engineered material with nanoscale diameters that can be used in food packaging as reinforcement. In addition, it was recently discovered that they may also exert powerful antimicrobial effects, which is explained by that the long and thin nanotubes can puncture bacterial cell walls, causing cellular damage. Indeed, *E. coli* cells died immediately upon direct contact with aggregates of carbon nanotubes (Kang *et al*., 2008).

When nanoparticles such as montmorillonites (MMTs) or LDHs are introduced into the polymer matrix they are assumed to decrease the diffusion of different gases through increasing the tortuosity of the path for gases penetrating in the films, thus enhancing the barrier properties in such way. These nanoparticles can also be used to decrease a release rate of certain volatile antioxidants and antimicrobials (Sanchez-Garcia *et al*., 2008). Due to their high specific surface area, however, they also tend to interact with gas molecules; they therefore may act as gas adsorbers.

Nanoscale technologies are also applied to improve traceability and to monitor the condition of food during transport and storage. Nanosensors, for instance, could be placed directly into the packaging material, where they detect metabolites released upon food spoilage. The main advantage of using nanosensors is that the time for pathogen detection can be reduced significantly when compared to traditional detection techniques. More detailed presentation of the different nanosensors is available in recent reviews (Sozer and Kokini, 2009; Mahalik and Nambiar, 2010).

It has to be emphasized that there might be potential risks of using nanomaterials in food-packaging materials. Although these nanomaterials from packaging are not intended to be

ingested or inhaled by consumers, their migration into food may occur. Currently, their potential risks to human health and to the environment are unknown. In addition, chemical and physical properties of nanoparticles may differ from those of their macroscale chemical counterparts, meaning that their toxicokinetic and toxicity profiles cannot be extrapolated from data on their corresponding non-nanoforms (Munro *et al.*, 2009). Detailed research to determine their safety, therefore, is greatly needed. The public perception of nanotechnology in general is another important factor that can influence the realization of such nanotechnology developments in the area of food packaging. These crucial issues are discussed elsewhere (Siegrist *et al.*, 2007; Chaudhry *et al.*, 2008; Das *et al.*, 2009).

2.6 FUTURE TRENDS

A&I packaging is an emerging area of food technology that can provide enhanced food preservation and extra convenience for the benefit of consumers. The effectiveness of these packaging concepts has been extensively studied both in model systems and in real food. Several examples of commercial applications indicate that A&I packaging is no longer merely a scientific curiosity. Interest in these areas and the number of food-related applications is expected to increase significantly in the near future. The main driving forces are the consumer preferences for minimally processed and naturally preserved foods and the willingness of the food industry to invest in product quality and safety. In these days more and more focus is placed on consumer concerns such as freshness, quality and information.

Although A&I packaging may provide many benefits to shelf-life extension and food safety, there are several issues to consider before implementing such packaging systems. Compliance with food-safety regulations, high production costs and consumer mistrust have been found to be potential challenges. Differences in legislation across the world, for instance, still have a major impact on the sales and market penetration. Although a legislation specific for A&I packaging exists now in Europe, delays in preparing that have hampered the introduction of A&I packaging in European food markets compared to Japan, USA and Australia. Another barrier is the generally conservative consumer attitude in Europe towards APs. A recent trend is to incorporate active compounds in the packaging film or in an adhesive label rather than using separate objects in packaging, and thus to avoid consumer resistance. Physical methods to modify polymer surfaces, such as electron- or ion-beam, plasma and laser treatments are also emerging and pose potential for functionalizing inert polymer film surfaces while providing an active function that is 'invisible' to consumers. The use of naturally occurring active substances and biopolymers from renewable sources is another key factor to increase consumer acceptance and to address recent demands for sustainability.

It seems very likely that future developments will combine the benefits of APs with those of IP systems and will eventually end up in the development of a truly interactive packaging that responds directly to the needs of the food. These will be packaging systems that sense the presence of microorganism in the food, triggering antimicrobial mechanisms as a response, in a controlled manner. The feasibility of such 'release-on-demand' packaging is approaching rapidly with the recent advances in the development in sensor technology including nanosensors/biosensors. Nanotechnology in general has a great potential to contribute to improved food safety and quality through A&I packaging.

In summary, A&I packaging can play an important role in reducing the risk of pathogen contamination and extending the shelf life of food, as well as improving convenience and traceability. It must not, however, be a substitute for good-quality raw materials and good

manufacturing practices, but should be used as a complementary technique for other non-thermal processes.

2.7 FURTHER SOURCES OF INFORMATION

It was not possible to cover all the matters of A&I packaging in this format. Further information on the subject is available elsewhere. The global market situation of such packaging systems, for instance, is presented in reviews by Danielli *et al.* (2008) and by Restuccia *et al.* (2010). These papers also discuss future trends of A&I with a special emphasis on safety concerns and risk assessment and provides information on the EU legislation and compliance testing of these novel food packaging technologies. Regarding the legislative status of food contact materials worldwide, including A&I packaging, readers can also consult with the following websites:

http://ec.europa.eu/food/food/chemicalsafety/foodcontact/index_en.htm
http://europa.eu/legislation_summaries/consumers/product_labelling_and_packaging/l21082a_en.htm
http://www.fda.gov/Food/FoodIngredientsPackaging/FoodContactSubstancesFCS/default.htm
http://www.food.gov.uk/foodlabelling/foodcontactmaterials2/
http://www.packaginglaw.com/1808_.shtml

For a recent reference to detailed discussion on the associated challenges and risk assessment of novel packaging systems, including A&I packaging, the reader is referred to Munro *et al.* (2009). Besides, several review papers and book chapters deal with the role of A&I packaging for maintaining the quality and safety of specific food product groups, including meat and meat products (Coma 2008), milk and milk products (Soares *et al.*, 2009), bakery products (Galić *et al.*, 2009), beverages (Paul, 2003), fruits and vegetables (Scully and Horsham, 2007) and fresh-cut produce (Lucera *et al.*, 2010).

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3 Modified-Atmosphere Storage of Foods

Osman Erkmen

Abstract: Safety and perishability are the two most important concerns in any food product. Safety is determined by the presence of a pathogen or toxic chemical in the food. Perishability is caused by microbial degradation, enzyme activity and chemical reactions. Foods vary in their perishability depending on their composition, processing, packaging and storage. Modified-atmosphere technologies are to extend shelf life of food products by minimizing the physiological, chemical and microbial decomposition of foods in an atmosphere that is different from the normal composition of air. Modified-atmosphere packaging is used for extending the shelf life of products, ensuring microbial safety, improving the product image, reducing the wastage of a wide range of chilled perishable foods and ambient stability of products. The choice of a particular packaging atmosphere depends on many considerations such as effect on microorganisms, retaining food stability, prevention of oxidative deterioration and inhibition of ripening. The packaging market has been a growing area with the recent packaging systems, such as new developments in packaging materials/machinery and food-product applications. There is a good potential for modified-atmosphere storage of meat, bakery, dried, dairy and poultry products, and vegetables and fruit.

Keywords: active packaging; modified atmosphere; packaging; safety; vacuum packaging

3.1 INTRODUCTION

The nutritional and organoleptic quality of foods will start to decline as a result of the food's own metabolic activities and microbial growth after handling or processing. Modified atmosphere (MA) is a preservation technique that may further minimize the physiological, chemical and microbial decomposition of foods by keeping them in an atmosphere that is different from the normal composition of air (78.08% nitrogen, 20.96% oxygen, 0.04% carbon dioxide, plus water vapor and traces of inert gases). MA involves the removal of air from the package and its replacement with a single gas or mixture of gases to enhance the shelf life of foods. This technology allows the storage of foods without use of any chemical additives by fewer processing treatments. The presence of oxygen is one of the major factors of food spoilage, since it can cause oxidation reactions (damaging vitamins and causing growth of aerobic microorganisms; Ahvenainen, 1996; Gorris and Peppelenbos, 2007; Erkmen and Bozoglu, 2008a). The gaseous atmosphere inside a MA pack changes continuously during storage due to absorption of gases by foods, respiration of certain products, microbial growth and exchange of gases through the package (Ooraikul, 2003; Gorris and Peppelenbos, 2007). MAs without oxygen are used to minimize oxidative deterioration reactions (such as brown discoloration of meat, rancidity of peanuts), reduce microbial

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growth, extend the shelf life of products, improve the product image, reduce the wastage of foods and produce stable products (Ahvenainen, 1996; Erkmen and Bozoglu, 2008a). Microorganisms differ in their requirement of gaseous atmospheres for growth (Day, 2008; Erkmen and Bozoglu, 2008b), as follows:

- Aerobic microorganisms require oxygen for growth, such as *Pseudomonas*, *Psychrobacter*, *Shevanella*, *Acinetobacter/Moraxella*, *Micrococcus*, some species of *Bacillus*, film yeasts, and molds. The growth inhibition of these microorganisms can be achieved by excluding oxygen from the MA.
- Microaerophilic microorganisms require low levels of oxygen for growth. Some may require increased levels of carbon dioxide for growth, such as *Campylobacter* and *Lactobacillus*.
- Facultative anaerobic microorganisms are able to grow in the presence or absence of oxygen, such as *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Brochothrix*, *Salmonella*, *Vibrio*, *Aeromonas*, some species of *Bacillus*, lactobacilli, and fermentative yeasts.
- Anaerobic microorganisms are inhibited or killed by oxygen, such as *Clostridium* and *Bifidobacterium*.

This chapter provides an overview of MA techniques, an application for food preservation and highlights the effects of MA on the spoilage and pathogenic microorganisms in foods.

3.2 MODIFIED ATMOSPHERE

3.2.1 Types of modified-atmosphere techniques

Modification of the atmosphere in a package involves a reduction of oxygen or an increase of the carbon dioxide/nitrogen concentrations, but in some cases an amount of carbon monoxide, ethylene, ethanol or other compounds in the atmosphere can also be used for shelf-life extension. MA can be created passively by the respiration of the product inside the package (product MA packaging) or actively by introducing the desired gas mixture (modified-atmosphere package, MAP). Other ways of obtaining MA are the use of gas generators and scrubbers to control levels of gases in the storage environment (controlled-atmosphere packaging, CAP), evacuation of air from packages (hypobaric storage or vacuum packaging, VP), and addition of chemical systems to packs that absorb or generate gases or volatile compounds (active packaging, AP) (Gorris and Peppelenbos, 2007; Erkmen and Bozoglu, 2008a). Another application of the concept of changing the gas environment is the carbonation of drinking water and soft drinks. Carbonation increases both the shelf life and the safety of the product (Molin, 2000). The choice of a particular packaging atmosphere depends on many considerations, such as effect on microorganisms, retaining food stabiliy, prevention of oxidative deterioration, inhibition of ripening, and protecting packaging of non-carbonated beverages from collapse. The concept of high carbon dioxide pressure (5–15 MPa) is used to inactivate microorganisms in liquid foods (Erkmen, 1997, 2001, 2002).

3.2.1.1 CAP

In CAP, the foods are placed into a room or container with a gas or mixture of gases after removal of air from the headspace by vacuum, and the levels of the gases are continuously

monitored and adjusted as required throughout storage. This method is used for bulk storage or transport of smaller or larger quantities of foods. Modification of the controlled atmosphere is an expensive technique and is used for long-term storage of foods to maintain their freshness and quality. CAP is used for transportation of foods, particularly fruit and vegetables, in atmospheres containing reduced oxygen (2–5%) and increased carbon dioxide (8–10%) in airtight chilled storage rooms, and shipment of chilled carcasses packed in aluminium foil laminate bags with an atmosphere of 100% carbon dioxide. This storage prevents the respiration and adverse changes to the sensory and textural properties of foods, and inhibits the growth of certain spoilage microorganisms (such as aerobic bacteria and molds). Growth inhibition is evident both in the extension of the lag phase and the reduction of maximal biomass formation (Erkmen and Bozoglu, 2008a).

3.2.1.2 MAP

In MAP, the gas composition within the package is not monitored or adjusted for changes during storage as CAP. One gas or a mixture of gases is flushed into the package before closing depending on the oxygen sensitivity, metabolic activity and stability of the products. The air in the package is removed by vacuum before gas flushing. Products sensitive to oxygen or products with a low level of respiratory activity are packed with a gas mixture composed of low oxygen and moderately high carbon dioxide. After closing the package, respiration of the product will decrease oxygen and increase carbon dioxide in the package (Gorris and Peppelenbos, 2007; Erkmen and Bozoglu, 2008a). The composition of the gas atmosphere changes during storage as a result of product and microbial respiration, dissolution of carbon dioxide into the aqueous phase, and gas diffusion through the foods and the package materials.

Respiration of a food depends on its physiological stage, temperature, oxygen and carbon dioxide partial pressures, relative humidity, and ethylene concentration. Gas diffusion through foods is affected by temperature, food mass and volume, respiration rate of food, cell-membrane permeability, gas diffusion path, maturity stage of the product, gas gradient across the film, and water-vapor gradient. Most plastic films do not have the proper oxygen/carbon dioxide permeability for specific foods. The gas permeability of the film depends on the concentation of gases; structure, pore size, thickness and surface area of film; temperature; and relative humidity (Gorris and Peppelenbos, 2007). MAP controls or reduces the growth of undesirable microorganisms (pathogenic or spoilage) in food, and retards enzymatic and respiratory activities of foods. The growth of aerobes is prevented in packed products while anaerobic and facultative anaerobic bacteria can grow unless other additional techniques are used to control their growth (Erkmen and Bozoglu, 2008a). MAP is used to increase the shelf life of many refrigerated foods, such as fresh pasta, bakery products, cooked poultry products, cooked egg products, fresh and cooked seafoods, sandwiches, raw meats, and fruit and vegetables.

3.2.1.3 VP

Whereas MAP and CAP mostly operate at ambient pressure (101 kPa), storage of foods at reduced atmospheric pressures is possible with VP. In VP, the air is removed from the headspace of the package by vacuum and the altered initial atmosphere is not controlled during storage (Gorris *et al.*, 1994). The initial gas composition is normal air but with oxygen present at about one-third of the normal amount due to its partial pressure (at an air pressure of

1–40 kPa). The lower oxygen content stabilizes the product quality by slowing down the metabolism of the product and the growth of spoilage microorganisms. VP strongly retards enzymatic browning of the cut food surfaces, such of as vegetables, fruit, and salad mixes (Gorris and Peppelenbos, 2007). The atmosphere which develops during storage is mainly the result of biological activity. VP is predominantly used for meat and related products. Another VP process is the sous-vide method. Sous-vide is a form of cooking in which food is placed into airtight plastic bags, vacuum sealed, immersed in hot water, and allowed to cook by heating for a period of time at a relatively low cooking temperature (usually around 60°C). The final minimally processed product may have a better flavor, color, texture, and aroma with minimal loss of juices while at the same time greatly improving food safety. The cooking temperatures are not to high enough to kill all microorganisms and spores (Erkmen and Bozoglu, 2008a), therefore foods packed with sous-vide must be stored at low temperature. *Clostridium botulinum* can grow in food in the absence of oxygen and produce the deadly botulinum toxin, so sous-vide cooking must be performed under carefully controlled conditions and chilling requirements to avoid botulism. Sous-vide is a safe and effective method of packaging for minimally processed food, and mostly used in restaurants to control the organoleptic properties of a product. In sous-vide, hermitic vacuum sealing of the plastic bags, heat treatment, and the oxygen barrier inactivate and slow the growth of most microorganisms, thus delaying spoilage (Creed, 1998).

3.2.1.4 AP

AP techniques can be defined as a packaging of the product together with an "active" material (chemical in absorbers/releasers) to prolong shelf life, enhance safety and sensory properties, and maintain the quality of the product. The atmosphere in packages can be controlled by the use of a gas or volatile compound-absorbing material or carbon dioxide-releasing material. The material in the package continuously modifies the gas environment by removing gases or adding gases to the package headspace. The active materials are either combined with the packaging material or placed in the package (Suppakul *et al*., 2003; Gorris and Peppelenbos, 2007; Erkmen and Bozoglu, 2008a). The amount of active compound added depends on the production rates of metabolites (carbon dioxide, ethylene), concentrations of gases to be reached, length of storage, and type of food, among others (Cameron *et al*., 1995; Gorris and Peppelenbos, 2007). Incorporation of chemicals into packaging materials minimizes negative consumer responses and offers a potential economic advantage through increasing shelf life of products, eliminating the risk of accidental rupture of the sachets, and inadvertent consumption of their contents (Suppakul *et al*., 2003). AP is used in packs for popcorn, fries, pizzas, pies, baked goods, etc. (Woods, 1993). Time-temperature indicators (TTIs) can be used on packages to display loss of shelf life and temperature-abuse conditions (Gorris and Peppelenbos, 2007). Possible future developments in AP include self-venting microwave packs in which a vent opens at a temperature and closes on cooling (Louis, 1998). AP systems include oxygen absorbers, carbon dioxide generators, preservative releasers (e.g. ethanol production), aroma releasers, moisture absorbers, odor and off-flavor or ethylene removers, TTIs, edible coatings, and the others.

3.2.1.5 Oxygen absorbers

Oxygen absorbers reduce ascorbic acid, photosensitive dyes, enzymes (such as glucose oxidase and ethanol oxidase), unsaturated fatty acids, rice extract, and immobilized yeast on a

solid substrate or in the packaging structure (Louis, 1998; Suppakul *et al*., 2003). The oxygen absorber can remove oxygen from the package headspace to a level of less than 0.01%. Oxygen causes oxidation of products and limits their shelf life. Oxidation can cause changes in flavor, color, and odor, and nutrient loss. The removal of oxygen from the package headspace, and from the solution in liquid foods, extends storage life of products (Fellows, 2000; Suppakul *et al*., 2003). An oxygen absorber in a food package can control growth of mold and aerobic bacteria in foods (such as cakes, dairy, and bakery products), delay oxidation and rancidity in fatty foods (such as vegetable oils), protect the color of packaged foods (such as meats), and slow down the rate at which baked products become stale (Rooney, 1995; Fellows, 2000).

3.2.1.6 Carbon dioxide generators

Carbon dioxide can inhibit or reduce microbial growth. A carbon dioxide generator may be incorporated into packaging material or added as a sachet in a food package (such as fresh meat, poultry, fish, cheese, and strawberries). Carbon dioxide can passes through most films more easily than oxygen. Carbon dioxide must be continuously produced to maintain the desired gas concentration within the package to control microbial growth (Erkmen and Bozoglu, 2008a).

3.2.1.7 Ethylene absorbers

Ethylene (C_2H_4) absorbers, in the form of sachets of potassium permanganate ($KMnO_4$) immobilized on an inert mineral substrate (such as alumina or silica gel) or activated carbon, are integrated into the packaging material (Fellows, 2000; Brennan and Day, 2006). A chemical reagent, incorporated into the packaging film, traps the ethylene produced during ripening of foods (such as fruit and vegetables). The color of system can be used as an indicator of the extent of reaction (the color changes from purple to brown) and indicates how much absorber has been used up. The sachet material is highly permeable to ethylene (Fellows, 2000; Suppakul *et al*., 2003).

3.2.1.8 Moisture absorbers

Condensation or sweating is a problem in most packaged foods, such as fruit and vegetables. When condensation wets the product, the nutrient leaks into the water which encourages rapid microbial growth. Moisture absorbers can control condensation inside packages and allow the food to remain dry (Suppakul *et al*., 2003). Moisture-absorbing polymers (such as polyacrylate salts and graft copolymers of starch) can control water activity (a_w) of foods, such as meat, fish, poultry, fruit, vegetables, and seafood (Rooney, 1995: Suppakul *et al*., 2003). Desiccants (such as silica gel, molecular sieves, calcium oxide, and natural clays in sachets) can be used to control moisture for a wide range of foods, such as cheeses, meats, chips, nuts, popcorn, candies, gums, and spices (Brody *et al*., 2001; Suppakul *et al*., 2003).

3.2.1.9 Antimicrobial migraters

Spoilage due to microbial growth in food can be limited by packaging with an antimicrobial-releasing scavenger, such as ethanol, ethylene, sulfur dioxide (SO_2), propionic acid, and the others. Ethanol scavengers are in the form of a sachet that releases ethanol vapor, which settles

on the surface of the food and prevents the growth of microorganisms. Ethanol scavengers are used in the food industry, for bread, cake, pizza, and other bakery products (Suppakul *et al.*, 2003). Sulfur dioxide is used to control mold growth on some processed foods (such as fruit and vegetables). Carton fumigation consists of a combination of quick-release and slow-release sachet systems which emit small amounts of sulfur dioxide (Suppakul *et al.*, 2003; Brennan and Day, 2006). Sachets containing iron powder and calcium hydroxide to regulate both oxygen and carbon dioxide are used to extend the shelf life of ground coffee.

3.2.1.10 Modified-humidity packaging

MAP, CAP, and VP focus on changing the metabolic gases oxygen and carbon dioxide. Modified-humidity packaging (MHP) is used to control both dehydration and condensation in packages which can cause the most important losses of quality. In most "closed" packages (such as MAP, CAP, and VP), the relative humidity is close to saturation due to the water exchange between the product and the headspace. The high humidity increases the probability of condensation and free water accumulation by the product (Shirazi and Cameron, 1992; Gorris and Peppelenbos, 2007). Reducing water loss is important. A lower relative humidity causes too much weight loss, while a higher relative humidity causes decay. MHP can be effectively used to minimize quality loss of a product due to changes in moisture level. Many commercially available packaging materials have favorable water-vapor-permeable characteristics. The in-pack relative humidity may be controlled through the use of packaging materials with high water-vapor-permeability or the inclusion of sachets containing water absorbers, such as $CaCl_2$, sorbitol, and xylitol, in the package (Beaudry, 1993; Gorris and Peppelenbos, 2007).

3.2.2 Gases used for modification of atmosphere

The gases used in the modification of atmosphere in storage rooms or in packages must be chosen to meet the needs of the specific food product. Nearly all products need individual gases or combinations of carbon dioxide, oxygen, and nitrogen. Sometimes other gases can also be used for special purposes.

3.2.2.1 Carbon dioxide

Carbon dioxide has bacteriostatic and fungistatic properties and will retard the growth of mold and aerobic bacteria. It has little effect on the growth of yeast and does not retard the growth of all types of microorganisms. The growth of lactic acid bacteria is improved in the presence of carbon dioxide with low oxygen content. The inhibitory effect of carbon dioxide is increased at low temperatures because of its enhanced solubility in water, forming carbonic acid (H_2CO_3). The absorption of carbon dioxide in a pack depends on the water and fat content of the product. Excess carbon dioxide absorption can reduce the water-holding capacity of meats. Some dairy products can be tainted, and fruit and vegetables can be physiologically damaged, at high carbon dioxide levels (Day, 2008).

3.2.2.2 Oxygen

In MA, oxygen levels are normally set as low as possible to inhibit the growth of aerobic spoilage microorganisms and to reduce the oxidative deterioration of foods. But oxygen is needed for fruit and vegetable respiration, color retention in red meats, and to avoid anaerobic

conditions in packs (Day, 2008). Oxygen is included in gas-flush mixtures in meat packaging to maintain the bright red appearance of oxymyoglobin. Oxygen is toxic to most microorganisms due to the formation of the superoxide radical. In the presence of oxygen, some microorganisms produce hydrogen peroxide that reacts with superoxide radicals, resulting in the formation of extremely reactive compounds, such as the hydroxyl radical. Different types of microorganisms are protected to various degrees against oxygen radicals by the enzymes such as superoxide dismutases, catalases, and peroxidases. They can remove hydrogen peroxide formed in the dismutation reaction (Molin, 2000).

3.2.2.3 Nitrogen

Nitrogen is an effective inert gas, and has a low solubility in both water and fat. In MA, nitrogen is used primarily to displace oxygen in order to retard aerobic spoilage and oxidative deterioration. Nitrogen can also be used as a filler gas to prevent pack collapse (Molin, 2000; Day, 2008).

3.2.2.4 Carbon monoxide

Carbon monoxide produces a stable, cherry-red color (carboxymyoglobin) in meat owing to it binding strongly to the muscle pigment deoxymyoglobin. Low concentrations of carbon monoxide (<0.5%) combined with anaerobic carbon dioxide atmospheres improve meat color; inhibit lipid oxidation, bone discoloration, and browning of meat and meat products; extend shelf life; reduce growth of certain spoilage and pathogenic microorganisms; and pose no toxic hazard to consumers. It is safe to use in food packaging (Sørheim *et al.*, 1997; Day, 2008).

3.2.2.5 Argon

A wide range of foods are MA packed in argon-containing gas mixtures. Argon effectively inhibits enzyme activity, microbial growth, and degradative chemical reactions in foods. Argon is a chemically inert gas and it has a similar atomic size to oxygen, and higher density and solubility in water compared with nitrogen and oxygen. Argon is more effective than nitrogen at displacing oxygen from cellular sites to inhibit oxidative deterioration reactions. Argon demonstrates some properties that are beneficial, but it is more expensive than nitrogen (Day, 2008).

3.2.2.6 Other gases

Other gases, such as ozone, nitrous oxide, ethylene oxide, helium, neon, propylene oxide, ethanol vapor, hydrogen, sulfur dioxide, and chlorine, can be used on a restricted commercial basis to extend the shelf life of a number of foods. The commercial use of these gases is limited owing to safety concerns, regulatory constraints, negative effects on sensory quality, and economic factors (Day, 1992, 2008).

3.3 EFFECTS OF MODIFIED GAS ATMOSPHERES ON MICROORGANISMS AND FOODS

3.3.1 Mechanism of effects

For many processed MA-packed products, main factors causing quality loss are ripening, oxidation, or senescence and microbial growth. The very low oxygen (<2–3%) and moderately

high carbon dioxide (5–20%) levels inside a package slow down the growth of aerobic microorganisms (Farber, 1991; Phillips, 1996). The growth-inhibitory effects of MAs vary for different microorganisms. MAs not only reduce microbial growth, oxidative reactions, and metabolic activity of the foods but also may impose changes in the composition of the microbial flora and improve the growth of some microorganisms. MA can discourage growth of aerobes (such as *Pseudomonas*, *Psychrobacter*, and *Shewanella*) and encourage growth of facultative anaerobes (such as Enterobacteriaceae, *Aeromonas*, *Photobacterium*, *Brochothrix*, and lactobacilli) and anaerobes (such as *Clostridium* and *Bifidobacterium*). Oxygen can allow initial growth of aerobes to produce carbon dioxide (Erkmen and Bozoglu, 2008a).

Plant parts, such as seeds, fruit, leaves, or roots, continue to respire after harvest. Respiration involves the consumption of oxygen and the production of carbon dioxide. Respiration of foods in packaging can be reduced by using low-temperature and limiting oxygen concentrations, and by altering gas compositions (Banks *et al*., 1993; Gorris and Peppelenbos, 2007). Oxygen has an effect on the activity of certain enzymes present in foods, such as polyphenol oxidase, which causes browning of foods (Nguyen-the and Carlin, 1994). The oxygen concentration may be chosen to be as low as possible with regard to product respiration without initiating fermentation reactions. Fermentation reactions in products lead to the production of compounds such as acetaldehyde, ethanol, lactic acid, and ethyl acetate. Alcoholic fermentation is always found in plant tissues exposed to an environment without oxygen. An increased concentration of ethanol and ethyl acetate is often related to quality problems, such as off-taste and off-odor (Larsen and Watkins, 1995; Gorris and Peppelenbos, 2007). The packaged foods susceptible to fermentation should have oxygen concentrations that are high enough to avoid fermentation (Gorris and Peppelenbos, 2007). Carbon dioxide levels above 20–50% significantly affect the growth of psychrotrophic pathogens that are relevant to MA-packaged products stored at low temperature (Bennik *et al*., 1995; Carlin *et al*., 1996). MA conditions reduce the growth of spoilage microorganisms that would be the competitors of such pathogens.

In addition to modification of atmosphere, other methods are necessary for effective control of microorganisms in the preservation of foods The antimicrobial action in MA-packed foods can be produced by the changes in redox potential (Eh) and carbon dioxide concentrations (Gorris and Peppelenbos, 2007; Barazi and Erkmen, 2010). Natural reducing components in foods (such as SH groups in protein foods, and ascorbic acid and reducing sugars in fruit and vegetables) can alter the Eh, and encourage growth of anaerobic and facultative anaerobic microorganisms while inhibiting aerobes. Thus just by changing the Eh it is not possible to control all microbial growth (Erkmen and Bozoglu, 2008a). Carbon dioxide increases the lag and exponential phases of microorganism growth. The antimicrobial effects of carbon dioxide increase with decreasing storage temperature due to the increasing solubility of carbon dioxide. From a microbiological point of view, carbon dioxide is the most important gas used in MA. Carbon dioxide may kill, inhibit, stimulate, or have no effect on the growth of microorganisms. The antimicrobial effect of carbon dioxide on microorganisms depends not only on the concentration of carbon dioxide but also on the a_w, acidity (pH), temperature, and numbers and age of the microorganisms (Day, 2008; Erkmen and Bozoglu, 2008a). Carbon dioxide has an important regulatory influence in the living cell. The antimicrobial effect of carbon dioxide is due to rapid cellular penetration, alteration in cell permeability, solubility of carbon dioxide in water to form H_2CO_3, reducing pH (environmental and cytoplasmic acidification), interaction with the cell membrane and other cellular compounds, and interference of carbon dioxide with the activities of several enzymes and biochemical reactions. Cell membranes are freely permeable to carbon dioxide and carbonic acid but impermeable to bicarbonate ions. The antimicrobial effect of carbon dioxide on

microbial growth occurs at the 10% level of the gas and increases as the concentration of carbon dioxide increases (Erkmen and Bozoglu, 2008a). The major factor affecting the antimicrobial characteristics of carbon dioxide lies in its ability to penetrate the bacterial membrane and causing changes to intracellular pH. Carbon dioxide is equally soluble in water per unit volume of the gas and water phases. In water, the carbon dioxide hydrates to form H_2CO_3, and then further dissociates to bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}). The amount of carbon dioxide dissolving in foods decreases with decreasing a_w and increases with increasing buffer capacity of foods (Molin, 2000; Erkmen and Bozoglu, 2008a).

Nitrogen has no direct inhibitory effect on microbial growth. Its indirect effect is due to its displacement of oxygen, and creating an anaerobic or microaerophilic environment. Facultative, microaerophilic, or anaerobic microorganisms may therefore grow in place of aerobic microbial flora (Day, 2008). Nitrogen is used as an inert filler that keeps flexible packs from collapsing (Day, 2008; Molin, 2000).

3.3.2 Effects of modified atmosphere on spoilage microorganisms

In order to extend the shelf life of foods by retarding microbial spoilage, carbon dioxide is used alone or with nitrogen and/or oxygen. Concentrations of carbon dioxide over 5% inhibit the growth of some food spoilage microorganisms. Inhibition of microorganisms increases linearly with increasing concentration of carbon dioxide depending on the food and microflora. Molds are sensitive, yeasts mostly resistant, and bacteria are highly variable in their sensitivity to carbon dioxide. The most affected bacteria are *Pseudomonas*, *Micrococcus*, *Bacillus*, and *Acinetobacter/Moraxella*, which grow rapidly, and produce off-odors and flavors in raw meats and other flesh foods. Lactobacilli are the most resistant bacteria to carbon dioxide but they do not readily spoil foods, unless present at very high levels (Day, 2008). The Gram-negative bacteria occasionally occur as a significant part of the spoilage flora of refrigerated fresh meat (*Aeromonas* and Enterobacteriaceae) and fish (*Aeromonas*, Enterobacteriaceae, and *Vibrio*) stored in MAs. Fresh fruit and vegetables normally have different spoilage microflora depending on handling conditions. The high acidity of many fruit (pH 4.6) limits spoilage to acid-tolerant molds, yeasts, and lactic acid bacteria. Vegetables generally have a pH around 6.0–7.0, and so lack this intrinsic protection and are spoiled by most microorganisms. In vegetables, pectinolytic Gram-negative bacteria of the genera *Pseudomonas* and *Enterobacter* are often involved in spoilage. Temperature abuse may be an important safety indicator. MA methods are used to extend product shelf life by suppressing spoilage microorganisms (Gorris and Peppelenbos, 2007). Low-temperature storage (0–6°C) not only decreases the growth rate of spoilage and pathogenic microorganisms on foods but also increases the inhibitory effects of MA by increasing the solubility of carbon dioxide. The depleted levels of oxygen (<2–5%) and elevated levels of carbon dioxide (>10%) used in MA generally inhibit the growth of aerobic spoilage bacteria and molds but can favor the growth of lactic acid bacteria, and anaerobic and facultative anaerobic bacteria are not effected. The effects of MA on yeasts are negligible since they are capable of both aerobic and anaerobic respiratory growth (Molin, 2000; Day, 2008).

3.3.3 Effects of modified atmosphere on microorganisms that cause food poisoning

High levels of carbon dioxide have an inhibitory effect on *S. aureus*, *E. coli*, *L. monocytogenes*, and *Salmonella*. The degree of inhibition increases as the temperature decreases.

There is a potential growth possibility of anaerobic *Clostridium* spp. under MA. Nearly all MA packed foods are refrigerated; therefore, it is mainly cold-tolerant pathogenic bacteria that survive and outgrow other species, such as *Yersinia enterocolitica*, *L. monocytogenes*, and *Aeromonas hydrophila* (Phillips, 1996; Gorris and Peppelenbos, 2007). Also, nonproteolytic *C. botulinum*, *E. coli*, *Shigella*, and *Salmonella* are able to grow and can cause potential health risks when present in MA pack products. Consequently, the use of additional barriers (such as acidification, use of preservatives, and/or a reduction in a_w) to prevent microbial growth is strongly recommended (Day, 2008).

3.3.3.1 Clostridium

C. botulinum and *Clostridium perfringens* are not affected by carbon dioxide and they can grow in anaerobic conditions. Strict temperature control prevents the growth of *Clostridium* spp. Proteolytic *C. botulinum* produces a deadly toxin but does not grow and produce toxin below 10°C and under pH 4.6 under MA storage (Day, 2008; Gorris and Peppelenbos, 2007). Nonproteolytic *C. botulinum* (such as types B, E, and F) can grow at temperatures as low as 3°C and over pH 5.0 (Lund and Peck, 2000; Gorris and Peppelenbos, 2007). *C. botulinum* is not markedly affected by the presence of carbon dioxide, and growth and toxin production is encouraged by the anaerobic conditions that may exist in MA (Gorris and Peppelenbos, 2007; Francis *et al*., 1999). *Clostridium* spp. can present in soils and thus come into contact with foods easily, such as fruit and vegetables. Fresh mushrooms and tomatoes can contain spores of *Clostridium* spp. from soil and therefore there is a possibility of botulism association with these MA-packed foods (Zagory, 1995). The initial level of oxygen used for high-respiring products (such as mushrooms) is very important, since it depletes more rapidly and results in an anaerobic environment (Farber *et al*., 2003; Day, 2008).

3.3.3.2 L. monocytogenes

This bacterium is widely present in the environment and able to grow at low temperatures (psychrotrophic). *L. monocytogenes* can grow to potentially harmful levels, at low temperatures from 4 to 15°C, during the extended shelf life of MA-packed products (Francis and O'Beirne, 1998; Erkmen and Bozoglu, 2008a). Carbon dioxide concentrations of 10–20% reduce the growth of spoilage microflora, whereas higher concentrations slightly increase the growth of *L. monocytogenes* (Gorris and Peppelenbos, 2007; Barazi and Erkmen, 2008; Day, 2008).

3.3.3.3 A. hydrophila

A. hydrophila is a psychrotrophic and pathogenic bacterium that is widespread in the environment, and associates with a wide variety of foods and water (such as drinking water, fresh and saline water, seawater, and sewage water). Cytotoxic strains can associate with seafood, meats, poultry, and vegetables (Gorris and Peppelenbos, 2007; Erkmen and Bozoglu, 2008b). *A. hydrophila* can grow in refrigerated foods, and growth is not affected by depleted oxygen (up to 2%) and/or elevated carbon dioxide (up to 50%) levels (Francis *et al*., 1999). Carbon dioxide levels above 50% inhibit the growth of *A. hydrophila* (Day, 2008).

3.3.3.4 Yersinia enterocolitica

Animals are the predominant natural source of *Y. enterocolitica.* This cold-tolerant pathogen can be present on vegetables, cheese, ice cream, raw meats, and seafoods (Erkmen and

Bozoglu, 2008b). MA cannot prevent its growth at refrigeration temperatures. Low oxygen (<1.5%) and high carbon dioxide (>50%) levels are required to reduce the growth of *Y. enterocolitica* significantly (Gorris and Peppelenbos, 2007).

3.3.3.5 *Bacillus cereus*

This bacterium, a common contaminant of vegetables, does not grow below 10°C. Some enterotoxigenic strains can grow at temperatures as low as 4°C and produce toxin at 8°C (Erkmen and Bozoglu, 2008b). *B. cereus* is susceptible to the antimicrobial effects of carbon dioxide and carbon dioxide-rich environments prevent spore germination (Gorris and Peppelenbos, 2007).

3.3.3.6 *Salmonella* species

This genus of bacteria is most commonly associated with animals and birds, and is only present on foods through cross-contamination (Erkmen and Bozoglu, 2008b). High levels of carbon dioxide retard the growth of *Salmonella*. Most *Salmonella* species are mesophilic, but many strains survive well in storage at 5°C (Gorris and Peppelenbos, 2007; Erkmen and Bozoglu, 2008b).

3.3.3.7 *Staphylococcus aureus*

S. aureus is a mesophilic bacterium and does not grow well under chill conditions or in the presence of competing microorganisms. The pathogen may be present on fresh product and ready-to-eat vegetable salads. It is passed onto foods by food handlers. Generally, carbon dioxide has an inhibitory effect on the growth of *S. aureus* when combined with low-temperature storage (Gorris and Peppelenbos, 2007).

3.3.3.8 *Escherichia coli*

E. coli is a mesophilic bacterium often used as an indicator of fecal contamination. Enterotoxigenic *E. coli*, the common cause of travelers' diarrhea, is regularly associated with raw vegetables (Erkmen and Bozoglu, 2008b). Some strains can grow at temperatures up to 6°C and produce toxin. Growth of this bacterium can be inhibited by high levels of carbon dioxide (Diaz and Hotchkiss, 1996; Gorris and Peppelenbos, 2007).

3.3.3.9 *Campylobacter jejuni*

This bacterium is one of the major causes of bacterial enteritis. Poultry and other animal foods, and fruit and vegetables are main sources. Optimal growth occurs at under atmospheres of reduced oxygen and high temperatures (42–45°C). The minimum growth temperature is 32°C, so the risk from consumption of refrigerated MA products is minimal (Gorris and Peppelenbos, 2007; Erkmen and Bozoglu, 2008a).

3.3.3.10 Other pathogenic microorganisms

Vibrio, *Aeromonas*, *Shigella*, and various enteric viruses (such as hepatitis A) can survive and grow (except viruses) on products, and they have been implicated in a few food poisoning

outbreaks. MA-packed products have an excellent level of food safety against these microorganisms (Amanatidou *et al*., 1999; Day, 2008). There are also psychrotrophic pathogenic (such as *Vibrio*, *Shigella*, and *Aeromonas*) and spoilage (such as *Citrobacter freundii* and *Enterobacter cloacae*) bacteria associated with MA-packed foods. Furthermore, a MA with a relatively low partial pressure of carbon dioxide can favor the growth of Enterobacteriaceae and *Aeromonas*. The best protection against *Aeromonas* and Enterobacteriaceae is the combination of low storage temperature with a MA that provides a high partial pressure of carbon dioxide (Doherty *et al*., 1995; Molin, 2000).

3.4 APPLICATION OF MODIFIED ATMOSPHERES FOR FOOD PRESERVATION

3.4.1 Meat and meat products

The major quality losses in meats are due to microbial growth, oxidation of the red oxymyoglobin pigment, and loss of exudates. The rate of oxidation of the bright red pigment of meat is controlled by reducing the oxygen level. Red meats provide an ideal medium for the growth of a wide range of spoilage and food-poisoning microorganisms. Modification of atmosphere within the package by reducing the oxygen and increasing the level of carbon dioxide and/or nitrogen significantly extends the shelf life of meats and meat products by retaining excellent color, microbiological quality, chemical composition, and organoleptic properties at chill temperature (Lee *et al*., 1995; Day, 2008; Erkmen and Bozoglu, 2008a). Saturation of carbon dioxide in meat tissue can lead to development of fissures upon cooking, but this does not affect the meat texture (Amanatidou *et al*., 1999; Wszelaki and Mitcham, 2000). During prolonged storage under vacuum or carbon dioxide, chilled red meats become tender and may lose their desired textural characteristics. The antimicrobial effect of carbon dioxide requires continuous contact with meat (residual effect); short exposure alone to high levels of carbon dioxide cannot provide a residual effect (Day, 2008). Low carbon monoxide ($<0.5\%$) concentrations along with altered carbon dioxide and oxygen improve the shelf life, stabilize color, and retard the oxidative rancidity of meat (Rao and Sachindra, 2002). High-barrier packaging material produces greater restriction of bacterial multiplication in MA-packed meat.

MA storage of food inhibits the growth of most initial food-spoilage bacteria. Storage of chilled meat in gas-impermeable packs restricts the growth of *Pseudomonas*, Enterobacteriaceae, *S. aureus*, *E. coli*, *Y. enterocolitica*, *Salmonella*, and lipolytic and proteolytic bacteria, while *Lactobacillus* and *Brochothrix thermosphacta* become the major components of the spoilage flora (Rao and Sachindra, 2002; Day, 2008). The growth of *L. monocytogenes* on MA-packed meats can be influenced by the type of meat and its pH value (Rao and Sachindra, 2002). The principal spoilage mechanisms in cooked, cured, and processed meat products are microbial growth, color changes, and oxidative rancidity. Meat products containing appreciable levels of unsaturated fat are spoiled by oxidative rancidity and the elimination of oxygen will inhibit this. The addition of nitrate and salt inhibits the growth of most food-poisoning bacteria including *C. botulinum* (Day, 2008). VP of meat products such as sausages can reduce or prevent the growth of yeasts and the dominant microorganisms will be *Lactobacillus* and *Leuconostoc*.

3.4.2 Seafoods

Spoilage affecting the quality of seafoods is principally the result of microbial and oxidative activities. The microbial flora of fresh seafoods is heterogeneous and includes the following bacteria: *Acinetobacter*, *Cytophaga*, *Flavobacterium*, *Micrococcus*, *Psychrobacter*, *Aeromonas*, *Shewanella*, *Pseudomonas*, and coryneforms. The dominant bacteria on seafoods stored under refrigeration are *Pseudomonas* (mostly *Pseudomonas fragi* and *Pseudomonas fluorescens*) and *Shewanella putrefaciens*, and to a lesser extent *Aeromonas* and *Psychrobacter* (Stenstrom and Molin, 1990; Molin, 2000). MA can extend the shelf life of seafoods depending on the species, fat content, initial microbial load, gas mixture, temperature of storage, and good hygiene and handling practices. MA is a very effective technique for delaying microbial spoilage and oxidative rancidity in seafoods. The inclusion of carbon dioxide in MA is necessary for inhibiting common aerobic spoilage bacteria on fish. Oxygen is necessary to prevent the growth of *C. botulinum* type E, color changes, and bleaching in MA packs. Oxygen is excluded from oily fish MA packs to prevent oxidative rancidity (Day, 2008). Fresh white fish, packaged in a mixture of oxygen, nitrogen, and carbon dioxide should be stored below 5°C. Increased concentrations of carbon dioxide reduce the proportion of *Sh. putrefaciens* in the flora more effectively than they reduce *Pseudomonas*. The antimicrobial effect of oxygen on the flora might be due to the ability of many lactobacillus strains to produce hydrogen peroxide in the presence of oxygen (Borch and Molin, 1989; Erkmen and Bozoglu, 2008c).

3.4.3 Dairy products

The principal mechanisms of spoilage affecting dairy products are microbial growth and oxidative rancidity. MA can significantly extend the shelf life of dairy products. Raw milk stored at low temperature is mainly spoiled by *P. fluorescens*, *P. fragi*, and *Pseudomonas lundensis* (Ternstrom *et al*., 1993). The bacteriological shelf life of refrigerated milk can be prolonged by increasing the level of carbon dioxide in packs. In cheese, the manufacturing process suppresses the spoilage flora of fresh milk, and normally the major threat is yeasts and molds on the surface. They can be suppressed by a MA containing low oxygen and increased carbon dioxide concentrations (Maniar *et al*., 1994; Molin, 2000). Hard cheeses are packaged by flushing with carbon dioxide before sealing, which is very effective at inhibiting mold growth. The gas will be absorbed by the cheese, creating a vacuum. To avoid collapse of the package, nitrogen may be included together with carbon dioxide. Soft cheeses are packed with carbon dioxide/nitrogen mixtures, which can inhibit bacterial spoilage and oxidative rancidity. MA is not recommended for mold-ripened cheeses since carbon dioxide/nitrogen gas mixtures would inhibit desirable mold growth. MA is used to improve the shelf life of fresh, sliced, and shredded cheeses. Cream is adversely affected by carbon dioxide-containing atmospheres. By excluding air, carbon dioxide/nitrogen inhibits aerobic microbial growth and oxidative rancidity (Day, 2008). Butter and yogurt are not usually MA-packed but would benefit from packaging under nitrogen (Mannheim and Soffer, 1996).

3.4.4 Bakery products

The principal spoilages in bakery products are mold growth, staling and moisture migration. Yeasts may cause problems in certain filled or frozen bakery products. The a_w of bakery products is generally less than 0.96 and bacterial growth is inhibited and rarely a problem.

However, it is possible that *S. aureus* and *Bacillus* spp. may be able to grow in certain bakery products. Use of MA can significantly inhibit these microorganisms. Molds are very effectively inhibited by carbon dioxide/nitrogen gas mixtures. Moisture migration from the pack is prevented by using barrier materials. MA appears to have little effect on the rate of staling (Day, 2008). The shelf life of bakery products can be significantly increased by packaging with carbon dioxide or carbon dioxide/nitrogen mixtures, such as fresh pasta, pizza, quiche, lasagne, and the others.

3.4.5 Dried food products

The principal mechanism of spoilage in dried foods containing unsaturated fatty acids (such as cereals, potato crisps, nuts, cocoa powder, and dried milk) and dried baby milk is oxidative rancidity. The low a_w of dried foods prevents the growth of bacteria, yeasts, and molds (Day, 2008). But many food-poisoning bacteria may survive on dried foods, particularly herbs and spices, and may pose a hazard when used as an ingredient in high-a_w foods. Microorganisms present before drying and by contamination during processing can survive for extend periods. This is most important with respect to pathogens if present before drying, since time and temperature abuse during drying and storage can allow them (such as *S. aureus* and *Salmonella*) to grow. Such pathogens can survive in chocolate, paste, dried milk, and eggs (Erkmen and Bozoglu, 2008b). Rancidity can be very effectively inhibited by MA with nitrogen. MA package materials must have very high moisture and gas barrier properties, such as metallized films. Residual oxygen level should be below 0.2% in the packs. In order to achieve very low residual oxygen levels, oxygen absorbers may be incorporated with MA packs (Day, 2008).

3.4.6 Fruits and vegetables

The principal mechanisms of spoilage in fruit and vegetables are microbial growth, ripening, oxidative rancidity, and moisture loss. Fresh products continue to respire after harvesting and any subsequent packaging must take into account this respiration activity. The rate of respiration of fruit and vegetables can be reduced by increasing the partial pressure of carbon dioxide and decreasing the partial pressure of oxygen. However, too high a level of carbon dioxide will result in tissue damage, similar to that caused by too low temperatures. Several processes in vegetables and fruit other than respiration are influenced by the gas atmosphere, such as ripening, production of ethylene, breakdown of chlorophyll, and structural changes due to the breakdown of pectin. If a product's respiration characteristics are properly matched to film permeability and a desired mixture of gases (such as oxygen, carbon dioxide, and nitrogen), a beneficial equilibrium MA can be established in the package (Day, 1998, 2008). Respiration in such packed products leads to a build-up of carbon dioxide and a reduction of oxygen. Product sealing in packaging film of insufficient permeability results in the development of anaerobic conditions (such as $<2\%$ O_2 and $>20\%$ CO_2) and leads to the production of off-odors and flavors (such as ethanol, aldehydes, and ketones), as well as creating conditions for the growth of anaerobic pathogenic bacteria (such as *C. botulinum*) (Amanatidou *et al.*, 1999; Day, 2008). The plant hormone ethylene can cause a marked increase in respiration rates as well as enhancing ripening and senescence. In some products, accelerated aging and the initiation of ripening can occur following exposure to very low levels of ethylene (Lee *et al.*, 1995; Amanatidou *et al.*, 1999). The production of ethylene, ripening of products, quality loss, and oxidation can be controlled or minimized by MA storage at chilled temperatures. In MA, carbon dioxide inhibits ethylene action and autocatalytic production of ethylene by products such as apples, pears, and tomatoes. But

carbon dioxide levels above 15–20% can cause undesirable physiological damage to whole-leaf plants (Wszelaki and Mitcham, 2000). Apples can be stored in a mixture of 5–10% CO_2 and 3% O_2 and N_2 as the filler, tomatoes can be kept in an atmosphere of 9% CO_2, 6% O_2, and N_2 as the filler (Molin, 2000); and a suitable atmosphere for strawberries can be 10–20% CO_2, with a low oxygen concentration and nitrogen as the filler (Molin, 2000; Rao and Sachindra, 2002). The microbiological spoilage on fruit and vegetables can be caused by Gram-negative bacteria (such as *Pseudomonas*, Enterobacteriaceae) and fungi (Molin, 2000).

3.5 FOOD SAFETY AND FUTURE OUTLOOK

Consumers can select any food according to the labeling requirements, storage temperature, achievable shelf life, principal spoilage microorganisms, possible food-poisoning hazards, types of packages, typical MA materials, and descriptions of legislation and regulations of specific relevance to MA (Day, 2008). At least four types of food safety principle are considered in the packaging of foods (Rooney, 1995). First, any need for food contact approval must be established before any form of packaging is used. Second, it is important to consider environmental regulations for packaging materials. Third, there may be a need for labeling in the cases where packaging may give rise to consumer confusion. Fourth, the effects of packaging on the microbial ecology should be considered. Packaging substances may be intentionally moved into the food or may be unintentionally removed from it. Intended migrants include antioxidants, ethanol, and antimicrobials from AP systems, but this would also require regulatory approval about toxicological effects. Unintended migrants include various metal or plastic compounds from packaging materials or AP substances.

Safety aspects of packaging foods under MAs may be associated with the hazards of botulism, in that the anaerobic conditions in MA-packed foods can facilitate growth and toxin formation by *C. botulinum*. This might be especially pronounced with temperature abuse. Increased partial pressures of carbon dioxide stimulate the germination of *C. botulinum* spores. A MA with 100% CO_2 suppresses toxin production by *C. botulinum* in comparison with 100% N_2 (Molin, 2000). Specific chemicals may be used to minimize the microbial safety risks of foods packed under reduced oxygen atmospheres (Brennan and Day, 2006). There are psychrotrophic pathogens that can reach high numbers in refrigerated MA packed foods, such as *L. monocytogenes*, *Y. enterocolitica*, *C. freundii*, *E. cloacae*, *Aeromonas*, and *Vibrio*. MA packages are increasingly being used successfully due to good control of the whole distribution chain from the moment of packaging the product until it is displayed on the retail shelf. Quality deterioration still occurs, and further improvements can still be achieved through selection of appropriate gas conditions for specific products, availability of more data on the interactions between product and gas composition, selection of suitable packaging systems for specific conditions (temperature, humidity), and preventing microbial hazards using improved MA techniques or new hurdles (Gorris and Peppelenbos, 2007; Erkmen and Bozoglu, 2008a).

3.6 CONCLUSIONS

The benefits of MA include:

- better utilization of labor and allowing longer runs of products to consumers;
- enabling distribution of products over greater distances;

- facilitating the purchase of larger quantities of raw materials;
- encouraging sales due to the attractive color and presentation of food products;
- preventing drips and odor losses from foods during storage and distribution;
- helping to increase markets for products that have retained their natural form;
- allowing less processing and requiring little or no preparation by the consumer;
- inhibiting the growth of aerobic microorganisms and oxidation reactions.

Against these benefits, the following disadvantages of MA must be considered:

- the capital cost of MA machinery and accessories;
- the cost of gases and specialized packaging materials;
- the cost of gas analysis and seal-integrity testing equipment;
- increased pack volume affecting transport costs;
- the requirements of different atmospheres for different products;
- the requirements of low-temperature storage during product distribution;
- the possibility of temperature abuse during storage;
- environmental concerns about packaging materials;
- the loss of package integrity due to improper sealing, holes, and other defects in the packaging materials.

Packaging can allow many preservation benefits on a wide range of food products due to recent advances in packaging, material science, biotechnology, and new consumer demands. The aim of this technology is to extend the shelf life of products by maintaining nutritional quality and ensuring microbial safety. The packaging market has been a growing area with recent packaging systems, such as new developments in packaging materials/machinery, and food-product applications. There is good potential for MA storage of vegetables, fruit, meat, bakery, dairy, and poultry products. The packaging techniques need further improvements in the future with the increasing application of MA techniques. Besides the effect on consumption quality, it is equally important to consider the safety aspects during extended storage times. The longer the storage time, the greater the risk of microbiological hazards. Application of packaging systems must be combined with advanced systems of microbial control and a knowledge of the microbial ecology of foods.

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4 Effects of Combined Treatments with Modified-Atmosphere Packaging on Shelf-Life Improvement of Food Products

Shengmin Lu and Qile Xia

Abstract: Raw, minimally processed and processed foodstuffs deteriorate in sensory quality and reduce in shelf life due to inner physiological metabolism, microbial spoilage, oxidation of lipids, and poor handling in temperature, moisture, sanitation and packaging management. Many treatments including physical, chemical and biological means have been employed to control postharvest diseases, microbial contamination, oxidative decomposition, and quality deterioration of food products. Modified-atmosphere packaging (MAP) is one of the most efficient preservation techniques. This chapter introduces the latest research progress on shelf-life improvement of food products rendered by MAP in combination with other treatments. They are classified into three categories: (i) physical treatments, including low temperature, high pressure, γ-irradiation, UV-C radiation, heat treatment, and films; (ii) chemical treatments, including sanitizers such as sulphite, chlorine, ozone, chlorine dioxide, lactic acid, hydrogen peroxide, peroxyacetic acid, and phosphate, etc., antibrowning agents such as EDTA, 4-hexylresorcinol, ascorbate/citrate, etc., antioxidants such as rosemary extract and ascorbic acid, natural products such as essential oils, and others such as oxygen scavengers; and (iii) biocontrol, including *Lactobacillus fermentum* inoculation, yeast antagonists, and nisin. Several treatments are presently considered to be promising. The benefits and restrictions as well as practical outlook for the use of conventional and innovative means are discussed.

Keywords: biocontrol; chemical treatment; food products; modified-atmosphere packaging; physical treatment

4.1 INTRODUCTION

There are three kinds of foodstuffs consumed in our lives: raw, minimally processed, and processed. Raw materials, of either plant or animal origin, can deteriorate easily due to the existence of spoilage microorganisms, as well as inner physiological and biochemical metabolisms. Such changes result in quality deterioration such as shrivelling, yellowing and decay in leafy green vegetables, browning and weight loss in fruit, and off-flavour and spoilage in animal foods, and therefore render a reduction of shelf life if proper handling and management have not been adopted.

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Minimally processed or fresh-cut fruit and vegetables are popular worldwide nowadays because of their great advantages for consumers such as convenience, high quality and nutritional value. However, the operations required for preparing minimally processed products result in an increase in the number of microorganisms, some of which may be potentially harmful to human health. Furthermore, these kinds of minimal processing operations destroy plant structure and therefore increase the rate of senescence of tissues and reduce their resistance to microbial spoilage. Physiological metabolisms such as ethylene production, respiratory activity and enzymatic and non-enzymatic browning accelerate in fresh-cut produce owing to physical damage to tissue and result in lowered quality and shorter shelf life compared to that from the whole intact product. The visual symptoms of deterioration of fresh-cut produce include flaccidity from loss of water, changes in colour (especially increased oxidative browning at the cut surface) and microbial contamination (Artés *et al*., 2007a). Processed foods also experience quality deterioration during storage. Such deterioration includes oxidation of lipids, non-enzymatic browning, etc. However, compared to raw and minimally processed foods, processed foods have a much longer shelf life due to sterilization during processing and packaging being extensively adopted.

Food safety, nutrition and sensory quality are required while providing extended shelf life and freshness. Fortunately, many treatments including physical, chemical and biological means have been employed to control postharvest diseases, microbial contamination, oxidative decomposition, senescence and deterioration in quality of food products, and thus prolong their shelf lives. However, as reviewed by Devlieghere *et al*. (2004), very few new preservation technologies such as high hydrostatic pressure, pulsed electric fields, natural preservatives and protective cultures have been implemented by the food industry until now. Modified-atmosphere packaging (MAP) is one of the most efficient preservation techniques. In this preservation technique the air surrounding the food in the package is changed to another composition in which the microbe loads on produce will be inhibited and respiration of fruit and vegetables will be reduced. In this way the initial fresh state of the product may be prolonged. The gases used in this technique are common and safe to most food types. At present, MAP has been found to be efficient in prolonging the shelf life of perishable products such as meat, fish, and fruit and vegetables, either raw or minimally processed. The MAP technology for preservation of fresh produce was recently reviewed by Sandhya (2009). However, the antimicrobial effect of MAP mainly caused by carbon dioxide is limited and its combination with other treatments must be considered to enhance its preservative role in perishable foods. This chapter introduces the latest research progress on shelf-life improvement of food products rendered by MAP in combination with other treatments. The benefits and restrictions as well as the practical outlook for the use of conventional and innovative means are discussed.

4.2 PHYSICAL TREATMENTS

4.2.1 Low temperature

Storage temperatures influence the rate of many deteriorative processes and transpiration because temperature is one of the most important factors in the maintenance of produce quality (Cliffe-Byrnes and O'Beirne, 2005). The quality parameters influenced include sensory attributes, vitamins and other nutrients in many fruit and vegetables (Gil *et al*., 2002). Generally, higher storage temperatures result in more rapid quality losses of produce,

including sensory changes, such as general appearance, firmness and odour; chemical changes, such as decreases in contents of ascorbic acid, soluble solid and titratable acid; and increases in respiration rate and ethylene, cell-membrane permeability and the symptoms of senescence (Jacxsens *et al*., 2002a; Maalekuu *et al*., 2006; Li *et al*., 2008). On the contrary, lower temperatures are usually beneficial, keeping storage quality and extending shelf life for most produce. The effectiveness of MAP is dependent on lower storage temperatures to increase the penetrable solubility of carbon dioxide in cellular water and lipids, thereby increasing the antimicrobial activity of the gas (Farber, 1991). However, chilling injury may occur for some chilling-sensitive crops at low storage temperatures, which also induces rapid losses in quality (Jacxsens *et al*., 2002b). Higher storage temperature (5°C) resulted in more rapid changes in the different quality parameters for stored mushrooms, whereas in the case of storage at 1°C chilling injury occurred (Li *et al*., 2008).

Modified atmosphere in terms of reduced oxygen and elevated carbon dioxide can extend the postharvest life of whole and pre-cut commodities by reducing their respiration rate as well as production of ethylene, minimizing metabolic activity, delaying enzymatic browning and retaining visual appearance (Kader, 1986); however, control of these processes is dependent on temperature control. MAP storage of fresh-cut or minimally processed fruit and vegetables along with low-temperature storage conditions have gained rapid popularity due to growing consumer preference towards ready-to-eat and convenient and nutritious produce (Shah and Nath, 2006). MAP fresh-cut peppers presented better visual quality, less leaked juice and had higher firmness than those stored under vacuum packaging (VP). Microbiological and quality analysis revealed a limit of shelf life of 14 and 21 days when fresh-cut peppers were stored at 10 and 5°C, respectively (González-Aguilar *et al*., 2004). MAP with silicon gum film windows at 3°C provided the best atmosphere for *Agrocybe chaxingu* mushroom as shown by the fact that the MAP packs with windows at 3°C were of better quality than the control (Li *et al*., 2008). MAP in low-density polyethylene film of carambolas held at 10°C markedly retarded the decline in tissue firmness and the development of fruit colour, restricted water loss, and suppressed the incidence of chilling injury (Ali *et al*., 2004). The retardation of fruit softening by MAP and/or low temperature, which correlates closely with delayed solubilization and depolymerization of the chelator-soluble polyuronides, may partly be attributed to suppression of the increase in activity of the major wall hydrolases. Suppression of the enzyme activities in fruit under MAP also appears to contribute to increased tolerance of the carambolas to chilling-injury incidence.

The success of MAP in extending seafood shelf life depends on many factors, including good initial product quality, good hygiene during slaughter, correct packing-material selection, packing equipment, appropriate gas mixture and gas-to-product volume (g/p) ratio for the product, and maintenance of the process temperatures (Sivertsvik, 2007), where the amount of carbon dioxide dissolved in the product and the storage temperature are the most critical factors (Rotabakk *et al*., 2008). Superchilling is another technique that helps to inhibit most autolytic and microbial reactions (Huss, 1995), and therefore extends the period of prime quality in fish. Several types of cooling system (4 to 0°C) have been used for superchilling of seafood products including flake ice or slurry ice (Zeng *et al*., 2005; Losada *et al*., 2006) and subzero temperatures during storage (−2°C) (Sivertsvik *et al*., 2003). The effect of natural additives, superchilling and MAP on the shelf life of Atlantic salmon (*Salmo salar*) fillets was investigated by Fernández *et al*. (2009). The greatest extension of shelf life was reached by a combination of superchilling and MAP. The samples with the highest carbon dioxide concentration (90%) and gas-to-product volume (g/p) ratio of 2.5 showed the highest shelf life: 22 days compared with 11 days (control sample).

However, MAP and low-temperature storage are usually not sufficient to extend the shelf life of pre-cut produce as the excessive physiological stress and increased susceptibility towards microbial spoilage caused by processing operations such as cutting and slicing reduce the shelf life significantly. Use of postharvest dip pretreatments consisting of certain chemical preservatives such as citric acid, ascorbic acid and calcium chloride at minimum levels, alone or in combination, during minimal processing has been found to be beneficial in minimizing stress-induced metabolism, reducing the browning reaction, maintaining firmness and improving organoleptic quality in various types of produce along with extension of shelf life under MAP at low-temperature storage (Martinez-Ferrer *et al.*, 2002; Soliva-Fortuny *et al.*, 2002).

Along the whole food continuum – that is, processing, storage, transportation and retailing – one needs to maintain optimum temperatures. However, maintaining proper storage temperatures is often most difficult at retail level.

4.2.2 High pressure

Derived from material science, high hydrostatic pressure processing (HPP) is the technology by which a product is treated at or above 100 MPa. In contrast to heat, HPP does not disrupt covalent bonds, thus maintaining the primary structure of proteins and thereby retaining appearance, flavour, texture and nutritional qualities of the unprocessed product (Murchie *et al.*, 2005). Changes in the tertiary structure, maintained by hydrophobic and ionic interactions, are usually observed for proteins in general above 200 MPa (Balny and Masson, 1993). Besides, temperature affects HPP efficacy. HPP has been applied to raw bivalve shellfish, fruit juices, cider, jams and jellies, drinkable yoghurt, smoothies, avocado products, chopped onions and ready-to-eat meat products (Kingsley *et al.*, 2005).

Many reports have demonstrated the inactivation effect of HPP on microorganisms, extending in this way the microbial shelf life and improving the microbial safety of food products. An overview of the sensitivity of microorganisms under several sets of processing conditions and in several substrates was given by Patterson (2000). Substantial count reductions (>4 $\log_{10}$ units) of most vegetative microorganisms are realized when a pressure treatment of 400–600 MPa at room temperature is applied. However, pressure treatment alone is often not sufficient for substantial reduction of viable spore counts (Hoover, 1993). Spores of some species survive pressures above 1000 MPa, when the temperature is not higher than 45–75°C (Cheftel, 1995). Pressure-induced germination of spores has been examined and subsequent pressure treatment of the germinated/germinating spores was shown to be an effective means of reducing spore counts (Knorr, 1995).

Combination treatments have been suggested from which the combination with an increased temperature was mostly investigated. Several studies indicate that it is possible to reduce bacterial spores through combinations of mild heat and HHP (Kowalski *et al.*, 1992; Seyderhelm and Knorr, 1992). *Bacillus stearothermophilus* was inactivated by six orders of magnitude by a treatment at 500 MPa at 70°C during six cycles of 5 min (Hayakawa *et al.*, 1994). Sojka and Ludwig (1997) demonstrated an eight orders of magnitude reduction of *Bacillus subtilis* spores when treated at 500 MPa at 70°C during 10 cycles of 1 min. A mild heat treatment can encourage spores to germinate, resulting in them being more susceptible to pressure treatments. Therefore, a preheat treatment followed by pressurization is generally more effective at inactivating spores than heating during pressurization (Patterson, 2000). Other proposed combination treatments are the use of HPP together with the addition of nisin (Roberts and Hoover, 1996; Stewart *et al.*, 2000), lactoperoxidase, or lysozyme. A combined

application of high pressure and gases (krypton, xenon, N_2O and CO_2) is effective in inactivating vegetative cells of bacteria *in vitro* (Thom and Marquis, 1984; Debs-Louka *et al*., 1999). ZoBell and Hittle (1967) reported that hydrostatic pressure increased the sensitivity of a range of microorganisms to oxygen. Carbon dioxide in supercritical state provides a promising technique for manufacturing heat-sensitive foods, although the prolonged pressure treatment required 24 h, which is a restraining factor for industrial application (Patterson *et al*., 1995). A disadvantage of HPP is that viral strains could develop resistance to this technology as was assumed by Smiddy *et al*. (2006). They noticed altered plaques of Qβ (a single-stranded-RNA coliphage) after HPP treatment whereby the altered shaped plaques persisted by sub-culturing. These phages with unusual plaque morphology might be more pressure-resistant but this hypothesis was not investigated further.

HPP at low temperatures combined with MAP was used for the preservation of salmon (Amanatidou *et al*., 2000). A shelf-life extension of 2 days was obtained after HPP treatment of 150 MPa for 10 min at 5°C compared to unpressurized, VP salmon. MAP storage (50% $O_2 + 50\%$ CO_2) alone extended the shelf life of salmon for 4 days at 5°C. When salmon had been subjected to HPP treatment in the presence of 50% $O_2 + 50\%$ CO_2, the threshold value for microbial spoilage of salmon (7.0–7.2 log colony-forming units (CFU)/g) was not reached for at least 18 days at 5°C. Spoilage microorganisms (lactic acid bacteria, *Shewanella putrefaciens*) as well as pathogens (*Listeria monocytogenes* Scott A, *Salmonella typhimurium*) spiked on salmon prior to the treatment were more susceptible to HPP in the presence of 50% $O_2 + 50\%$ CO_2. The mode of action of compressed gases is probably related to intracellular formation of reactive oxygen species as well as to phase-transition phenomena. Although microbial growth on salmon was retarded, the combined HPP and MAP treatments, at the settings used in this study, promoted a detrimental effect on colour and changes in the balance of oxidative rancidity.

Lopez-Caballero *et al*. (2000) investigated the application of high pressures (200 and 400 MPa) in chilled prawn tails, both conventionally stored (air) and VP. VP and high-pressure treatment did extend the shelf life of the prawn samples, although it did affect muscle colour very slightly, giving it a whiter appearance. The viable shelf life of 1 week for the air-stored samples was extended to 21 days in the VP samples, 28 days in the samples treated at 200 MPa, and 35 days in the samples pressurized at 400 MPa. VP inhibited the onset of blackening, whereas high-pressure treatment aggravated the problem. From a microbiological point of view, batches that were conventionally stored reached about 6 log CFU/g or even higher at 14 days, while similar figures were reached in total number of bacteria in VP samples and in 200 MPa pressurized samples at 21 days. When samples were pressurized at 400 MPa, total numbers of bacteria were below 5.5 log CFU/g at 35 days of storage. Consequently, a combination of VP and HPP would appear to be beneficial in prolonging freshness and preventing spotting in chilled prawn tails.

The industrial equipment used at present for HPP are discontinuous (from 10 to 500 L of capacity); for solid, viscous and particulated foods batch processing and semicontinuous are used (from 1 to 4 tonne h^{-1} of production); for liquid foods bulk processing is used (Yuste *et al*., 2002). The processing cost of some recent equipment has been estimated at 10–15 Eurocents per kg of product, inclusive of investment and operation costs (Anonymous, 2002). Commercial production of pressurized foods has been reported for fruit jams, jellies, sauces, juices, rice wine, cake, avocado pulp, guacamole and cooked ham. For dairy applications, the technique has been studied, among others, to improve the shelf life of goat's cheese (Capellas *et al*., 1996), to reduce the ripening time of cheese to 3 days at 250 MPa (Yokoyama *et al*., 1992) and to prevent over-acidification of yoghurt, increasing the shelf life to more than

2 weeks at 4°C when treated at 200 MPa for 15 min at 20°C (De Ancos *et al*., 2000). Olsen *et al*. (2003) demonstrated the possibility of using HPP to reduce milk allergenicity of milk by specific hydrolysis of β-lactoglobulin at 250 MPa.

4.2.3 Radiation

Although chemical treatments are effective in reducing surface microbial counts, they do not sanitize tissue crevices (Beuchat, 1992). Low temperature is highly effective in conjunction with chemical treatment in reducing metabolic activity and microbial proliferation (Zagory, 1988), but is not a good defence against psychrotrophic microorganisms such as *L. monocytogenes*, *Aeromonas*, etc., which tend to cause foodborne illness (Sumner and Peters, 1997). Ionizing irradiation is a promising technology to maintain the quality of minimally processed produce due in part to its efficiency in controlling both spoilage and pathogenic bacteria (Hines, 2000). Application of γ-radiation is a well-known method to eliminate/inactivate the spoilage-causing and pathogenic microorganisms with no adverse effects on nutritional and sensory quality of foods. Its use is gradually increasing worldwide (WHO, 1999; Bidawid *et al*., 2000). An irradiation dose up to 1.0 kGy is permitted for fresh produce by the US Food and Drug Administration (FDA, 1995).

γ-Irradiation at doses of 2–4 kGy are often used for bacterial control in particular food products and its effect on foodborne viruses was reported by several investigators. Bidawid *et al*. (2000) found that 3 kGy was needed in order to achieve 1 log reduction of hepatitis A virus on lettuce or strawberries. Mallett *et al*. (1991) reported that 2.0 kGy was able to reduce hepatitis A virus by 1 log in oysters and clams but 2.4 kGy was needed to achieve this for rotavirus. Coxsackievirus B2 was reduced by 1 log in ground beef when treated with 7 kGy (Sullivan *et al*., 1973). A dose of 200 Gy reduced canine calicivirus and feline calicivirus respectively by 2.4 and 1.6 log (De Roda Husman *et al*., 2004). γ-Irradiation was found to be greatly affected by the presence of proteins (De Roda Husman *et al*., 2004). The authors assumed that free OH radicals, induced by γ-irradiation, which normally interact with nucleic acids and the virus coat, were scavenged and induced therefore less inactivation.

The use of γ-irradiation to enhance the shelf life of minimally processed fruit and vegetables and to ensure the microbiological safety is increasing (Howard *et al*., 1995; Hagenmaier and Baker, 1997; Prakash *et al*., 2000; Zhang *et al*., 2006). Baskaran *et al*. (2007) investigated the effect of irradiation dose, citric acid concentration and potassium metabisulphite concentration on the quality characteristics of minimally processed potatoes by response surface methodology (RSM). A sensory score above 6.0 at the end of the storage period of 4 weeks was obtained at the optimum conditions (γ-irradiation dose 1.0 kGy, citric acid concentration 0.33% and potassium metabisulphite concentration 0.55%).

The combination of γ-irradiation with other treatments such as chlorination (Hagenmaier and Baker, 1997) and MAP (Prakash *et al*., 2000) for reducing the microbial population and maintaining the nutritional quality of fresh-cut lettuce during preservation at low-temperature storage has been reported. Ahn *et al*. (2005) investigated the effects of irradiation, in combination with a MAP, on minimally processed salted Chinese cabbage used to manufacture Kimchi, a representative Korean salted and fermented vegetable. Their results suggested that irradiation at 1 kGy or above can be used to enhance the microbial safety of cut Chinese cabbage without a significant loss in the quality attributes. A combination of irradiation (1.0–2.0 kGy) with MAP retained the physicochemical and microbiological quality of fresh-cut Chinese cabbage for a period of 3 weeks at 4°C. In case of pre-cut peppers and carrots the irradiation dose of 1.0 kGy reduced the microbial

load and extended shelf life and sensory quality (Farkas *et al.*, 1997). The application of γ-irradiation (1.0 kGy) resulted in a shelf life of fresh-cut celery of 6 days for the samples stored at 4°C (Lu *et al.*, 2005). The irradiation dose of 2.0 kGy was found to be effective in maintaining the textural, sensorial and microbiological quality of minimally processed carrots for 14 days at 5°C (Chaudry *et al.*, 2004). However, irradiation-induced softening was reported for a number of fruit and vegetables by many researchers (Kovacs *et al.*, 1997; Prakash *et al.*, 2002; Rastogi and Raghavarao, 2004; Rastogi, 2005). Irradiation and MAP can be combined to prevent the regrowth of *L. monocytogenes* during post-irradiation refrigerated storage, thereby improving product safety of fresh-cut endive (Niemira *et al.*, 2005). After irradiating with a dose of more than 0.3 kGy, no *Escherichia coli* were detected in pre-cured carrots under MAP during the whole storage, while a level of 1–2 log CFU/g of *E. coli* was detected in those under air after 5 days of storage (Lacroix and Lafortune, 2004). A combined application of MAP and γ-irradiation was also found in extending the shelf life of fresh aqua-cultured sea bass (*Dicentrarchus labrax*), a kind of highly perishable seafood (Reale *et al.*, 2008).

It has long been reported that UV-C light at 190–280 nm wavelength affects several physiological processes in plant tissues and, more importantly, that UV-C rays damage microbial DNA (Lucht *et al.*, 1998). Therefore, cells which are unable to repair radiation-damaged DNA die and sublethally injured cells are often subject to mutations (Lado and Yousef, 2002). UV radiation has been recommended as best used in combination with other preservation techniques, since the accumulative damage due to microbial DNA appears effective in decreasing the overall number of bacteria cells, but does not result in complete sterilization (Rame *et al.*, 1997). Therefore, a combination of preservation treatments is recommended, because it provides the required level of protection while concomitantly retaining the organoleptic attributes of the product such as colour, flavour, texture and nutritional value (Brul and Coote, 1999). The Code of Federal Regulations (Title 21, Part 179) of the USA permits the use of UV radiation (wavelengths of 220–300 nm with 90% of emission at 253.7 nm) on food products to control surface microorganisms (Rhim *et al.*, 1999). The combined use of chilling, MAP and UV-C radiation in fresh processed Red Oak Leaf lettuce was reported by Allende and Artés (2003). They found that UV-C was effective to reduce growth of most of the microbial groups (i.e. psychrotrophic and coliform bacteria as well as yeast) without adversely affecting sensory quality of Red Oak Leaf lettuce. The effect of the UV-C radiation on the microbial growth of minimally processed pomegranate arils was unclear (López-Rubira *et al.*, 2005). Some of the applied UV-C treatments reduced mesophilic, psychrotrophic, lactic acid and Enterobacteriaceae counts. However, microbial counts were not systematically reduced throughout the shelf life. In addition, UV-C-treated arils showed higher bacterial counts in a few cases. Yeasts and moulds were unaffected by the UV-C treatments.

The lack of a residual compound on the surface of the fruit and the low cost make this alternative technique attractive for the minimally fresh processing industry, although more research is needed to ensure the optimal use of UV-C radiation on plant products.

4.2.4 Heat treatment

Postharvest heating is a non-contaminating physical treatment that delays the ripening process, reduces chilling injury and controls activity of pathogens. Due to these beneficial effects, heat treatments are currently used commercially for quality control of fresh products (Ferguson *et al.*, 2000).

Heat treatment causes changes in fruit ripening, such as inhibition of ethylene synthesis and action of cell-wall-degrading enzymes, due to changes in gene expression and protein synthesis (Paull and Chen, 2000). Heat treatment increased postharvest life by delaying softening in plums (Tsuji *et al.*, 1984), pears (Maxie *et al.*, 1974) and tomatoes (Biggs *et al.*, 1988), improving the flavour of a number of fruit (Shellie and Mangan, 1998) without affecting soluble solids concentration in apples (Klein and Lurie, 1990), nectarines (Lay-Yee and Rose, 1994) or strawberries (Garcia *et al.*, 1995). In addition, titratable acidity was reduced in apples (Klein and Lurie, 1990) or remained unaffected in tomatoes (Lurie and Sabehat, 1997) and grapefruit (Miller and McDonald, 1992) after hot air treatments. Heat treatment of peaches at 40°C for 70 min before cutting resulted in no significant effect on the instrumental colour evaluation and specific lightness values (L^*), which was used as an indicator of browning intensity (Steiner *et al.*, 2006). On the other hand, higher temperatures ($\geq$45°C) for shorter time periods have been effectively used to reduce browning in fresh-cut products such as lettuce, celery and Chinese water chestnut (Saltveit, 2000; Loaiza-Velarde *et al.*, 2003; Peng and Jiang, 2004). A clear beneficial effect of 4 h pre-cutting heat treatment at 50°C for 10 min on postharvest quality of fresh-cut peach was found (Koukounaras *et al.*, 2008).

Hot-water treatments are also used as alternative methods to control postharvest diseases. Ripe fruit is highly susceptible to disease infection, and therefore some means of controlling it are necessary. Peaches subjected to 46°C hot-water treatment for just 2–8 min were found to have substantially decreased disease susceptibility (Margosan *et al.*, 1997).

In recent years, the use of combined techniques in the postharvest handling of fresh products has increased and numerous authors have obtained good results using a combined treatment. The combination of hot-water treatments and lower oxygen had a more substantial effect in delaying colour development of mature green cherry tomato (*Lycopersicon esculentum*, cv. Coco) fruit than the individual treatments (Ali *et al.*, 2004). Hot-water treatments (46°C hot water containing 200 mM NaCl for 25 min) did not cause any damage to nectarine and peach fruit based on external observations, specific conductivity and total phenol content evaluations, but reduced firmness loss (possibly in combination with MAP) especially in the white-flesh nectarines and kept the cellular membranes functioning better (Malakou and Nanos, 2005). Hot water combined with MAP during storage resulted in good-quality fruit after 1 week for postharvest handling.

4.2.5 Films

In order to improve MAP conditions, improved permeability of packaging films has been studied and developed successfully (Garcia *et al.*, 1998; Fonseca *et al.*, 1999; Van der Steen, 2002; Lee *et al.*, 2003). Because respiration rates differ greatly among different vegetable products, packaging films with a wide range of permeabilities for oxygen and carbon dioxide are necessary to meet preservation requirements (Jacxsens *et al.*, 1999). Variations in permeability of packaging films such as polyethylene, polypropylene (PP), shrink film, etc. used for MAP also affect the produce quality. Use of selective permeable packaging films such as silicon membrane as a window on MAP could also be beneficial in extending the shelf life of the produce, where atmosphere inside the package gets modified by respiration of the produce along with selective passage of the respiratory gases across the membrane (Stewart *et al.*, 2005; Li *et al.*, 2007).

Silicon membrane has been studied and developed successfully as a packaging material for storing vegetables and fruit (Vigneault *et al.*, 1992; Stewart *et al.*, 2005) because of its high

permeability to gases. Silicon gum film windows with a given window size of 0.9 cm^2 at an initial concentration of 50 mL/L O_2 and 100 mL/L CO_2 have shown an extension of the storage life of the mushroom *A. chaxingu* at a selected temperature of 3°C (Li *et al*., 2007). Silicon membrane systems with modified atmospheres were effective for storing bananas, maintaining the fruit with a harvest-fresh appearance, good colour and excellent marketability after 42 days of storage (Stewart *et al*., 2005). Compared to the packages without the silicon gum film windows, the packages with the windows were more effective for keeping the quality of the stored mushrooms. This window could keep the gas compositions of the packages at levels which avoided anaerobic respiration and resulting off-odours. Among three different modified-atmosphere systems, the packages with the silicon gum film window with initial gas concentrations of 5% O_2 and 10% CO_2 were the most effective for maintaining mushroom quality (Li *et al*., 2008).

Packaging of horticultural crops within plastic films creates a modified atmosphere higher in carbon dioxide and water and lower in oxygen than ambient levels, in response to the respiration of and moisture loss from the commodity. It has been shown that elevated carbon dioxide and water and reduced oxygen are beneficial in alleviating chilling injury symptoms in chilling-sensitive crops. However, the low water transmission rate of the films commonly used in combination with the high transpiration rate of some crops such as mushroom results in rapid saturation of the package atmosphere. These conditions may cause the growth of *Pseudomonas tolasii*, responsible for browning or yellowing of the sporophore surface, known as bacterial blotch, also making the package unattractive (Jin *et al*., 1994). The use of moisture absorbers such as sorbitol, sodium chloride, propylene glycol and polyvinyl alcohol have resulted in better colour of the mushrooms (Anantheswaran and Sunkara, 1996). Lower relative humidity was observed in packages containing silica gel and this did not affect the quality of the mushrooms. However, increasing amounts of silica gel increased weight loss in *Pleurotus* mushrooms, and high weight loss detected in PP packages made them unacceptable (Villaescusa and Gil, 2003). Recently developed Xtend® film (XF), has a higher water vapour transmission rate than polyethylene film and so has better potential to reduce humidity in the package. This XF film has been found to be beneficial for several fruit commodities, including cherry and nectarine (Lurie and Aharoni, 1998) and sweetcorn (Aharoni and Richardson, 1997). Rodov *et al*. (1997) found that microperforated film was more beneficial for mango packaging, as it avoided the accumulation of dangerous levels of carbon dioxide that can cause off-flavours and peel injury. A modified atmosphere ($\approx$5% CO_2 and $\approx$10% O_2) created by using XF film when storing mango (*Mangifera indica* L. cvs. Tommy Atkins and Keitt) fruit were effective in reducing chilling injury symptom and the level of sap inside the package due to the lower relative humidity in the XF film ($\approx$90%) compared with that of PP packaging ($\approx$99%) (Pesis *et al*., 2000).

4.3 CHEMICAL TREATMENTS

4.3.1 Chemical sanitizers and preservatives

Chemicals containing SH groups as sulphites and chlorine-based agents are commonly employed in the processing of of fresh-cut produce such as potatoes to prevent browning and to sanitize the produce. However, there is a concern over the application of these compounds in fresh-cut commodities as they might affect human and environmental safety and this has created the need to investigate alternatives.

4.3.1.1 Chlorine-based agents

Chlorine-based chemicals, particularly liquid chlorine and sodium hypochlorite (NaOCl), are probably the most widely used sanitizers for decontaminating fresh produce. Chlorine compounds are usually used at levels of 50–200 ppm free chlorine and with typical contact times of less than 5 min (Watada and Qi, 1999). Although chlorine is more effective in solution at acidic pH levels, in order to minimize the corrosion of processing equipment, chlorine-based sanitizers are usually used at pH values between 6.0 and 7.5.

Washing with chlorinated water has been traditionally applied to decontaminate vegetables and fresh-cut produce. The antimicrobial treatment consisting of washing for 5 min with agitation in a chlorine solution (100 ppm available chlorine) combined with MAP for improving the quality of dry coleslaw mix was investigated by Cliffe-Byrnes and O'Beirne (2005). Washing with chlorine was found to significantly improve sensory scores, particularly at 4°C, with better acceptability of appearance, colour and aroma. Microbial loads were significantly reduced by the use of chlorine and these reductions were substantially greater at 4°C. Chlorine had no effect on tissue pH. Overall, chlorine washing had significant effects on the quality and storage life of MAP dry coleslaw mix of the treatments studied. Chlorine gas (Cl_2) produced by a salt mixture and combined with MAP for 25 days of storage at 0°C significantly reduced *Botrytis* decay in artificially inoculated table grapes of the Flame Seedless, Thompson Seedless and Ribier cultivars (Zoffoli *et al.*, 1999), suggesting it as a sound alternative to sulphur dioxide for postharvest control of decay in table grapes.

Currently, most produce is washed with chlorinated water (50–200 ppm of active chlorine) to reduce levels of microorganisms, but this treatment results in a microbial reduction of less than 2 log CFU/g on fruit and vegetables (Beuchat, 1992; Brackett, 1992; Taormina and Beuchat, 1999; Lee *et al.*, 2004a). Several reports have questioned its efficacy (Adams *et al.*, 1989; Zhang and Farber, 1996). Hypochlorite has been shown to induce darkening of mushrooms at concentrations as low as 50 mg/L (Choi and Sapers, 1994). Also, treatment with strong solutions of chlorine may produce harmful by-products such as chloramines and trihalomethanes, which are potential carcinogens (Fawell, 2000). Future regulatory restrictions on the use of chlorine are likely and will require the development of functional alternatives. In some European countries including Germany, The Netherlands, Switzerland and Belgium the use of chlorine in ready-to-use products is prohibited. Therefore, alternative treatments that are more efficacious than chlorine for reducing or eliminating human pathogens from fresh produce are needed (Lee *et al.*, 2004a).

4.3.1.2 Chemicals containing SH groups as sulphites

Sulphites are widely used as additives for food preservation because they are greatly efficient in preventing oxidation and bacterial growth as well as controlling enzymatic or nonenzymatic reactions. By providing stabilizing and conditioning functions, they improve the appearance and maintain the quality of foods and wines. Globally, sulphur dioxide fumigation is currently still used to overcome decay caused by fungi, in litchi (lychee), table grape, and kiwi fruit (Harvey and Uota, 1978; Cheah *et al.*, 1993; Sivakumar *et al.*, 2008).

However, it has drawn much attention recently because of its allergenic effect on those individuals who are hypersensitive. Sulphur dioxide fumigation can also cause undesirable residues, altered fruit taste and health hazards for consumers and packhouse workers (Lonsdale and Kremer-Köhne, 1991). Nowadays, many countries have set strict limits on the residual amount of sulphite in different types of food. The strict standards enforced on

fruit imports by the European Community permit a maximum sulphur residue concentration of only 10 μg g^{-1} in the edible portion of the fruit (Sivakumar *et al.*, 2010). The consumer demand for 'sulphur dioxide-free fruit' has necessitated the development of alternative postharvest treatments to maintain overall quality during storage and transportation. The effects of MAP (bioriented PP, BOPP-1 or BOPP-2) in combination with the antimicrobial agents *Bacillus subtilis* (10^7 CFU mL^{-1}), ethylenediaminetetra-acetic acid (EDTA), calcium disodium salt hydrate (0.1%) or 4-hexylresorcinol (4-HR) (0.15%) on postharvest decay control and quality retention of litchi cultivar McLean's Red were assessed as possible replacements for commercial sulphur dioxide fumigation (Sivakumar *et al.*, 2008). (See section 4.8.1 for more on the use of *B. subtilis* as a control agent.)

The use of sulphur dioxide is registered in the USA with a tolerance of 10 mg kg^{-1} but frequently residues exceed tolerance limits (Federal Register, 1986, 1989). Several conventional fungicides (e.g. benzimidazoles, dicarboximides) used to control postharvest *Botrytis* decay on other fruit are difficult to apply to table grapes due to practical difficulties in achieving full coverage of the cluster of berries. Chlorine (as hypochlorous acid) is an effective and economical biocide that has been extensively used and is recognized as safe in many countries (Spotts and Peters, 1980; Brown and Wardowski, 1984; Eckert and Ogawa, 1988; Sholberg and Meheriuk, 1991; Boyette *et al.*, 1993; Zoffoli *et al.*, 1996). Chlorine gas produced by a salt mixture and combined with 25 days of storage at 0°C significantly ($p < 0.05$) reduced *Botrytis* decay in artificially inoculated table grapes (cultivars Flame Seedless, Thompson Seedless and Ribier) and no deleterious effect due to chlorine gas generation was observed (Zoffoli *et al.*, 1999) and indicated that this is a sound alternative to sulphur dioxide for postharvest control of decay in table grapes.

4.3.1.3 Chlorine dioxide and acidified sodium chlorite

Chlorine dioxide (ClO_2) has received attention as a decontaminant for produce, largely because its efficacy is less affected by pH and organic matter and it does not react with ammonia to form chloramines, as do liquid chlorine and hypochlorites (Beuchat, 1998). It is a strong oxidizing agent that has broad and high biocidal effectiveness. Application of this sanitizing agent has recently received attention due to its potential advantages over chlorine-based sanitizers, and because the FDA has allowed the use of aqueous chlorine dioxide in washing fruit and vegetables in 1998. Several recent studies reported that chlorine dioxide in gaseous or aqueous phase was effective in killing vegetative cells and spores of foodborne pathogens and spoilage microorganisms (Reina *et al.*, 1995; Han *et al.*, 2001a, 2001b; Lindsay *et al.*, 2002; Lee *et al.*, 2004a, 2004b, 2006; Rodgers *et al.*, 2004; Popa *et al.*, 2007).

Different factors can influence the lethality of a chlorine dioxide gas treatment. Han *et al.* (2001a) reported in a study about the inactivation of *E. coli* O157:H7 on green peppers that the order of significance of the factors from the most important to the least was chlorine dioxide gas concentration, time, relative humidity and temperature; moreover, a synergistic effect was found between gas concentration and relative humidity. Studies on the efficacy of chlorine dioxide to inactivate microorganisms inoculated onto fruit and vegetable surfaces have focused on pathogens such as *E. coli* O157:H7, *L. monocytogenes*, and *S. typhimurium* (Singh *et al.*, 2002; Han *et al.*, 2004). Comparatively few studies have been devoted to the effect of chlorine dioxide gas on the spoilage microflora or sensory properties of the treated fruit and vegetables. Singh *et al.* (2002) reported the decoloration of lettuce leaves after treatment, which may have been due to oxidation of chlorophyll.

Lee and Baek (2008) investigated the efficacies of sodium hypochlorite and chlorine dioxide in eliminating *E. coli* O157:H7 on commercial spinach and the use of MAP following treatment with chemical sanitizers on the growth of *E. coli* O157:H7 on commercial spinach during refrigerated storage. Treatment with 100 ppm ClO_2 or 100 ppm NaOCl for 5 min at room temperature significantly decreased levels of *E. coli* O157:H7 by 2.6 and 1.1 log CFU/g, respectively. There were significant differences (about 3–4 log) of *E. coli* O157:H7 populations between samples packed in air and other packaging methods following treatment with chemical sanitizers after 7 days' storage. These results suggest that the combination of treatment with chlorine dioxide and packaging methods such as VP and MAP may be useful for improving the microbial safety of spinach against *E. coli* O157:H7 during storage.

Acidified sodium chlorite (ASC) is a highly effective antimicrobial that is produced by lowering the pH (2.5–3.2) of a solution of sodium chlorite ($NaClO_2$) with any 'generally recognized-as-safe' (GRAS) acid (Warf, 2001). The FDA has recently approved ASC (0.5–1.2 g L^{-1}) for spray or dip application on various food products, including fresh and fresh-cut produce (Code of Federal Regulations, 2000). Inatsu *et al.* (2005) demonstrated the same sanitation efficacy of different organic acid-activated ASC solutions. Currently, ASC is commercially supplied as a kit containing citric acid and sodium chlorite. When combined these chemicals produce active chlorine dioxide, which is more soluble than sodium hypochlorite in water and has about 2.5 times greater oxidizing capacity than hypochlorous acid (HOCl) (Inatsu *et al.*, 2005). A number of reports have described the strong efficacy of ASC in the FDA-approved application concentration range of 0.5–1.2 g L^{-1} on inactivation of pathogens, including *E. coli* O157:H7 and *Salmonella* spp. (Gonzalez *et al.*, 2004; Ruiz-Cruz *et al.*, 2007). However, a negative impact on organoleptic quality of red meat and shredded carrots occurred when ASC was used within the approved concentration range (Bosilevac *et al.*, 2004). Therefore, it is critical to find the concentration of ASC that will optimize microbial safety while maintaining quality of fresh-cut produce. Both ASC and sodium chlorite at concentrations below the FDA-approved range significantly reduced aerobic mesophilic bacteria, yeast and moulds and *E. coli* O157:H7 populations in fresh-cut cilantro (Allende *et al.*, 2009).

4.3.1.4 Ozone

With the increasing concerns about the residual by-products of chlorine in foods, the food industry is in search of safe disinfectants that are effective against common and emerging pathogens. Ozone may be a good alternative sanitizer for fresh fruit and vegetables (Liew and Prange, 1994; Zhao and Cranston, 1995; Kim and Yousef, 1999; Han *et al.*, 2002). Ozone destroys microorganisms by the progressive oxidation of vital cellular components. Ozone oxidizes polyunsaturated fatty acids or SH and amino acids of enzymes, peptides and proteins to shorter peptides. The degradation of unsaturated lipids in the cell envelope results in cell disruption and subsequent leakage of cellular contents. In Gram-negative bacteria, the lipoprotein and lipopolysaccharide layers are the sites of destruction, resulting in increases in cell permeability and eventually cell lysis (Victorin, 1992; Kim and Yousef, 1999). Cellular death can also occur due to the potent destruction and damage of nucleic acids.

Ozone has been extensively applied for sanitation of drinking water with efficacy against bacteria, moulds, viruses and protozoa (Korich *et al.*, 1990; Restaino *et al.*, 1995). Furthermore, ozonated water has reduced microbial populations and extended the shelf life of some fresh-cut fruit and vegetables (Beuchat, 1998; Kim *et al.*, 1999). The decrease in pathogens including *S. typhimurium*, *Yersinia enterocolitica*, *Staphylococcus aureus*,

L. monocytogenes and *E. coli* O157:H7 has also been described (Restaino *et al*., 1995; Singh *et al*., 2002). Therefore, the use of ozonated water has been suggested as an interesting alternative to traditional sanitizers due to its efficacy at low concentrations and short contact times as well as the breakdown to non-toxic products (Graham, 1997; Rice, 1999).

Besides the effectiveness on microorganisms, excessive use of ozone may change the surface colour of some fruit and vegetables such as peaches (Bediani *et al*., 1996), carrots (Liew and Prange, 1994) and broccoli florets (Lewis *et al*., 1996).

There have been several studies on *Salmonella enteritidis* in fruit and vegetables. These studies widely focus on the risk of contamination of *Salmonella* to the fresh produce during the preharvest period (Wood *et al*., 1991; Guo *et al*., 2001, 2002) and rarely on the growth and survival of these bacteria under different storage atmospheres, on produce such as diced tomatoes (Drosinos *et al*., 1999) and shredded carrots, cabbage and lettuce (Finn and Upton, 1997; Kakiomenou *et al*., 1998). The studies coincide at a common point, which is the ability of these bacteria to survive and/or grow in low-pH produce such as tomatoes (Asplund and Nurmi, 1991; Tassou and Boziaris, 2002). For ozone applications on fresh produce, mostly aqueous solutions of ozone with or without bubbles and rarely gaseous ozone applications have been studied. Apples (Bazarova, 1982), blackberries (Barth *et al*., 1995), lettuce (Kim and Yousef, 1999), carrots (Liew and Prange, 1994) and green peppers (Han *et al*., 2002) have been treated with gaseous ozone to analyse the effect of ozone on moulds and bacteria, including *Salmonella*. Gaseous ozone treatment (5–30 mg/L ozone gas for 0–20 min) has a bactericidal effect on *S. enteritidis* inoculated onto the surface of the tomatoes, and can be used for surface sanitation of *S. enteritidis* on tomatoes before storage at different conditions (Daş *et al*., 2006). Ozone gas treatment (10 mg/L) with different time intervals of 5 and 15 min was found to be effective respectively on low- and high-dose inoculum levels of *S. enteritidis* attached for 1 h. Another variable considered during ozone treatment was the 4 h attachment time.

Ozone treatment can also inhibit the browning of potato strips under VP or MAP (Beltrán *et al*., 2005). After 14 days of storage there was no evidence of browning in fresh-cut potatoes dipped in ozonated water or ozone/Tsunami (Tsunami is a commercial brand of peroxyacetic acid; Tsunami Ecolab, Mendota Heights, MN, USA) and stored under vacuum and these treatments maintained initial texture and aroma. However, the use of ozonated water alone was not effective in reducing total microbial populations. Treatment with ozone/Tsunami resulted in the most effective treatment to control microbial growth, achieving 3.3, 3.0 and 1.2 log reductions for lactic acid bacteria, coliforms and anaerobic bacteria, respectively. Therefore, although microbial growth was not slowed down by ozone alone, the combination of ozone/Tsunami resulted in an efficient and promising treatment for controlling microbial growth and maintaining sensory quality of potato strips under vacuum.

4.3.1.5 Hydrogen perioxide and peroxyacetic acid

Hydrogen peroxide (H_2O_2) is a powerful bactericide (including spores) and oxidant, being able to generate other cytotoxic oxidizing species such as hydroxyl radicals (Khadre and Yousef, 2001). The efficacy of hydrogen peroxide washing, similar to that of NaClO, has been demonstrated in extending shelf life and reducing native microbial and pathogen populations, including *E. coli*, in whole grapes, prunes, apples, oranges, mushrooms, melons, tomatoes, red bell peppers and lettuce, and in fresh-cut cucumber, zucchini, bell peppers and melons (Sapers, 2003; Artés *et al*., 2007b). Although hydrogen peroxide is permitted for other uses in food processing and packaging because it leaves no potentially harmful residues, it is not yet

approved by the FDA as a sanitizing agent for fresh produce (Artés *et al*., 2007b). The methods for monitoring hydrogen peroxide and handling and safety issues are still in discussion (Taormina and Beuchat, 1999; Sapers, 2003). The use of a dilute hydrogen peroxide solution was promising for disinfection of fresh-cut commodities, although results were inconsistent. In fact, washing with 5% H_2O_2 was more effective than with 1000 ppm NaClO and sodium phosphate (Na_3PO_4) in terms of reducing the microbial load on cantaloupe melon rinds, thus improving microbial quality and shelf life (Sapers and Simmons, 1998). Treatment with 3% H_2O_2 for up to 60 s prior to slicing followed by a spray application of 4% sodium D-isoascorbate monohydrate or 1% H_2O_2 and subsequent storage under modified atmospheres at 4°C maintained quality and enhanced shelf life of sliced mushrooms (Cliffe-Byrnes and O'Beirne, 2008).

However, browning of shredded lettuce increased after dipping in a hydrogen peroxide solution (Parish *et al*., 2003), while hydrogen peroxide treatments (0.15–0.9%) suppressed surface discoloration and decay of fresh-cut Chinese water chestnut (Peng *et al*., 2008). A vapour treatment of hydrogen peroxide also appeared to reduce microbial counts, extended shelf life and maintained quality of fresh-cut green bell pepper, cucumber and zucchini (Sapers, 2003).

Peroxyacetic acid is a combination of peracetic acid (CH_3CO_3H) and hydrogen peroxide, usually commercialized as a liquid. Its breakdown products – acetic acid, oxygen, carbon dioxide and water – are not particularly harmful for the ecosystem. It is applied for surface cleaning in concentrations ranging from 85 to 300 ppm, and the FDA (FDA, 1997) has set a minimum of 85 ppm peracetic acid for cleaning hard surfaces where food is handled. Stampi *et al*. (2001) indicated that for cleaning the surface of foods 50 ppm is commonly enough, while, by comparison, concentrations used in the environmental and medical areas range from 1200 to 2600 ppm.

Because of peroxyacetic acid tolerance to several factors such as temperature, pH (from 1 to 8), hardness and soil contamination, its current main area of application is in fruit and vegetable processing (Artés *et al*., 2007b). For the treatment of plant surfaces, recommended formulations combine 11% H_2O_2 and 15% CH_3CO_3H, at 80 ppm, followed by rinsing with tap water (Suslow, 1997). It has been reported that this was effective for controlling *E. coli* and *L. monocytogenes* in fresh-cut products (Rodgers *et al*., 2004). *Enterobacter sakazakii* counts decreased 5 log units in lettuce with applications of peroxyacetic acid (Kim *et al*., 2006a). Compared to 150 ppm NaClO, 68 ppm peroxyacetic acid reduced psychrotrophic counts by 2 log units and mesophilic counts by 1 log unit in fresh-cut Galia melon, resulting in the fruit pieces having a shelf life of 10 days at 5°C (Silveira *et al*., 2007).

Research on the efficacy of peroxyacetic acid to inactivate microorganisms has produced varying results. One group of researchers has demonstrated the effectiveness of peroxyacetic acid (the Tsunami brand) at reducing *E. coli* O157:H7 populations on the surface of cantaloupe melons, but this treatment were not effective on asparagus spears (Park and Beuchat, 1999).

4.3.1.6 Organic acids and other preservatives

Organic acids such as citric acid, lactic acid and ascorbic acid have been applied largely for the prevention of enzymatic and non-enzymatic browning (Sapers, 1993) and microbial growth (Yildiz, 1994) at levels that did not adversely affect taste and flavour of plant commodities. They are more effective for bacteria than for moulds and yeasts due to the low pH (between 2.1 and 2.7) at which they are applied.

Kim and Klieber (1997) reported that citric acid (10 g L^{-1}) repressed petiole sprouting (black speck) development of fresh-cut Chinese cabbage and prolonged shelf life from 10 days (control) to 14 days at 5°C. However at 0°C storage life was not extended by citric acid, ascorbic acid or calcium chloride dips, and no microbial spoilage occurred after 35 days at 0°C or 21 days at 5°C under any treatment, but citric acid improved quality by reducing black speck.

Minimizing product contamination and delaying or inhibiting growth of spoilage and pathogenic organisms in the product are major keys for improving fresh meat shelf life and increasing consumer safety (Sallam and Samejima, 2004). Lactic acid is an antimicrobial agent commonly used in meat and meat products for decontamination (Jimenez-Villarreal *et al*., 2003; Shrestha and Min, 2006). European Directive 95/2/EC with regard to food additives classifies lactic acid as a generally accepted additive for food products (EC, 1995). However, discoloration continues to be a major problem associated with organic acid decontamination of meat cuts, especially at higher levels, and improving the colour stability of meat is a matter of concern to retailers (Smulders and Greer, 1998; Naveena *et al*., 2006). Addition of lactic acid (2% or 5%) to minced beef under modified atmosphere (70% O_2 and 30% CO_2) at chilled storage was associated with a pH drop, which increased drip loss and roasting loss (Friedrich *et al*., 2008). Although application of lactic acid inhibited the growth of aerobic microorganisms, it discolored the samples while sodium ascorbate seemed to improve colour stability. In general, various salts of lactic, acetic or other organic acids have also demonstrated antimicrobial activity in bacteriological media or food products under laboratory. Artificial preservatives such as sodium acetate, sodium lactate and sodium citrate have been used to avoid microorganism proliferation on refrigerated salmon slices (Sallam, 2007).

In a report by Djenane *et al*. (2003a) both the 40% CO_2 atmosphere and 1.5% lactic acid treatment was found to significantly ($p<0.05$) inhibit growth of the lactic acid bacteria *Brochothrix thermosphacta* and *Pseudomonas* spp. Neither carbon dioxide in the pack atmosphere or treatment with lactic acid, or a combination of both, affected formation of thiobarbituric acid reactive substances, myoglobin oxidation or International Commission on Illumination (CIE) a^* values. However, treatment with antioxidants (0.1% rosemary extract and 0.05% ascorbic acid) significantly ($p<0.05$) delayed oxidation of both myoglobin and lipids, and so extended the storage life of beef steaks.

Potassium sorbate and sodium benzoate are widely used as preservatives for foods. Their antibacterial effects are pH-dependent. Sorbic acid and its salts have several advantages as food preservatives. Initially thought to have only antimycotic activity, they are now known to also inhibit a wide range of bacteria, particularly aerobic catalase-positive organisms. Effective concentrations do not normally alter product taste or odour. Although these preservatives are considered harmless, in the case of some foodstuffs other than meat, maximum levels of use are also referred by European Directive 95/2 EC (EC, 1995). Potassium salt is commonly used because it is stable. Furthermore, its greater solubility extends the use of sorbate to solutions appropriate for dipping and spraying (González-Fandos and Dominguez, 2006). Potassium sorbate has been shown to inhibit potent spoilers in the natural flora of beef including *Pseudomonas* spp., *B. thermosphacta*, *Lactobacillus* spp. and Enterobacteriaceae (Zamora and Zaritzky, 1987). Medium- or high-moisture foods such as prunes and raisins will spoil at room temperature due to the growth of *Aspergillus niger* and *Zygosaccharomyces rouxii*. Prunes and raisins adjusted to a water actvity (a_w) of 0.84–0.87 in the presence of carbon dioxide atmospheres (40 and 80% CO_2) did not support growth of *A. niger*. However, *Z. rouxii* spoiled the fruit samples, both in air and under carbon dioxide

conditions. Modified atmospheres (40% CO_2/60% N_2 or 80% CO_2/20% N_2) combined with the addition of 417 and 343 ppm potassium sorbate or 383 and 321 ppm sodium benzoate accomplished complete growth inhibition of *Z. rouxii* and extended the shelf life of high-moisture prunes and raisins at 30°C for at least 6 months (El Halouat *et al.*, 1998).

4.4 QUALITY-IMPROVING AGENTS

Although MAP is widely used as a supplement to ice or refrigeration to delay spoilage and extend the shelf life of fresh fishery products, loss in water-holding capacity of fish stored under MAP generally occurs (Masniyom *et al.*, 2002). Thus, quality-improving agents such as phosphate compounds are usually adopted to improve functionality before MAP is applied.

Addition of polyphosphates to seafood products reduced the drip loss in fish stored under MAP (Alvarez *et al.*, 1996), inhibited the growth of bacteria in fish stored in ice (Zaika *et al.*, 1997) and retarded the oxidation of unsaturated fatty acids in seafood products (Dziezak, 1990). Increased water-retention ability by phosphates is achieved through muscle-fibre expansion (swelling) caused by electrostatic repulsions, which allows more water to be immobilized for the myofibril lattices (Offer and Trinick, 1983). Changes in pH and the ability to chelate metal ions essential for bacterial metabolism determine the antimicrobial effectiveness of various ortho-, pyro- and polyphosphates (Molins, 1991). Inhibition of oxidative changes may be through the chelation of prooxidative metal ions by phosphates (Matlock *et al.*, 1984). The effectiveness of phosphates on functional properties of meat products depends on the type of phosphate, the amount used and the specific food products (Lindsay, 1996). Masniyom *et al.* (2005) investigated the synergistic effect of phosphate pretreatment including trisodium phosphate, sodium pyrophosphate and sodium tripolyphosphate with MAP (80% CO_2, 10% O_2, 10% N_2) on reduction of microbiological, chemical and sensory deterioration of seabass slices. Pretreatment with sodium pyrophosphate resulted in the retarded protein denaturation as evidenced by the reduced changes in SH content and surface hydrophobicity during the extended storage. Increase in water-uptake ability accompanied by the decreased exudate loss was observed in samples pretreated with phosphates, especially pyrophosphate. No marked autolytic degradation in samples kept under MAP with and without phosphate pretreatment was observed throughout storage, as indicated by no changes in trichloroacetic acid soluble peptide. Therefore, the effective retardation of microbiological, chemical and sensory deterioration of seabass slices stored under MAP could be achieved by pretreatment with pyrophosphate (Masniyom *et al.*, 2005).

Phosphate types used in enhancement solutions will influence meat characteristics in MAP. Higher levels of phosphates and use of sodium tripolyphosphate (STP) or tetrasodium pyrophosphate rather than sodium hexametaphosphate (SHMP) improved water retention and yield in overwrapped beef biceps femoris (Baublits *et al.*, 2005a). However, colour was not improved with phosphate addition compared with non-enhanced steaks (Baublits *et al.*, 2005b). Chops enhanced with STP and packaged in carbon monoxide MAP experienced the least amount of purge loss while chops in high-oxygen MAP with both STP and STP/SHMP blend had the most purge (Wicklund *et al.*, 2006). Colour striping (two-toning) was less with the STP/SHMP blend than STP at 0.4% enhancement levels, but cook yields were higher with STP (Wicklund *et al.*, 2006). Removing phosphate from enhancement solutions containing potassium chloride, sodium chloride and sodium acetate did not affect colour of beef rib steaks in high-oxygen MAP (Knock *et al.*, 2006a) while sodium acetate and potassium lactate improved sensory attributes of injection-enhanced beef (Knock *et al.*, 2006b).

Colour and microbiological shelf life of pork with pH higher than 5.75 and enhanced with sodium acetate were improved compared with other chops (Livingston *et al.*, 2004). Colour of beef stored for 9 days and displayed for 5 days in high oxygen could be stabilized with 2.5% potassium lactate, which would replenish NADH via lactate dehydrogenase activity (Kim *et al.*, 2006b). Sodium lactate decreased metmyoglobin formation, which might explain improved colour stability of lactate injection-enhanced beef (Mancini and Ramanathan, 2008). Use of enhancement solutions containing carbon monoxide improved colour of pork chops in 80% N_2/20% CO_2 and VP, while shear force of chops in 80% O_2/20% CO_2 and in air-permeable packaging was less than for chops in anoxic packaging after storage for 4 weeks. All gaseous enhancement solutions containing carbon dioxide improved pork shelf life in MAP compared with pork enhanced with no gases and air-permeable packaging (Guerra *et al.*, 2007). Use of carbon monoxide in enhancement solutions with ammonium hydroxide and salt increased colour stability of triceps brachii in high-oxygen MAP and biceps femoris and rectus femoris in low oxygen (100% CO_2) during 7 days of simulated retail display after 7 weeks of dark storage, but oxidation values of triceps brachii were very high and total plate counts were higher in injected than non-injected steaks (Hamling *et al.*, 2008).

4.5 ANTIBROWNING AGENTS

Browning is the main physiological disorder that impairs sensory properties and discourages consumer purchase of fresh-cut produce. Enzymatic browning reactions in fruit are primarily catalysed by polyphenol oxidase (PPO) in the presence of oxygen (Martinez and Whitaker, 1995). Extensive research has been focused on control of browning in fresh-cut produce and several approaches to browning inhibition have been explored.

Inhibitors of enzymatic browning fall into six categories based on their mode of action as reviewed by McEvily *et al.* (1992). These six groups are reducing agents, chelating agents, complexing agents, acidulants, enzyme inhibitors and enzyme treatments. Most inhibitors are reducing agents and acidulants; the inhibitory effects of oxidizing agents and alkaline substances on browning of apple slices were recently reported by Lu *et al.* (2006, 2007). The inhibitors of enzymatic browning most frequently used in industry include acid or brine dips, ascorbic acid and various forms of sulphite-containing compounds. The latter have applications for a broad range of produce and are strong antibrowning and antimicrobial agents. However, in addition to causing off-flavours, sulphites pose health risks to allergic individuals and, consequently, their application on fresh and fresh-cut produce was banned by the US FDA (1986). Ascorbic acid and its derivatives are frequently added to acidic dips used for the pretreatment of peeled or sliced fruit to prevent the oxidative browning of fruit juice prior to pasteurization (Walker, 1995). However, browning proceeds after the depletion of ascorbic acid (McEvily *et al.*, 1992). Furthermore, ascorbic acid and its derivatives, unlike sulphiting agents, do not have antimicrobial activity, and thus a sanitizer should be used in conjunction with the antibrowning agent to reduce the potential pathogen contamination and spoilage microorganisms developed during the storage of produce. Unfortunately, most sanitizers used in the produce industry are incompatible with most antibrowning agents because they tend to be oxidizing agents, whereas most browning inhibitors tend to be reducing agents. Consequently, in combination they usually cancel out each other's desired effects, jeopardizing product safety. To maintain food safety and quality of fresh-cut produce, a sanitizer that is compatible with the currently widely used antibrowning solution or, better

yet, a solution that can provide dual control of browning reaction and microbial growth is urgently needed.

The sanitizers frequently used in produce and fresh-cut industry might have some degree of inhibitory effects on enzymatic browning besides effective inhibition to microorganism spoilage. Brecht *et al.* (1993) showed that 17.5 ppm hypochlorite concentration was effective in inhibiting enzymatic browning in apple and potato tissue. Hydrogen peroxide treatments (0.15–0.9%) suppressed surface discoloration and decay of fresh-cut Chinese water chestnut (Peng *et al.*, 2008). Ozone treatment inhibited the browning of fresh-cut potato strips under VP or MAP during 14 days of storage (Beltrán *et al.*, 2005). Lu *et al.* (2006) provided evidence that sodium chlorite, a sanitizer used as ASC to rinse fresh produce for sanitation, can inhibit apple PPO activity, thus preventing browning of fresh-cut apple. Lu *et al.* (2007) further investigated efficacy of sodium chlorite as an inhibitor of enzymatic browning in apple slices. However, these sanitizers with potent oxidative abilities might have some damage to tissue of produce; further experiments must be carried out in terms of dose of the sanitizer, time of treatment and rinse after treatment, etc. for a specified fruit or vegetable before its application in production.

EDTA calcium disodium salt hydrate is a chelating agent permitted for use in the food industry as a chemical preservative and has been approved for use as a food additive by the FDA (Anonymous, 1992). Another permitted additive, 4-HR, has a competitive inhibitory effect on PPO activity (McEvily *et al.*, 1992). EverFresh™, a patented product (US Patent 5,049,438), which consists of 4-HR as the active ingredient and sodium chloride as the carrier agent, has been shown as an alternative to sulphites in the control of enzymatic browning, or blackspot, in crustaceans (Lambrecht, 1995). 4-HR was also known as an effective anti-browning agent for some fruit and vegetables (Monslave-Gonzales *et al.*, 1995). Furthermore, 4-HR controlled browning and antimicrobial activity in combination with ascorbic acid and cysteine on minimally processed litchi (Shah and Nath, 2008). Combination treatments EDTA, 4-HR or *B. subtilis* in BOPP (16% O_2/6% CO_2) inhibited PPO and significantly reduced browning in pericarp of the litchi cultivar McLean's Red (Sivakumar *et al.*, 2008).

For MAP itself, it helps to reduce browning of produce, besides controlling postharvest diseases, maintaining a high-humidity environment for produce inside the sealed plastic film and preventing cross-contamination during transportation and storage (Kader, 1994). However, when combined with antibrowning agent pretreatment, the quality of fresh or fresh-cut produce can be kept better and longer than MAP alone.

4.6 NATURAL PRODUCTS

Synthetic preservatives have been used in foods for decades; however, an increasing perception by consumers that synthetic compounds may lead to negative health consequences has led to a reduced acceptance for their use in foods. Plant-derived spices are generally used in foods for flavouring and medicinal purposes. However, a number of studies have demonstrated that compounds existing in many spices also possess antimicrobial activity. Examples of such spices are cassia, clove, garlic, sage, oregano, pimento, thyme, rosemary, scutellaria and *Forsythia suspense* (thunb).

Essential oils (EOs) are aromatic oily liquids obtained from plant organs: flower, bud, seed, leaf, twig, bark, herb, wood, fruit and root. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or phenol derivatives. Table 4.1 shows some of the most common EOs as well as the major components used in the food industry with

Table 4.1 Some common EOs and their components used as flavouring in the food industry that exhibit antioxidant, antifungal and antibacterial activity in *in vitro* systems. Reprinted from Serrano *et al*. (2008), © 2008 Elsevier.

Common name	Latin name	Source	Major components	Antioxidant	Antifungal	Antibacterial	References
Clove	*Syzygium aromaticum*	Bud, leaf	Eugenol	Lipid	*Penicillium*, *Aspergillus*	*Lysteria monocytogenes*, *Lactobacillus sakei*	Gill and Holley (2004); Lee and Shibamoto (2001); Suhr and Nielsen (2003)
Eucalyptus	*Eucalyptus globulus*	Leaf, wood	Eucalyptol, eucalyptone	Thiobarbituric acid, DPPH	Moulds and yeasts *in vivo*	Pathogenic bacteria	Amakura *et al*. (2002); González *et al*. (2004a); Ponce *et al*. (2004)
Mint	*Mentha canadensis*	Leaf	Menthol	$ABTS^{++}$	*Botrytis*	Pathogenic bacteria	Bouchra *et al*. (2003); Iscan *et al*. (2002); Shan *et al*. (2005)
Oregano	*Origanum vulgare*	Leaf, flower	Eugenol, carvacrol, thymol	Peroxidase	*Botrytis*, *Fusarium*, *Clavibacter*	*Shigella* spp.	Bagamboula *et al*. (2004); Daferera *et al*. (2003); Milos *et al*. (2000)
Thyme	*Thymus vulgaris*	Leaf	Carvacrol, *p*-cymene, thymol	Aldehyde/carboxylic acid	*Aspergillus*	Pathogenic bacteria	Lee *et al*. (2005); Ozkan *et al*. (2002); Rasooli and Abyaneh (2004)

$ABTS^{++}$, 2,2′-azino-di(3-ethylbenzthiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl.

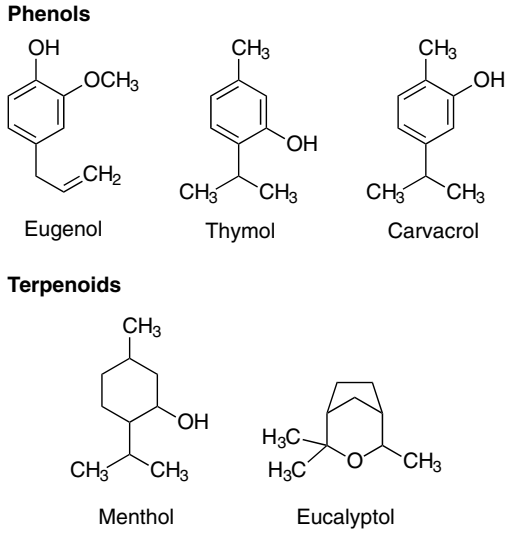

Figure 4.1 Chemical structures of pure essential oils. Reprinted from Serrano *et al.* (2008), © 2008 Elsevier.

descriptions of antioxidant or antimicrobial properties *in vitro* (Serrano *et al.*, 2008). These natural compounds belong to the genera *Thymus*, *Origanum*, *Syzygium*, *Mentha* and *Eucalyptus*. The whole EOs show antioxidant activity, but their fractionation has indicated that the main component responsible for the antioxidant effect is carvacrol for oregano (Milos *et al.*, 2000), thymol for thyme (Lee *et al.*, 2005), eugenol for clove (Lee and Shibamoto, 2001), menthol for mint (Shan *et al.*, 2005) and eucalyptol for eucalyptus (Amakura *et al.*, 2002). The chemical structures of these natural EOs are shown in Figure 4.1 (Serrano *et al.*, 2008), in which eugenol, thymol and carvacrol are phenols, while eucalyptol and menthol are terpenoids. Among these natural compounds, the antifungal activity of several EOs belonging to genera *Thymus*, *Syzygium*, *Mentha* and *Eucalyptus* is well documented (see review of Appendini and Hotchkiss, 2002). Until recently, EOs have been used as food flavourings due to their flavour and fragrance, but nowadays EOs and their pure components are gaining increasing interest from the point of view of their safe status, wide acceptance by consumers and their exploitation for multipurpose uses (Cowan, 1999). The utilization of natural antioxidants as substitutes for those from chemical synthesis has encouraged the search of new sources of EOs (Ruberto and Baratta, 2000; Capecka *et al.*, 2005). In addition, the antimicrobial properties of EOs derived from many plant organs have been empirically recognized for centuries, but only came to scientific attention recently (Appendini and Hotchkiss, 2002; Burt, 2004).

In food products, these EOs have been used in bakery (Nielsen and Rios, 2000), cheese (Vázquez *et al.*, 2001), meat (Quintavalla and Vicini, 2002) and fruit (Lanciotti *et al.*, 2004), among others. The advantage of EOs is their bioactivity in the vapour phase, a characteristic that makes them useful as possible fumigants for protection of stored commodities.

Oregano is a characteristic spice of the Mediterranean cuisine, obtained by drying leaves and flowers of *Origanum vulgare* ssp. *hirtum* plants, well known for its antioxidative and antimicrobial activity (Burt, 2004). Oregano oil is widely used in raw or cooked foods yielding a distinct but pleasant aroma and taste. Oregano EO has been studied for its antimicrobial and antioxidant activity in various commercial or model foods, e.g. raw and cooked chicken, beef, fish, fish oil, sunflower oil and egg yolk (Mejlholm and Dalgaard, 2002;

Wong and Kitts, 2002; Harpaz *et al*., 2003; Burt, 2004; Kulisic *et al*., 2004; Skerget *et al*., 2005). The combined effect of oregano EO (0.1 and 1% w/w) and MAP (30% CO_2/70% N_2 and 70% CO_2/30% N_2) on shelf-life extension of fresh chicken meat stored at 4°C was investigated by Chouliara *et al.* (2007). Microbial populations were reduced by 1–5 log CFU/g for a given sampling day, with the more pronounced effect being achieved by the combination of MAP and oregano EO. On the basis of sensory evaluation a shelf-life extension of breast chicken meat by approximately 3–4 days for samples containing 0.1% oregano oil, 2–3 days for samples under MAP and 5–6 days for samples under MAP containing 0.1% of oregano oil was attained. Thus oregano oil and MAP exhibited an additive preservation effect.

The four major constituents of oregano EO as a percentage of total content are the phenols carvacrol and thymol and the monoterpene hydrocarbons *p*-cymene and *c*-terpinene (Baydar *et al*., 2004; Burt, 2004). Carvacrol and thymol comprise the main antimicrobial and antioxidant components while possible synergistic antibacterial action has been attributed to the terpenes (Wong and Kitts, 2002; Burt, 2004; Skerget *et al*., 2005). Also, the flavonoids of oregano EO are a group of compounds with antioxidant activity (Skerget *et al*., 2005). Goulas and Kontominas (2007) investigated the combined effect of MAP (40% CO_2/30% O_2/30% N_2) and oregano EO on the shelf life of lightly salted cultured sea bream (*Sparus aurata*) fillets stored under refrigeration. For salted sea bream fillets stored under MAP the inhibition in the total volatile basic nitrogen (TVBN) and trimethylamine nitrogen (TMAN) values was evident in the order, from least to most, of MAP followed by MAP/0.4% (v/w) oregano oil followed by MAP/0.8% (v/w) oregano oil, indicating the preservative effect of oregano oil. Salting had a noticeable preservative effect but produced an increase in 2-thiobarbituric acid values while oregano oil had a strong antioxidant activity giving the lowest 2-thiobarbituric acid values. All raw sea bream fillet samples received acceptable sensory scores during the first 15–16 days of storage. The salted samples remained acceptable up to approximately 20–21 days while the MAP salted samples up to about 27–28 days of storage. The addition of oregano oil in MAP salted samples yielded a distinct but pleasant flavour and contributed to a considerably slower process of fish spoilage given that the fillets treated with 0.8% (v/w) oregano oil were still sensorily acceptable after 33 days of storage.

Mixtures of cinnamon and clove oils were capable of suppressing the growth of major spoilage microorganisms of intermediate-moisture foods (Matan *et al*., 2006). Clove oil contains eugenol, which strongly inhibits the growth of *L. monocytogenes*, *S. enteritidis*, *E. coli* and *S. aureus* in various agar mediums (Cressy *et al*., 2003; Mytle *et al*., 2006). Oussalah *et al.* (2007) evaluated 28 EOs for their antibacterial properties against four pathogenic bacteria (*E. coli* O157:H7, *L. monocytogenes*, *S. typhimurium* and *S. aureus*) and showed that most of these EOs possess antibacterial activity. The individual extracts of clove, rosemary, cassia bark and liquorice were found to have strong antimicrobial activity, but the mixture of rosemary and liquorice extracts was the best inhibitor against all four common meat spoilage and pathogenic bacteria (*L. monocytogenes*, *E. coli*, *Pseudomonas fluorescens* and *Lactobacillus sake*) either cultured in media or inoculated in MAP fresh pork as well as VP ham slices (Zhang *et al*., 2009). The results demonstrated strong potential of mixed rosemary and liquorice as a natural preservative in fresh pork and ham products.

Rosemary extracts have been demonstrated to strongly inhibit hydroperoxide formation (Frankel *et al*., 1996). The antioxidant activity of rosemary extracts has been associated with the presence of phenolic compounds, which break free-radical chain reactions by hydrogen atom donation (Basaga *et al*., 1997). A number of researchers have reported their antioxidant properties in various meats (Sánchez-Escalante *et al*., 2001; Djenane *et al*., 2002). Vitamin C

has been used together with various natural antioxidants (α-tocopherol, herb extracts) for preventing food oxidation (Shahidi and Wanasundara, 1992). In this sense, vitamin C acts as a singlet oxygen ($^1O_2$) quencher. However, depending on conditions, vitamin C can act as a prooxidant or an antioxidant (Elliott, 1999). The use of the antioxidant mixture of rosemary and vitamin C together with the absence of UV radiation significantly reduced the rates of metmyoglobin formation and lipid oxidation, as well as microbial growth, and extended the display life of fresh beef steaks packaged in 70% O_2 + 20% CO_2 + 10% N_2 and displayed at $1 \pm 1\,^{\circ}C$ from about 10 to about 20 days (Djenane *et al.*, 2003b). Lund *et al.* (2007) investigated the effect of two antioxidant systems (rosemary extract and ascorbate/citrate) in a ratio 1:1 in combination with MAP on protein and lipid oxidation in minced beef patties during storage in the dark for up to 6 days at 4°C. A high level of oxygen in the packaging atmosphere was found to increase both lipid and protein oxidation during storage. Both antioxidant systems tested were found to inhibit lipid oxidation but not protein oxidation. In contrast, ascorbate/citrate was found to promote protein oxidation. Rosemary extract was found to regenerate or protect α-tocopherol whereas the packaging atmospheres had no effect on α-tocopherol stability. In high-oxygen atmospheres both antioxidants protected the fresh red meat colour with ascorbate/citrate being more efficient than the rosemary extract, whereas no effect of antioxidant on meat colour was found in beef patties stored in 100% nitrogen.

Siqueira *et al.* (1997) reported that taurine acted as an antioxidant by preventing or delaying oxidations. Keys and Zimmermann (1999) also studied the antioxidant activity of taurine; they found that taurine, combined with other compounds, showed greatest protection against lipid oxidation. Surface application of antioxidant combinations resulted in an effective delay of oxidative deterioration of fresh beef steaks packaged in modified atmosphere. Both combinations of vitamin C with either rosemary or taurine significantly ($p<0.01$) extended the shelf life of fresh beef steaks by about 10 days. Rosemary was the most effective in delaying oxidation processes. The combination of vitamins E and C was significantly ($p<0.01$) less effective than those combinations in delaying meat oxidation (Djenane *et al.*, 2002).

Combined use of EOs with MAP to maintain the overall quality of fruit has been recently reviewed by Serrano *et al.* (2008). The use of MAP in fruit has been found to be effective on quality maintenance, but the carbon dioxide concentration inside packages could not be high enough to act as fungicide or bactericide. In this sense, the use of EOs or their components in combination with MAP in fruit received great attention on some fruit such as sweet cherry and several cultivars of table grapes such as Crimson, Autumn Royal and Aledo (Serrano *et al.*, 2005; Valverde *et al.*, 2005; Valero *et al.*, 2006; Guillen *et al.*, 2007). With this active packaging, the overall quality of products can be improved in terms of maintenance of organoleptic and functional properties together with safety. This active packaging led to reduced softening and colour evolution compared to those control fruit under MAP conditions. Thus, the combination of MAP and EOs delays the evolution of these parameters related to postharvest ripening more than MAP alone and these effects could be attributable to the EOs added. The effect of EOs on increasing or maintaining total phenolics and total antioxidant activity has been also found using thymol in table grapes (Valero *et al.*, 2006) or thymol, eugenol and menthol in strawberry (Wang *et al.*, 2007). The use of pure EOs (eugenol, thymol or menthol) in combination with MAP is an innovative and useful tool as an alternative to the use of synthetic fungicides in fruit and vegetables, especially for those which are highly perishable and have a reduced shelf life. Given the phenolic nature of some of the EOs, such as eugenol, the bioactive compounds with antioxidant activity were also enhanced during prolonged storage.

Although the whole EOs show antioxidant activity and antimicrobial activity, the main disadvantages for the use of those natural compounds fractionated from EOs are related to persistence of strong aromas which sometimes affect the organoleptic properties of food adversely, particularly in fruit. Thus the use of EOs as preservatives is limited although there is little evidence. For these reasons the preservative effect of EOs may be achieved by using lower concentrations of EOs in combination with other preservation technologies such as low temperature, low-dose irradiation, HHP, MAP or edible coatings. In future, strong development of this technology is required for commercial application, since active packaging is an emerging and exciting concept in food technology conferring many benefits, which fulfils consumer demand for safe products avoiding the use of chemicals as a means of preservation. Further studies are needed to better understand the mechanism(s) by which these EOs affect the fruit physiology modulating the ripening process as well as their ability to kill microorganisms.

4.7 OTHER METHODS, SUCH AS OXYGEN SCAVENGERS AND COATINGS

MAP, as a method where the normal gas atmosphere is changed by a package headspace as close as possible to optimal conditions, has been a challenge for scientific research due to its contribution to extending the shelf life of packed products. Moreover, the barrier properties of the polymeric material will further influence the movement of gases and moisture into and out of the package. Despite the advantages of MAP, such as improvement of product presentation and reduction of the need for food additives, this method has several drawbacks, including problems with leak detection and environmental impact (Ahvenainen *et al.*, 1995).

MAP can be a passive (before sealing) or an active operation (during storage), using a gas absorber or emitter. The latter is a recent technological innovation which involves the use of chemical scavengers (e.g. ethylene, oxygen, water) or which can release a specific gas (carbon dioxide, or microbial inhibitors such as ethanol or sulphur dioxide). With respect to atmospheric conditions in the package, the main reason for using a modified atmosphere inside the package container is to achieve the atmospheric medium that can better avoid or delay lipid oxidation of high-lipid foods such as potato crisps during storage. The most effective conditions in order to avoid or at least delay lipid oxidation were storage at room temperature with an oxygen scavenger (Silva *et al.*, 2004).

Undesirable changes in green asparagus, a highly perishable vegetable, can be reduced by rapid cooling after harvest, keeping at low temperatures (0–5°C) and use of controlled- or modified-atmosphere storage (Lipton, 1990), but Lipton (1990) and Siomos *et al.* (2000) also indicated that controlled atmospheres provided little added benefit when used to retard deterioration of asparagus during cool storage. Like many other physiological processes, senescence is also strongly regulated by plant hormones. The cytokinins have long been known to inhibit the senescence of leaves and some floral tissues (Wingler *et al.*, 1998). Exogenous application of cytokinins to plant tissues results in a variety of responses including delay in senescence, maintenance of chloroplast activity, decline in chlorophyll degradation, the production of protein and nucleic acid synthesis and mobilization of nutrients into the cytokinin-treated area (Clarke *et al.*, 1994; Wingler *et al.*, 1998). Cytokinin was also effective in delaying chlorophyll degradation and decreasing the rate of respiration in harvest broccoli and stimulating mass flow in the phloem, attracting substances from untreated tissue and directing them towards the site of application. Fresh green asparagus

(*Asparagus officinalis* L.) spears treated with 6-benzylaminopurine (6-BA; 20 ppm, 10 min) had a better colour, firmness and overall appearance, retained more chlorophyll and ascorbic acid, and had fewer fibres in both packaging treatments (passive MAP or 10 kPa $O_2 + 5$ kPa CO_2) at the end of a 24-day storage period (An *et al.*, 2006).

Coating vegetables and fruit with semi-permeable film has the beneficial effect of delaying ripening and prolonging the storage life of fresh produce (El Ghaouth *et al.*, 1992a; Park *et al.*, 1994). Chitosan appears to be an ideal preservative coating for fresh produce because of antifungal activity and film foaming properties (Hirano and Nagao, 1989). Chitosan coating has been used to extend the shelf life of tomatoes, cucumbers, bell peppers and strawberries (El Ghaouth *et al.*, 1992a, 1992b; Zhang and Quantick, 1997). Alginate and calcium-alginate films were used as coating materials for mushrooms by Nussinovitch and Kampf (1993) and Hershko and Nussinovitch (1998). Polyvinyl chloride (PVC) wrap and polyolefin films are used to pack whole and sliced fresh mushrooms after coating with calcium chloride and chitosan (Kim *et al.*, 2006c).

4.8 BIOCONTROL

Postharvest diseases limit the storage period and marketing life of fruit such as peaches. The major postharvest pathogens of peaches are *Botrytis cinerea* Pers. and *Penicillium expansum* Link. Losses due to decay are estimated to be 5–10% when postharvest fungicides are used; without fungicide treatment, losses may reach 50% or more (Lurie *et al.*, 1995; Margosan *et al.*, 1997). Recently, fludioxonil and azoxystrobin were registered in the USA for postharvest application to control decay in peaches. However, postharvest use of these fungicides in European Union countries is prohibited due to fungicide regulatory issues. In addition, public demands to reduce pesticide use, stimulated by greater awareness of environmental and health issues, as well as the development of resistance of some pathogens to fungicides, limits the postharvest application of chemicals to agricultural products. The lack of an effective postharvest treatment against postharvest decay of fruit highlights the need for developing new control methods.

Various non-chemical approaches have been investigated or proposed in recent years. Several studies have shown that hot-water treatments have the potential to control postharvest diseases of peaches (Smith and Redit, 1968; Wells and Harvey, 1970; Margosan *et al.*, 1997; Karabulut *et al.*, 2002). In addition, biological control of postharvest diseases of stone fruit has been persued actively by using bacteria (Pusey and Wilson, 1984; Wilson *et al.*, 1987; Smilanick *et al.*, 1993) and yeast antagonists (Lurie *et al.*, 1995; Hong *et al.*, 1998; Spotts *et al.*, 1998, 2002). Furthermore, several studies have demonstrated the inhibitory effect of MAP on postharvest pathogens (Prusky *et al.*, 1997; Spotts *et al.*, 1998, 2002; Karabulut *et al.*, 2001).

4.8.1 Bacterial antagonists

The biocontrol agent *B. subtilis*, developed as Avogreens for the control of postharvest diseases in avocado, is a registered biocontrol product recommended for commercial application in South Africa. This bacterial species was previously found to be effective in controlling postharvest decay in litchi cultivar Huaizhi under cold storage (Jiang *et al.*, 2001). The mode of action of *B. subtilis* was reported as being due to the antibiotic action of lipopetides, bacillomycin, iturin A and fengycin (Romero *et al.*, 2007).

The efficacy of biological control and two types of MAP alone and in combination was evaluated under cold storage as well as simulated market-shelf conditions to control decay and pericarp browning on litchi cultivar McLean's Red by Sivakumar *et al.* (2007). Fruit treated with *B. subtilis* + PP or prochloraz + PP and stand-alone PP treatment did not show decay or browning at 2°C. Decay and browning were controlled significantly after 2 days at 14°C in *B. subtilis* + PP or prochloraz + PP treatments. The stand-alone PP treatment (14% O_2/5% CO_2) showed 11.3% decay due mainly to *Alternaria alternata* and *Cladosporium* spp. at 14°C. The effectiveness of the MAP was improved at 14°C when *B. subtilis* was combined with PP, controlling decay and pericarp browning and retaining the fruit colour and quality. Stand-alone low-density polyethylene (LDPE) (3% O_2/10% CO_2) and combination treatments *B. subtilis* + LDPE or prochloraz + LDPE failed to control decay and pericarp browning. Higher yeast populations were observed in LDPE or *B. subtilis* + LDPE at both 2 and 14°C.

Bacterial antagonists were also found application in meat products. Because lactic acid bacteria are GRAS in food production (Schillinger *et al.*, 1996), in recent years the use of either their bacteriocins or the bacteriocin-producing lactic acid bacteria starter cultures has received special attention as a new preservation method to control pathogenic bacteria (Holzapfel *et al.*, 1995; Jack *et al.*, 1995; Ennahar *et al.*, 1999). Preservation of meat with bacteriocin-producing *Pediococcus* spp. and *Lactobacillus* spp. has already been described (Campanini *et al.*, 1993; McMullen and Stiles, 1996; Muriana, 1996; Stiles, 1996). However, it was reported that inoculation with *Lactobacillus fermentum* had little or no effect on the shelf life of ground chicken breast meat while the combination of ethanol rinsing and high-nitrogen packaging extended gound chicken quality compared with meat rinsed in water and packaged in high oxygen (Keokamnerd *et al.*, 2007). With regard to enterococci, their bacteriocins (enterocins) have the potential to inhibit the growth of a narrow range of strains closely related to the producer microorganism: Gram-positive foodborne pathogens and spoilage bacteria, and Gram-negative species. Bacteriocin-producing *Enterococcus* strains with strong anti-*Listeria* activity have been isolated from dairy products, fermented sausages, fish, vegetables, fermented olives and silage. The ability of enterococci to inhibit *L. monocytogenes* may be explained by the fact that enterococci and *Listeria* are phylogenetically closely related (Devriese and Pot, 1995). Since enterococci are common in various food systems and their technological and probiotic benefits are widely recognized (Giraffa *et al.*, 1997), these microorganisms might be good candidates for potential application of bacteriocin-mediated antagonism against *L. monocytogenes* in foods (Muriana, 1996).

Nisin, a bacteriocin produced by *Lactococcus lactis* ssp. *lactis*, is active against Gram-positive organisms including bacterial spores, but it is not generally active against Gram-negative bacteria, yeasts and fungi. The efficiency of killing most Gram-positive bacteria by nisin and its high safety render it a broad and potential application in meat products, dairy products and seafoods (Chen and Hoover, 2004). Nisin Z in crude form (6.54×10^{10} units of bacteriocin activity (BU)/g) and purified form (8.13×10^{23} BU/g) delivered a shelf-life extension of brined shrimp to 31 days, compared to 10 days in the control treatment (Einarsson and Lauzon, 1995). So far, nisin is the only bacteriocin licensed in Europe as a food preservative (E234) (Gálvez *et al.*, 2007) and has also been approved by the FDA in the USA as GRAS.

Studies have been published by a number of authors on the use of nisin as an antimicrobial in a wide variety of food products (Delves-Broughton *et al.*, 1996). Application of antimicrobial treatments using nisin and EDTA to raw poultry products in combination with MAP or VP has received great attention. EDTA, a chelator, can have an antimicrobial effect by

limiting the availability of cations and can act to destabilize the cell membranes of bacteria by complexing divalent cations which act as bridges between macromolecules, such as lipopolysaccharides. Nisin and EDTA increased shelf life of fresh chicken meat by a minimum of 4 days when packaged under aerobic conditions and a maximum of 9 days when VP (Economou *et al*., 2009). These studies confirmed that a combination of nisin and EDTA treatment and VP, significantly increase the shelf life of raw poultry. The use of MAP in combination with nisin/EDTA (500 IU/g/50 mM EDTA) antimicrobial treatments resulted in an organoleptic extension of refrigerated, fresh chicken meat by by 13–14 days, maintaining acceptable odour attributes even up to 24 days of storage (Economou *et al*., 2009).

Lu (2009) investigated the effects of different bactericides and MAP on shelf life of Chinese shrimp (*Fenneropenaeus chinensis*) during cold storage and found that the Chinese shrimp at $2 \pm 1°C$, either whole or decapitated, treated with MAP (40% CO_2/30% O_2/30% N_2) and 100% CO_2 after soaking with compound bactericide (1 g/L 4-HR, 500 IU/mL nisin and 5 g/L sodium dehydroacetate) showed a shelf life of 13 and 17 days, respectively.

4.8.2 Yeast antagonists

Biological control of postharvest diseases has made great advances, especially during the past decade, during which the usefulness of this approach has been proven under commercial conditions (Janisiewicz and Korsten, 2002). The commercial products Aspire™ (Ecogen, Langhore, PA, USA) based on the yeast *Candida oleophila* (Droby *et al*., 1998) and BioSave™ 100 and 110 (JET Harvest Solutions, Longwood, FL, USA) containing saprophytic strains of *Pseudomonas syringae* (Janisiewicz and Jeffers, 1997) were registered by the USA Environmental Protection Agency for application to pome and citrus fruit in 1995. The use of BioSave has been continually increasing and the original registration for postharvest application to apples, pears and citrus fruit has been extended to cherries, potatoes and sweet potatoes (Stockwell and Stack, 2007). On pears it was reported to be the most effective postharvest treatment in integrated management trials and was comparable to or better than a standard fungicide (Sugar, 2006). Although this product has been very effective in various systems and under a variety of conditions, as with any biocontrol agent it has its limitations, especially those imposed by environmental conditions. Other biocontrol products for postharvest application that are on the market include YieldPlus™ (Anchor Yeast, Cape Town, South Africa) containing *Cryptococcus albidus* in South Africa, and Shemer™ (AgroGreen, Asgdod, Israel) containing *Metschnikovia fructicola* in Israel (Kurtzman and Droby, 2001; Karabulut *et al*., 2003). Both are registered in their respective countries for control of postharvest decays on several fruit including grapes, pome, stone and citrus fruit.

A mixture of two yeast antagonists, *Metschnikowia pulcherrima* and *Cryptococcus laurentii*, originally isolated from apples and apple cider, which exhibited greater biocontrol activity against blue mould of apple than either yeast applied alone, were used in combination with sodium bicarbonate in a pilot test in which treated fruit were stored under commercial citric acid storage conditions (Janisiewicz *et al*., 2008). An integrated approach was investigated by Karabulut and Baykal (2004) for the control of postharvest diseases of peaches including application of a yeast antagonist (*Candida oleophila*), hot-water treatment at 55°C for 10 s and storage in modified atmosphere at 0°C. They found that hot water and yeast antagonist as stand-alone treatments were not effective in controlling *Botrytis cinerea* and *Penicillium expansum* infections on wound-inoculated peaches. In contrast, MAP alone significantly reduced lesion diameters caused by the infections of both pathogens after 45 days' storage at 0°C and 5 days at 24°C. The biocontrol activity of yeast antagonist

Table 4.2 The important works carried out in the field.

	Methods	Important works	References
Physical treatments	Low temperature	Lower temperature usually beneficial in keeping storage quality and extending shelf life for most produce.	González-Aguilar *et al*. (2004)
	High pressure	HPP does not disrupt covalent bonds, thus maintaining the primary structure of proteins and thereby retaining appearance, flavour, texture and nutritional qualities of the unprocessed product.	Murchie *et al*. (2005); Kingsley *et al*. (2005); De Ancos *et al*. (2000); Olsen, *et al*. (2003)
	Radiation	Ionizing irradiation is a promising technology to maintain the quality of minimally processed produce due in part to its efficiency in controlling both spoilage and pathogenic bacteria.	Hines (2000); WHO (1999); Bidawid *et al*. (2000); Lado and Yousef (2002); López-Rubira *et al*. (2005)
	Heat treatment	Heat treatment causes changes in fruit ripening, such as inhibition of ethylene synthesis and action of cell-wall-degrading enzymes. Hot-water treatments are also used as alternative methods to control postharvest diseases.	Paull and Chen (2000); Steiner *et al*. (2006); Saltveit (2000); Loaiza-Velarde *et al*. (2003); Peng and Jiang (2004); Malakou and Nanos (2005)
	Film	Use of selective permeable packaging films like silicon membrane could also be beneficial in extending the shelf life of the produce, where atmosphere inside the package gets modified through the respiration of the produce along with selective passage of the respiratory gases across the membrane.	Li *et al*. (2007); Stewart *et al*. (2005); Li *et al*. (2007); Stewart *et al*. (2005); Villaescusa and Gil (2003)
Chemical treatments	Chlorine-based sanitizers and preservatives	Washing with chlorine was found to significantly improve sensory scores, with better acceptability of appearance, colour and aroma. Microbial loads were significantly reduced by the use of chlorine and these reductions were substantial.	Cliffe-Byrnes and O'Beirne (2005); Beuchat (1992); Brackett (1992); Lee *et al*. (2004a); Taormina and Beuchat (1999)
	Sanitizers and preservatives containing SH groups as sulphites	Sulphites are widely used as additive for food preservation, with their great efficiency in preventing oxidation and bacterial growth as well as controlling enzymatic or non-enzymatic reactions.	Sivakumar *et al*. (2008); Cheah, Hunt and Lorentz (1993); Lonsdale and Kremer-Köhne (1991); Sivakumar *et al*. (2010)

(*Continued*)

Table 4.2 (*Continued*)

	Methods	Important works	References
Chemical treatments (*continued*)	Chlorine dioxide and ASC	ClO_2 in gaseous or aqueous phase was effective in killing vegetative cells and spores of foodborne pathogens and spoilage microorganisms. ASC showed strong efficacy on inactivation of pathogens, including *E. coli* O157:H7 and *Salmonella* spp.	Han *et al*. (2001a, 2001b); Lee *et al*. (2004a, 2004b, 2006); Lindsay *et al*. (2002); Popa *et al*. (2007); Reina *et al*. (1995); Rodgers *et al*. (2004); González *et al*. (2004b); Ruiz-Cruz *et al*. (2007)
	Ozone	Ozone has been extensively applied for sanitation of drinking water with efficacy against bacteria, moulds, viruses and protozoa. Furthermore, ozonated water has reduced microbial populations and extended the shelf life of some fresh-cut fruit and vegetables.	Korich *et al*. (1990); Restaino *et al*. (1995); Beuchat (1998); Kim *et al*. (1999); Beltrán *et al*. (2005)
	Hydrogen perioxide and peroxyacetic acid	The efficacy of H_2O_2 washing demonstrated in extending shelf life and reducing native microbial and pathogen populations, including *E. coli*, in whole grapes, prunes, apples, oranges, mushrooms, melons, tomatoes, red bell peppers and lettuce, and in fresh-cut cucumber, zucchini, bell peppers and melons.	Sapers (2003); Artés *et al*. (2007b); Taormina and Beuchat (1999); Sapers (2003); Cliffe-Byrnes and O'Beirne (2008)
	Organic acids and other preservatives	Organic acids such as citric acid, lactic acid and ascorbic acid have been applied largely for the prevention of enzymatic and non-enzymatic browning and microbial growth at levels that did not adversely affect taste and flavour of plant commodities.	Sapers (1993); Yildiz (1994); Jimenez-Villarreal *et al*. (2003); Shrestha and Min (2006); Friedrich *et al*. (2008); Sallam (2007)
	Quality-improving agents	Addition of polyphosphates and other agents to seafood and other products reduced the drip loss in fish stored under MAP, inhibited the growth of bacteria in fish stored in ice and retarded the oxidation of unsaturated fatty acid in seafood products.	Alvarez *et al*. (1996); Zaika *et al*. (1997); Dziezak (1990); Masniyom *et al*. (2005)
	Antibrowning agents	Antibrowning agents help to reduce browning of produce besides controlling postharvest diseases, maintaining a high-humidity environment for produce inside the sealed plastic film, and preventing cross-contamination during transportation and storage.	Lu *et al*. (2006, 2007); Peng *et al*. (2008); Beltrán *et al*. (2005); Lu *et al*. (2006); Sivakumar *et al*. (2008)

	Natural products	Compounds existing in many spices such as cassia, clove, garlic, sage, oregano, pimento, thyme, rosemary, scutellaria and *Forsythia suspense* (thunb) have demonstrated antimicrobial activity.	Lee *et al*. (2005); Serrano *et al*. (2008)
	Others such as oxygen scavenger and coatings	The most effective conditions to avoid or at least delay lipid oxidation were storage at room temperature with an oxygen scavenger. Coating vegetables and fruits with semi-permeable film has the beneficial effect of delaying ripening and prolonging the storage life of fresh produce.	Silva *et al*. (2004); Siomos *et al*. (2000); El Ghaouth *et al*. (1992a); Park *et al*. (1994); Kim *et al*. (2006c)
Biocontrol	Bacterial antagonists	The biocontrol agent *B. subtilis*, developed as Avogreens for the control of postharvest diseases in avocado, is a registered biocontrol product recommended for commercial application in South Africa. Nisin, a bacteriocin produced by *L. lactis* ssp. *lactis*, is active against Gram-positive organisms including bacterial spores.	Romero *et al*. (2007); Sivakumar *et al*. (2007); Keokamnerd *et al*. (2007)
	Yeast antagonists	A mixture of two yeast antagonists, *M. pulcherrima* and *C. laurentii*, originally isolated from apples and apple cider, exhibited greater biocontrol activity against blue mould of apple than either yeast applied alone.	Janisiewicz *et al*. (2008); Karabulut and Baykal (2004)

was improved when yeast treatment was combined with MAP. The results also indicate that all of the treatments significantly reduced the decay incidence caused by natural infections after 45 days of storage at 0°C followed by 5 days at 24°C. The highest efficacy was achieved by the combination of all three tactics and none of the treatments caused surface damage to fruit or impaired quality.

A summary of the important works carried out in the fields covered in this chapter is given in Table 4.2.

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5 Coating Technology for Food Preservation

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Abstract: The potential use of coating technology for food preservation has been well known for thousands of years. Even though surface coating is a very old practice, the research into this field is expected to continue for the foreseeable future. In this chapter recent progress in the field of food preservation with coating technologies is presented. An overview of the development and application of coating technologies is presented in terms of both experimental and commercial implementation. Emphasis is placed on surface coating techniques and different types of coating component used to protect foods from environmental influences (e.g. light, oxygen, microorganisms) and the generation of desirable functionality on the surface of food or food contact materials. Finally a discussion on effectiveness, drawbacks, and future trends in the technology is included in anticipation of extensive research interest in food applications of the technology.

Keywords: active agents; coating material; encapsulation; food preservation; packaging; surface coating

5.1 INTRODUCTION

Fruit and vegetables naturally adapt themselves in several ways to survive natural fluctuations in their surrounding environment, such as temperature, humidity, light, and microbial attack. This is achieved by a naturally occurring protective layer on the surface of the fruit or vegetable, the most susceptible area to changes in the environment. For example, fruits are capable of retaining moisture and freshness with the aid of a layer of waxy crystals on their surface (Kolattukudy, 1984). This self preservation is generally known as a "built-in coating." Long before the development of modern chemistry, the advantages of such coating technologies were well known and the Chinese made use of wax coating to extend the shelf life of fruit during its transportation across the country.

Coating technology for food preservation can be classified as either macroscale or microscale coating. Bulk foods are protected from deterioration by applying a coating layer directly to the food surface, food contact surface, or packaging on the macroscale. On the other hand, deteriorations occurring with food ingredients on the microscale are retarded by a coating methodology referred to as encapsulation.

Extensive development in coatings of food surfaces, food contact surfaces, and food packaging has long been nurtured from traditional coating using only biodegradable coating materials from natural sources such as lipids, proteins, carbohydrates, and resins, to synthetic polymers either alone or in combination as composites, and to modern coatings in which

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traditional coatings are functionalized. In functionalized coatings the coating material forms a shelter and acts as a carrier for protection and delivery of active agents capable of preserving food quality. Such agents include biocontrol agents, antioxidants, gas absorbers, and antibrowning agents. These active agents are applied to the coating by incorporation into a film matrix or coating directly onto the film of coating material. A range of active agents used in coating is reported. These include the use of a generic antibacterial agent, the direct coating of antimicrobial agents onto polymeric films (e.g. polyvinyl chloride, linear low-density polyethylene, nylon) which are proposed to inhibit microbial growth in foods, and innovative hygienic photocatalytic titanium dioxide (TiO_2) to transform the surface of food packaging and food-processing plants into an antibacterial and antifungal active surface.

Encapsulation used on the microscale can be defined as packages or micro- and nanoscale capsules in which the active ingredient is protected inside a coated polymer wall. Great benefits are obtained by applying encapsulation to the delivery of food ingredients and additives. It gives shelter to sensitive vitamins, minerals, nutrients, and additives during exposure to unfavorable conditions such as changes in pH, oxygen, moisture, light, and heat, and adverse exposure to enzymes, during processing, handling, and storage. Furthermore, it shields the often undesirable aroma, flavor, and taste of nutritious food ingredients (such as fish oil) during consumption. Moreover, controlled release of the encapsulated ingredients or additives at a specific place, time, and rate is controllable depending on wall polymer formulation by selecting appropriate lipids, proteins, and carbohydrates. For example, Saenz and co-workers (2009) reported an increase in the stability of antioxidants and colorants using maltodextrins or inulin as wall polymers. Fragile vitamin C in fruit juice is effectively protected using maltodextrin and gum arabic (Dib Taxi *et al.*, 2003). Additionally, an innovative use of empty yeast cells as a thermostable wall for entrapment of beef flavor was reported by Dardelle and colleagues (2007) and showed a superior long-lasting flavor release compared to traditional spray-dried encapsulated beef flavor.

An approach exists where both macroscale and encapsulation techniques are merged, in which active substances are protected by encapsulation prior to being incorporated into a macroscale coating. Protection and controlled release of active substances are benefits from this approach as demonstrated in nanolaminate coatings and edible coatings (Weiss *et al.*, 2006; Sorrentino *et al.*, 2007; Lee, 2010).

5.2 PROGRESS IN RELEVANT MATERIALS AND THEIR APPLICATIONS IN COATING

A high demand for food safety and quality has lead to strong growth in research and development of coating technologies of food surfaces or food contact surfaces. The progress in coating technology highlighted here includes active agents for coating, controlled-release coating, multifunctional surface coating, and nutraceutical coating.

5.2.1 Active agents for coating

For preservative coatings the active agents generally include natural antimicrobial extracts, chemical preservatives, antioxidants, and antibrowning agents. Several novel active agents, such as chitosan glucose complex, plant essential oils, and wood smoke, and nanoscale metals and metal oxides (Holley and Patel, 2005; Kanatt *et al.*, 2008; de Azeredo,

2009), have recently been suggested. The active agents with applications in the area of coating are now described.

5.2.1.1 Chitosan

Chitosan is a linear polysaccharide polymer of D-glucosamine and *N*-acetyl-D-glucosamine mainly derived from deacetylation of chitin. The antimicrobial properties of chitosan are more pronounced against yeasts and molds followed by Gram-positive and Gram-negative bacteria (Aider, 2010). This antimicrobial effect of chitosan coating has been proven to be very effective for food preservation when it is in direct contact with the food surface. Additionally, a chitosan coating can also induce host-defense responses of coated fruits (Zhao, 2005). Significant antibrowning activity of chitosan was observed when used in the form of a chitosan–glucose complex (Kanatt *et al.*, 2008). Due to the film-forming ability of chitosan and its compatibility with other active agents, chitosan is also used as a coating carrier for other antimicrobials such as lysozyme and potassium sorbate to achieve a significant synergistic antibacterial activity (Zhao, 2005).

5.2.1.2 Nisin

Nisin is a polycyclic antimicrobial peptide produced by *Lactococcus lactis* ssp. *lactis* (Delves-Broughton, 1990). Nisin is a US Food and Drug Administration (FDA)-approval food additive with E number E234. Nisin is known to exert a strong antibacterial activity against Gram-positive bacteria and lactic acid bacteria. The antibacterial activity of nisin can also be extended to Gram-negative bacteria when used in a combination with ethylene-diaminetetra-acetic acid (EDTA). Nisin is effective against bacteria through the specific assembly between nisin and lipid II, a key molecule in peptidoglycan synthesis embedded in the exterior site of the plasma membrane (Hasper *et al.*, 2006). The binding complex results in the formation of pores across the plasma membrane which induces a rapid outflow of small cytoplasmic compounds (Breukink and de Kruijff, 2006; Hasper *et al.*, 2006). Nisin is widely used, either by direct addition or by incorporation into a coating. The use of nisin-containing coatings on the surface of polymeric films has the potential for delivery and transfer of nisin compounds to the surface of foods. Nisin-coated films have been reported to effectively inhibit and reduce the risk of microbial contamination on the surface of foods.

5.2.1.3 Metal and metal oxides

Metals and metal oxides including titanium dioxide (TiO_2), copper (Cu), zinc oxide (ZnO), gold (Au), magnesium (Mg), and silver (Ag) are capable of inhibition of microorganisms. At low concentrations their inhibition of microorganisms is mainly due to their extremely small size, which facilitates: (i) an ability to penetrate the cell wall of target pathogens; and (ii) a higher surface-area-to-volume ratio for interaction with target molecules. In order to utilize these materials in coating, they can be incorporated into substrates, adsorbed or coated onto substrates, or linked via ionic or covalent bonds to the surface functional groups of substrates. The terms nanomaterials and nanocomposites are used to describe metal and metal oxides on the nanoscale and the coating materials which are used in combination with nanomaterials, respectively.

The antimicrobial property of silver has been known since ancient times when it was used to treat water and to extend the shelf life of milk. Inert crystalline silver (Ag^0)

becomes antibacterially active when it is ionized (Ag^+). The higher surface area of silver nanoparticles (Ag^0) allows them to release active silver ions rapidly, with effective antibacterial activity. It has been suggested that the antimicrobial effect of silver is related to its ability to bind strongly to biomolecules containing electron donor groups such as sulfur, oxygen, nitrogen, and phosphorus, which leads to a breakdown of the biomolecules. Respiratory enzymes and DNA are found to be the most favorable target molecular sites for silver ions. Recently, silver coatings on surfaces and equipment used in food processing have been shown to have potential for reducing foodborne pathogens and to enhance food safety.

Photocatalytic coatings with the ability to self-sanitize food-processing plants and food packaging are available now. The use of titanium dioxide for processing-plant interior coatings and food packaging has received great attention from the food industry due to its safe, non-toxic, environmentally friendly, maintenance-free, and cost-effective nature. Photocatalytic coating technology was discovered over 30 years ago in Japan and is broadly described as the opposite of photosynthesis (Fujishima *et al.*, 1999). Photocatalysts, such as titanium dioxide and zinc oxide, upon illumination with UV light (<385 nm), generate an electron-hole pair on the titanium dioxide surface. The hole (h^+) in the surface of titanium dioxide subsequently reacts with water or OH groups adsorbed on the surface to form a hydroxyl radical ($OH^\bullet$) and the electron (e^-) reduces oxygen to produce superoxide ions (O_2^-). The resulting reactive oxygen species and $OH^\bullet$ radicals then decompose organic compounds such as odors, volatile organic compounds, microorganisms, and biofilm into water and carbon dioxide. Titanium dioxide is typically a white pigment used in food contact material and used as a color additive as approved by the FDA (food additive E171) with the restricted level at 1% by weight. Another photocatalyst is zinc oxide which is often used in foods as a source of zinc and antimicrobial agent. The antimicrobial mechanisms of zinc oxide are attributed to both photocatalysis and zinc ions (Wang *et al.*, 2004; Qin *et al.*, 2006).

5.2.2 Controlled release of active agents

Coating, a thin film applied onto surfaces of food or packaging, is generally recognized as a barrier against transfer of gases, moisture, and microorganisms, which are termed passive characteristics. Concurrently, the microscopic porous or network structure of the coating allows for additional active characteristics to be realized as the incorporated active agent can be subsequently released through the pores onto the food surface in a controllable manner. Hence any reformation in the microscopic structure by means of physical or chemical treatments also has a tendency to alter diffusion behavior (LaCoste *et al.*, 2005; Wongsasulak *et al.*, 2006). This diffusion behavior begins with the relaxation of the polymer film, resulting from swelling of the film after the sink's solvent diffuses through, which allows for subsequent diffusion of incorporated agent (Armand *et al.*, 1987; Ouattara *et al.*, 2000). It should be noted that the addition of active agents to a coating can be done by an incorporation into the film while the film is being fabricated, or by coating onto the film to reduce interaction or degradation that may occur during film fabrication (Cooksey, 2005). Various different types and ratios of coating materials, lipids, plasticizers, and incorporated active agents and different target applications have been formulated and applied. The diffusion coefficient (D) or diffusion as a function of time of active substance is basically determined based on the Fick's laws of diffusion (Crank, 1975; Ouattara *et al.*, 2000). The release medium is adjusted to have similar characteristics to the target application

including pH and water activity (a_w) typically achieved through addition of dextrose or glycerol (Chirife *et al.*, 1980; Franssen *et al.*, 2004; Sanjurjo *et al.*, 2006). A steady slow release of antimicrobial agents has been reported to provide a greater antimicrobial effect compared to the direct addition of agents (Chung *et al.*, 2001; Sanjurjo *et al.*, 2006; Jin, 2010). This can be explained by the protection of the agent with a coating matrix which prevents non-specific binding to food components and enzymatic degradation. Furthermore the ability of coating matrix to prolong the release of the agent over time leads to the death of surviving dormant or recovering injured microbial populations (Zhang *et al.*, 2004; Jin, 2010). This is true when the initial release concentration of incorporated agent in its active form is at least at the minimum inhibitory concentration (MIC) or high enough to kill most of the contaminated microbial population, leaving only a small number of dormant or injured or even healthy cells to be killed by the subsequent slow release of agent. Importantly, controlled-release coating is used to protect active agents from non-specific binding and to prevent degradation, allowing the agent's activity to be extended and hence increase product shelf life. For the controlled release of antimicrobials, a multistage release or multi-hurdle approach is preferable for reducing the initial level of contamination to a level at which the antimicrobial agent can be effective.

5.2.2.1 Starch-based carriers

The use of starch as a carrier for controlled release of flavor and aroma compounds in foods, pharmaceutical drugs, and plant nutrients in agriculture has long been established (Mikkelsen, 1994; Elvira *et al.*, 2002; Madene *et al.*, 2006). The physicochemical characteristics of a starch coating film are unique and dependent upon the source of starch, modification process, preparation conditions, and additives used (Jobling, 2004; Singh *et al.*, 2007; Abbas *et al.*, 2010). A difference in the final microscopic structure, which is tuned by controlling the physicochemical properties of the film, can dramatically alter the release pattern of an active substance. A higher degree of crystallization limits the mobility of solvent and hence active agent inside the starch matrix and will result in a slow release rate, for example in sorbate-incorporated tapioca starch films (Flores *et al.*, 2007b). Alternatively, the addition of glycerol as a plasticizer into sago starch films has been shown to facilitate dispersibility of lemongrass oil and hence increase the rate of release (Maizura *et al.*, 2007). An increase in diffusion was also observed when the pH in the sink was adjusted to increase the solubility of sorbate and chitosan (Flores *et al.*, 2007a; Shen *et al.*, 2010). Electrostatic interaction between oppositely charges or chemical bonding retards the release of active agent as demonstrated in the case of a carboxylic group of sorbate and an amino group of chitosan embedded in a tapioca starch film where hydrogen bonding between chitosan and starch was observed (Vásconez *et al.*, 2009). An inclusion complex formed from the insertion of small active agent molecule into the helical molecular structure of amylose diminishes the rate of release and is considered as an obstruction to an effective release rate of the agent (Ofman *et al.*, 2004).

5.2.2.2 Chitosan-based carriers

Chitosan coating not only provides a wide range of antimicrobial properties but also acts as a carrier for controlled release of other active agents from its matrix. A chitosan coating provides effective antimicrobial activity on a surface only in direct contact without diffusion whereas incorporated antimicrobials can diffuse through the chitosan matrix to the surface

and further into the food matrix (Brody *et al.*, 2001; Pranoto *et al.*, 2005). As reported in the case of a chitosan-starch film, the electrostatic interaction between amine groups of chitosan and carboxyl groups of acid-species preservatives (e.g. sorbate), as confirmed by Fourier transform infrared (FTIR) spectrum, is a key issue in retarding the release of the preservatives (Pranoto *et al.*, 2005; Vásconez *et al.*, 2009). The highly electron-dense benzene ring in tetrahydrocurcuminoid enhanced the electrostatic interaction with the chitosan matrix, resulting in a slower release compared to another species of tetrahydrocurcuminoid containing no phenol and methoxy electron-donating groups (Portes *et al.*, 2009). A deviation of the diffusion pattern from usual Fickian behavior to non-Fickian behavior was also observed when the coating absorbed a higher load of solvent, resulting in a highly swollen film as in the case of chitosan and other extreme hydrophilic polymers (Ouattara *et al.*, 2000). The presence of a lipid component in hydrophilic-type films affect the microscopic structure of the film and causes a reduction in hydrophilic solvent absorption and hence slows down the diffusion rate of incorporated hydrophilic agent (Outtara *et al.*, 2000). Increasing the temperature during release also increases the solubility of incorporated agents due to their increased kinetic energy (Daniels and Alberty, 1972; Outtara *et al.*, 2000).

5.2.2.3 Protein-based carriers

Incorporation of a hydrophilic compound into a coating leads to an increase in film hydrophilicity. This results in a higher coating-solvent interaction which speeds up a relaxation process of the film and consequently increases the diffusion coefficient (Ozdemir and Flores, 2001). The non-Fickian diffusion coefficient of sorbate in whey protein film is found to be 10^{-11} m^2/s in which one- and 10-fold higher than in wheat gluten and low-density polyethylene (LDPE) films, respectively (Ozdemir and Flores, 2001). Zinnoviadou and co-workers (2009) demonstrated a superior inhibition of oregano oil released from whey protein isolate against bacteria on fresh beef compared to use in its free form. An increase in glycerol and sorbitol used as a plasticizer in whey protein isolate film as well as an increase in release temperature was found to facilitate the diffusion of natamycin, sorbate, hypothiocyanate, and thiocyanate (Franssen *et al.*, 2004; Min *et al.*, 2007). The concentration of thymol did not show a significant increase in the diffusion rate from a zein film but caused phase separation between thymol and the film structure (Del Nobile *et al.*, 2008). Regarding difference in amino acid contents and charge density of different types of protein used in making the film, hydrophilicity and swelling rate of a zein film are higher compared to a wheat gluten film (Dawson *et al.*, 2003). Lipid content such as beeswax was found to significantly retard the diffusion of sorbate in wheat gluten film but have little effect in whey protein isolate film when used at 20–40% by weight (Franssen *et al.*, 2004). An incorporated agent with a bigger molecular size diffuses slower than with a smaller molecule (Franssen *et al.*, 2004).

5.2.2.4 Cellulose-derivative carriers

Methylcellulose (MC), hydroxypropylcellulose (HPC), hydroxypropyl methylcellulose (HPMC), carboxymethylcellulose (CMC), cellulose acetate (CA), and ethylcellulose (EC) are derivatives of cellulose commonly used for coating. Their hydrophilicity can be ranked from highest to lowest in the order of CMC > HPMC > MC > HPC > CA (Drumel and Lindsay, 1976). In an attempt to control the release rate, Gemili and co-workers (2010) prepared a cellulose acetate film using a dry-phase inversion technique with different

conditions in order to have films with various amounts of porosity and degrees of asymmetry. A highly macroporous film prepared using a low concentration of CA showed high diffusion coefficients of L-ascobic acid and L-tyrosine whereas denser film with small pores prepared from a higher concentration of CA showed a slower release. Fabrication of multilayer EC/HPMC/EC films has been proposed by the Guiga research group (Guiga *et al*., 2010). Their films exhibit prolonged release of nisin for up to 20 h with a desorption coefficient of 10^{-5} m/s compared to a 3 h release at 10^{-3} m/s derived from a two-layer EC/HPMC film.

5.2.2.5 Other carriers

Alginate films for sorbate release, formed by ionic gelation using Ca^{2+}, were found to have decreased swelling ability with increasing Ca^{2+} concentration (Zactiti and Kieckbusch, 2006). This was found to correlate with the case of a collagen film in which the release rate decreased when the concentration of glutaraldehyde cross-linking was increased (Ho *et al*., 2001). Nisin immobilized on activated alginate film was also found to be protected from degradation and to be released but with less activity (Millette *et al*., 2007). Tunç and Duman (2011) demonstrated a different release rate of carvacrol, an antimicrobial extract from spices from MC/montmorillonite. Montmorillonite clay dispersed in the MC delayed the diffusion of carvacrol thus providing a slower release compared to MC film alone. The blending of multicoating materials to improve barrier properties and control the release pattern have been reported as different ratios of starches, proteins, chitosan, cellulose derivatives, and other carbohydrates are formulated (Li *et al*., 2006; Vásconez *et al*., 2009).

5.2.3 Multifunctional surface-coating materials

Multifunctional surfaces can be found in nature, for example plant cuticles. Cuticles are protective, hydrophobic, waxy coverings produced by the epidermal cells of plant organs. Cuticles serve as a transpiration barrier, as self-cleaning, anti-adhesive, signaling, protective layers, and control the surface temperature of the plant (Bargel *et al*., 2006). The main structural components of plant cuticles are cutin impregnated with wax.

Such innovative surfaces of food and food packaging could be achieved through surface engineering and material technology to develop novel coatings. For example, chitosan, a cationic polysaccharide derived from chitin, provides a multifunctional property to a surface of coated food and food packaging. Chitosan could theoretically be used as a preservative for coating fruit. The chitosan coating both exhibits antimicrobial activity and induces defense responses of coated fruit such as chitinase, glucanase, phenylalanine ammonia-lyase, and phytoalexin (Zhao, 2005). Chitosan polymers could also function as permeability barriers for oxygen and water to decrease respiration, transpiration and delay ripening.

Another example is a photocatalytic coating of titanium dioxide on food packaging. Photocatalytic coatings provide a multifunctional active packaging that is more economical than one functional active packaging coated with traditional materials such as an ethylene absorbent or antimicrobial agent. The multifunctional actions of titanium dioxide-coated oriented polypropylene film have reported to block UV and visible light, and function as an ethylene scavenger, odor-removal agent, and antimicrobial agent (Chawengkijwanich and Hayata, 2008a, 2008b; Chawengkijwanich *et al*., 2008; Zhou *et al*., 2008). The self-cleaning, superhydrophilic, and antifogging efficiency of titanium dioxide photocatalytic coating

(Fujishima *et al.*, 1999) makes it a promising alternative material for surface treatment of food contact materials.

5.2.4 Nutraceutical coatings

The application of nutraceutical coatings to fruits, vegetables, and other foodstuffs has recently increased due to increased consumer demand for vitality and good health. This trend indicates a preference for "incorporation" of nutraceuticals into an edible coating formulation to enhance the nutritional value of some fruits and vegetables after harvest (Rojas-Graü *et al.*, 2009).

Calcium gluconate is a calcium salt of gluconic acid which is used in the treatment of calcium deficiencies. The research group of Hernández-Muñoz *et al.* (2006, 2008) and Han *et al.* (2004) demonstrated an increase in calcium content in strawberries and raspberries when an incorporated calcium gluconate–chitosan coating was applied.

Vitamin E is a fat-soluble antioxidant that stops the production of reactive oxygen species when fat undergoes oxidation. The α-, β-, γ-, and δ-tocopherols all have vitamin E activity. Development of an edible film and coating carrying calcium and vitamin E has been reported several times in the literature. Chitosan-based coatings containing high levels of calcium or vitamin E were found to increase their levels significantly in fresh and frozen strawberries and red raspberries (Han *et al.*, 2004). However, the coating appeared to reduce a glossiness in strawberries, which later developed into a waxy and white appearance on the surface, causing a decrease in the product's consumer acceptance.

Ascorbic acid is a sugar acid with antioxidant properties with the most commonly known form being vitamin C. Tapia and co-workers (2008) reported that the addition of ascorbic acid to alginate- and gellan-based edible coatings helped to preserve the natural ascorbic acid content in fresh-cut papaya, thus maintaining its nutritional quality throughout storage.

Probiotics are live microorganisms thought to have various health benefits for humans. Lactic acid bacteria and bifidobacteria are the most common types of probiotic. The addition of probiotics to obtain functional edible coatings has gradually increased within recent years. Tapia's research group (Tapia *et al.*, 2007) developed the alginate or gellan film-forming solution containing viable bifidobacteria for probiotic coatings on fresh-cut apple and papaya. Their results demonstrated that these coatings could carry and support viable probiotics on fresh-cut fruit. Higher than 10^6 colony-forming units (CFU)/g *Bifidobacterium lactis* were maintained for 10 days during refrigerated storage of both papaya and apple pieces.

5.3 PROGRESS IN COATING METHODOLOGY

To date, direct coating of active substances onto food surfaces by means of dipping, spraying, brushing, or fluidizing is still active as it is simple and effective for certain coating applications; for example, in fruit, vegetables, nuts, and meat products. Food surfactants listed in the FDA Code of Federal Regulations Title 21 (21 CFR; www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm) are selectively used to increase adherence of active substances to the coated food surface. The use of substances or extracts derived from natural sources is a continuing preference of food manufacturers and consumers. Grapefruit seed extract alone or in combination with chitosan was found to effectively reduce fungal rot in table grapes with delayed browning and dehydration (Xu *et al.*, 2007). Treatment of frankfurter sausages with liquid smoke extracts showed an inhibitory effect to *Listeria*

monocytogenes for up to 10 weeks when sufficient dipping time was used (Gedela *et al*., 2007). In addition, cinnamon bark extract for use against *Escherichia coli* and *Listeria innocua* (Muthuswamy *et al*., 2008) and hop extracts against *Listeria* and *Clostridium* (Seman *et al*., 2004; Durham, 2008) were reported as coating solutions.

The direct-coating approach has been overtaken by more effective coating technologies in order to overcome limitations of conventional direct coating. As active substances can migrate freely after being directly coated onto the food surface, they rapidly diffuse from the surface, where they are intended to be, further into the food. Moreover, application of an active substance in its free form may lead to a dilution of the substance, a non-specific binding to food components such as fats, proteins, and carbohydrates, thus causing neutralization, and degradation by enzymes, adverse pH, and temperature changes (Karel and Langer, 1988; Ferreira *et al*., 2007; Chollet *et al*., 2008). In order to overcome these limitations without using a higher dose of active substance, several types of food-grade coating materials are applied into the coating solution. In 1966 the FDA CFR described materials with generally-recognized-as-safe ("GRAS") status that are used for coating in the FDA 21 CFR Parts 181, 182, 184, and 186, including lipids (such as oils and waxes), proteins from plant and animal sources (such as zein, soy protein, and collagen), carbohydrates (such as cellulose, pectins, gums, chitosan, and starch), and resins (such as shellac and rosin). After the coating process, a thin film of material on the food surface helps maintain an effective concentration of active substance at the surface of the food, where cross-contamination with microorganisms can occur. At the same time, the film itself acts as a barrier to limit the accessibility of nutrients to the cross-contaminating microorganisms.

Selective use of coating materials provides different barrier properties to the final product; hydrophilic-based materials including polysaccharides and proteins are a good barrier against gas permeation whereas lipid- or hydrophobic-based materials offer protection against moisture. In order to confine permeation of both gases and moisture at the same time, a combination of hydrophilic- and hydrophobic-based materials is applied by means of emulsion coating. Oil-in-water (O/W) coating provides a thinner coating film compared to water-in-oil (W/O) coating. O/W coating is additionally claimed to retard the growth of molds since its water phase is exposed to air, thus creating an unfavorable aerobic environment for molds (Thomson, 1987). Conventional emulsions with microscale droplets has been generally used to coat fruit and vegetables (Avena-Bustillos *et al*., 1994; Hagenmaier and Baker, 1994). Recently, advances have allowed large microscale droplets to be ruptured down to nanoscale droplets by the use of extreme shearing forces through ultrasonic and microfluidic processes. The technology of nano-emulsions may provide better coating properties as it offers unique characteristics compared to microscale emulsions, including near optical transparency, higher stability, strong elasticity, effective mass transfer, and yet further properties still to be investigated (Mason *et al*., 2006; Weiss *et al*., 2006). As an alternative to emulsion coating, hydrophilic- and hydrophobic-based materials can be combined through the use of lamination, in which two or more layers of coating materials are physically or chemically bound to the food surface, layer by layer. The lamination coating can also be innovatively prepared into extremely thin layers ranging between 1 and 100 nm per layer. The deposition or adsorption of coating materials via dipping or spraying typically occurs through electrostatic interaction between opposite charges of materials or additives (McClements *et al*., 2005). With this approach, active agents can also be added into the system for additional functionalized coating (Tarver, 2006).

Parameters influencing the final quality of coated products can be: (i) *surface characteristics of food* such as roughness, wettability, functional group, and electrostatic charge;

(ii) *coating solution properties* such as pH, temperature, viscosity, and surface tension as well as functional group and electrostatic charge of active substance present in coating solution; and (iii) *dipping or spraying process parameters* including pre-treatment of food surface, dipping time, speed of withdrawal, draining time, drying technique, and flow rate, droplet size, and distance to the pneumatic nozzle in the case of spraying (Schoff, 1992; Park, 1999; Cisneros-Zevallos and Krochta, 2003; Skurtys *et al*., 2010). In the dipping process, viscosity of coating is optimized to be high enough to overcome gravity but low enough to facilitate capillarity-driven leveling (Peressini *et al*., 2003). Spreadability of coating on the food surface relies on close values of surface tension between coating and food surface (Tzoumaki *et al*., 2009). The higher hydrophobicity of whey protein isolate compared to soy protein isolate, CMC, and wheat gluten gave greater protection against *Salmonella* spp. as determined by a dye-penetration assay in eggs (Xie *et al*., 2002). For spray coating, pneumatic pressure applied to the nozzle head is optimized to be high enough to obtain tiny droplet size. However, mechanical shear stress from high pneumatic pressure applied to the nozzle head during spraying causes irreversible structural damage and changes in the barrier properties of coatings made from polysaccharide-lipid materials (Bravin *et al*., 2006).

Electrostatic interaction can be used to enhance the powder coating process. Positive or negative charges are optionally generated in the coating powder prior to spraying onto food surface. Coating efficiency is improved with the application of charge that is identical to the initial charge of the powder, and the use of small less-aggregated powder, with a higher surface area, is available for charge deposition (Bailey, 1998; Sumawi and Barringer, 2005). The technique reduces tremendously the consumption of active agents and coating materials, with a more uniform distribution of coating powder over the target surface. Binding between the charged coating and the surface occurs through electrostatic interaction, hence providing a stronger bond than surface or friction attraction in typical coating methodologies. The electrostatic coating system is commercially available for a wide range applications such as snacks, confectionary, bakeries, cheeses, and meats.

Among coating materials, chitosan coating appears to have multifunctional properties including: (i) film-forming ability giving clear, flexible, and durable film; (ii) acting as a barrier to permeation of gases and moisture, thereby lowering the respiration rate of fruit and vegetables, reducing weight loss and discoloration; (iii) an antimicrobial action against bacteria, yeasts, and molds; and (iv) as an inhibitor delaying enzymatic activity in fresh produce (Allan and Hadwiger, 1979; Standford, 1989; Rhoades and Rastall, 2000; Jiang and Li, 2001; Jeon *et al*., 2002; Eissa, 2007; Vásconez *et al*., 2009).

Complementing properties of non-multifunctional coating materials or intensifying properties of multifunctional coating materials are accomplished through the combined use of coating materials and active agents. A number of reports include the use of antibrowning agents with carrageenan and whey protein concentrate (Lee *et al*., 2003); acid or salt preservatives with MC or alginate (Olivas *et al*., 2003; Mitrakas *et al*., 2008); lactoperoxidase or *p*-aminobenzoic acid or sorbic acid with whey protein isolate (Cagri *et al*., 2006; Seacheol *et al*., 2006); natural antimicrobials or extracts with alginate or chitosan or CMC or casein (Ponce *et al*., 2008; Raybaudi-Massilia *et al*., 2008); lauric, malic, or lactic acids or nisin with soy-based protein (Dawson *et al*., 2002; Eswaranandam *et al*., 2006); green tea powder with pectin (Kang *et al*., 2007); and oleic acid with chitosan (Vargas *et al*., 2006).

Beyond the "passive" functional properties derived from the combined use of active agents with coating material, coating material also acts as a carrier providing an "active" property in controlled release of active agents during the extended period of handling and storage of food products (Stading, 2003). The release kinetics of an active substance rely on the

characteristics of food, the active substance molecule, and the coating material, such as pH, temperature, viscosity, pressure, electrostatic interaction, ionic osmosis, and structural changes of coating induced by the presence of active substance (Masaro and Zhu, 1999; Pérez-Pérez *et al*., 2006; Petersson *et al*., 2007). Flores and co-workers (2007a, 2007b) demonstrated the different release kinetics of potassium sorbate from starch-glycerol film as affected by the gelatinization and drying steps of film preparation and the pH of aqueous media. Release characteristics of nisin-incorporated starch film were studied at different concentrations of nisin and different pH release conditions, and the impact on *L. innocua* inhibition monitored (Sanjurjo *et al*., 2006). The obtained results demonstrated that a gradual release of nisin from the film can inhibit growth of the bacteria more effectively than direct use of nisin. Mastromatteo and co-workers (2009) revealed the slow release of thymol when multilayer films and higher fiber contents were applied to zein film.

Existing coating processes on packaging materials to produce a functional coating roughly fall into three conventional techniques: spraying, printing, and casting. These processes can feasibly be scaled-up to a production line at reasonable cost. The active compounds can be included in coatings in either an aqueous or solvent base and are placed directly on the surface of coated packaging materials. Meanwhile, vacuum plasma coatings for deposition of active materials, which can be made from inorganic metal oxide (MeOx) or metal (e.g. Ag), are complex and costly to produce.

Although multiple processes are available to create functional surface coating, printing process and printable inks with active compounds have the advantages of flexibility, simplicity of use with existing process equipment, and lower cost (Scottland, 2006). Printable solutions simplify packaging operations compared to other processes. Innovation in active materials based in printable solutions is a key tool for the development of applications related to food packaging with the aim of extending the life of perishable foods. Such ink as well as specific printing methods would be critical in delivering high-performance active packaging.

Additionally, recent emphasis and interest in the development of coating technology have been focused on composite coatings. For example, a team of researchers in the European Union has started to develop the combination of nano- and enzyme technology for barrier coating on food packaging. Enzymes such as glucose oxidase, which has a potential for use as an oxygen absorber, are immobilized on nanoparticles and then embedded in water-borne dispersing coatings.

5.4 FUTURE TRENDS IN COATING TECHNOLOGY

The deterioration of coated fresh produce occurs through mass transfer phenomena. Predictions of mass transfer using mathematical modeling and manipulation of coating at the micro-, nano-, or molecular scales are expected to lead to improvements in coating effectiveness and reduce the amount of required active substances. Furthermore, development will allow for tunable and controllable release kinetics of active substances over the period of a food's shelf life. Apart from fast progress in coating technologies, supreme coating performance, and some novel scientific breakthroughs, development has been hampered due to missing knowledge about the actual mass transfer phenomenon. The migration rate of active substances in coatings is crucial for determining exactly the right concentration needed for specific preservation purposes. Guillard and co-workers (2009) made a prediction based on a distribution profile of sorbic acid on the surface/food interface based on validated equations created in the study. This work allowed for the use of less (100s-fold less sorbic

acid was reported) preservatives in edible films while still providing an effective kill concentration for the model microorganism. Such mathematical modeling is beneficial to the use of novel materials in coatings such as extracts and nanomaterials when fewer accessible data can be obtained from experimentation. The migration kinetics of these materials from non-edible films to foods is significant in the evaluation of toxicity and determination of safety levels for consumption (de Azeredo, 2009; Lee, 2010).

Along with increased market demand for fresh and minimally processed fruits and vegetables, edible coatings with unique functionality will certainly become more important in the future. Future needs in coatings for fruits and vegetables include: (i) a design of coating material and formulation with appropriate permeability for the purpose of preserving food; (ii) incorporation of active ingredients and nutrients into coatings to enhance food quality and safety; (iii) improvement of moisture-barrier properties of hydrophilic coating materials for high-moisture foods; (iv) improvement of coating coverage, adhesion, and durability on the food surface especially on a wet surface; (v) investigation into sensory quality and consumer acceptance of coated products; and (vi) seeking of more feasible coating application systems to scale-up coating operations to the industrial scale.

On the other hand, using nanotechnology such as nanoencapsulation and nanolaminate systems provides a new generation of edible coating technology. Nanoencapsulation of bioactive compounds with edible coatings helps to control their release and protect them from the environment (López-Rubio *et al.*, 2006). The use of nanolaminate layer-by-layer (LBL) coating offers promise for the formation of multinanolayer structures on coated foods (Weiss *et al.*, 2006). Coating foods with nanolaminates involves either dipping or spraying a series of solutions containing target substances to food surface layer by layer (McClements *et al.*, 2005). The LBL electrodeposition technique could be used to coat highly hydrophilic food systems such as fresh-cut fruit and vegetables including further vitamins and antimicrobial agents (Vargas *et al.*, 2008).

It is worth noting that the future trend for coating of food packaging and food contact material includes: (i) hygienic coating of food-processing plant and interiors; (ii) environmentally compatible bio-based coating materials; (iii) a nanocomposite coating formulation and process to reduce oxygen levels and control release of flavorings and preservatives; and (iv) development of surfaces which are able to respond to an external stimulus and able to react automatically. For example, Slaghek (2008) reported on the development of a delivery system using natural polymers such as polysaccharides and proteins as a capsule to entrap active ingredients such as biocides against specific microorganisms. The microorganisms recognize the outside of the capsule as a source of food. Subsequently the microorganisms will excrete enzymes which hydrolyze the biopolymers, resulting in release of the biocide. Furthermore, printed quality indicators are a distinct need as labels for food packages to monitor product quality throughout the supply chain.

5.5 CONCLUSIONS

From traditional coatings mimicking the natural self-coating of fruit and vegetables to active or functionalized coating, it is anticipated that more effective and controllable coatings are on their way to being discovered and utilized. A number of innovative coating prototypes are in the pipeline, making use of newly proposed natural extracts or innovative hygienic photocatalytic agents as active agents, combined or modified coating materials for controllable release of active agents, and coating techniques in which interacted parameters are controlled

at the molecular or nanoscale. For safety reasons, supporting data on migration of active agents and coating materials, their degradation, reaction products, and their toxicological properties are required as an effective dose of active agent must be at non-toxic exposure levels complying with relevant regulation (Restuccia *et al*., 2010). Evaluation of migration thereof can be carried out by conventional migration tests in foods or simulation conditions according to food properties or using validated mathematical modeling (Castle, 2007; Guillard *et al*., 2009; Restuccia *et al*., 2010). Today, micro-nanotechnology in active coating can be effectively combined with existing coating techniques (e.g. spraying, dipping, printing) to reduce the permeation of gases, generate surface functionality, and control the release of active ingredients, including automatically responding to external stimuli to monitor the storage life of foods.

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Part II Novel Decontamination Techniques

6 Biological Materials and Food-Drying Innovations

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Abstract: Parallel with the worldwide increase in technology and the cultural habits of societies, changes occur in eating habits as well. In addition to this, agricultural products go through many different process stages from harvesting to reaching the consumer and are transported long distances to enable widespread trade. In accordance with these advancements, some contemporary and new applications are being put into use in agriculture and food production processes. However, research into the efficiency of the energy used for drying, drying kinetics, timing, environmental effects and the quality of the dried product has been started and is still in progress. As a result of this research, several new drying methods such as microwave, radio frequency drying, infrared drying and Refractance Window™ drying, along with new dryers, have been developed and some of these have been commercialized. Others are in the prototype stage or the research stage.

Keywords: drying kinetics; infrared drying; microwave drying; new drying methods; radio frequency drying; Refractance Window™ drying

6.1 INTRODUCTION

Even though drying of food and agricultural products is known to be the oldest method of preservation and thought to be a simple method, actually it is fairly complex (from the point of view of heat and mass transfer, chemical reactions, changes in quality and dependence on material and drying medium) and development continues in terms of drying method and dryer design.

Drying is one of the most effective methods of prolonging the shelf life of food and agricultural products. In addition to the dried product having a decreased weight and volume, drying also significantly decreases the storage and transport costs since the dried product does not require cooling. In many countries (especially in countries that have enough hours of sunshine), drying is done in the open air under the sun and is completed by different processes based on the fruit or vegetable to be dried (3–12 days). This classic method brings with it some basic disadvantages. Among these disadvantages the non-uniformity of the drying of products laid out onto the drying area, the need for large areas for drying, the difficulty in controlling these large areas, the long drying time, high labor costs, undesirable climate effects, and the fact that both the area and the products cannot be protected from environmental pollution may be listed, and, in addition to these, since sunlight has different wavelengths the chemical structure, color, and hygiene of the dried good may be affected

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(Öztekin *et al*., 1999; Ertekin and Yaldız, 2004; Doymaz, 2007). Artificial drying methods have been started to be developed due to these undesired results. Convective hot-air drying and dryers have been developed and have become traditional after playing an important role in industrial drying. The main reason for the slowness of the hot-air drying process is the low heat-transfer characteristics of food and agricultural products and the main problems of convective hot-air drying methods are enzymatic browning, weak drying characteristics, and loss of food value (Hebbar *et al*., 2004).

Parallel to the change in lifestyles throughout the world, there have been changes in eating habits and this change still continues. Especially the changes in ready-to-serve food habits increase the demand for dried food and agricultural products that make up the ingredients used in the preparation of such foods. Depending on this increase there is a serious ongoing competition in agricultural products and food-drying markets throughout the world.

In the processing of biological metarials drying is seen as an important process which is very quality-sensitive. In industrial applications drying is a process stage that requires extensive energy consumption (Ratti and Mujumdar, 2006), which is why new drying systems need to be developed. Likewise, new dryers need to be designed, the usage of new energy sources needs to be researched, energy inputs must be decreased, and methods that do not pollute the environment should be sought.

Water activity, glass transition temperature, drying mechanism and theories, and chemical and physical changes should be recognized as key elements for any food-drying operation, and absolute distinctions can't be drawn in the selection of drying method and dryer type: there is no universal dryer that can dry all types of product (Vega-Mercado, 2001). Developments or innovations in drying technology and dryer design occur in two ways. Innovations may be revolutionary or evolutionary. Mujumdar (2004) and Mujumdar and Huang (2007) offered a checklist for innovations replacement of existing products, operations, or processes in drying technology. This list includes new product or process not made or invented heretofore, higher capacities than current technology permits, better quality and quality control than currently feasible, reduced environmental impact, safer operation, better efficiency (resulting in lower cost), and lower cost (overall; i.e., lower investment and running costs). And, they stated, innovations may include one or more of the checklist items.

Vega-Mercado *et al.* (2001) go through historical development of drying technology that evolved from the simple use of solar energy to current technology, and divided methods into four groups or generations in the development of drying technology: cabinet- and bed-type dryers in the first generation, spray and drum dryers for drying of slurries and purees in the second generation, freeze and osmotic drying in the third generation, and high-vacuum, fluidization, Refractance Window™ drying technologies and use of electromagnetic energy (microwaves, radio frequency, infrared radiation, etc.) in the fourth generation.

Developments in dryers (spray, fluidized bed, spouted bed, superheated stream, heat pump, etc.), which use heated air to improve energy efficiency and quality and to eliminate overdrying, have been discussed by various researchers (Mujumdar, 2004; Chua and Chou, 2005; Mujumdar and Huang, 2007; Raghavan and Orsat, 2007). Over the past few decades, Refractance Window drying and use of electromagnetic energy for drying of food and agricultural products have attracted intensive attention as potential drying technologies with rapid, more uniform heating, better energy efficiency, and high-quality final products. In this chapter new drying methods and technologies, which have been used recently in the drying of food and agricultural products – that is, microwave, radio frequency, infrared, and Refractance Window drying types – either in research or on a commercial scale, will be discussed.

6.2 MICROWAVE DRYING

Microwaves are defined as electromagnetic radiation with a microwave frequency between 300 and 3000 MHz and a wavelength between 1 mm and 1 m. Microwaves can be transmitted, reflected, or absorbed by the material they contact (Scaman and Durance, 2005). Electromagnetic energy absorbed by the product generates heat inside the product; the polar molecules of water are used in the generation of heat. Since microwaves affect the product as a whole, creating volumetric heating. When an alternating electromagnetic field is applied, it transforms into heat depending on the change in the direction of the electric field due to the vibration of molecular dipoles and ionic species (Strumillo and Kudra, 1986). The amount of energy absorbed by the product is analyzed by the loss factor (dielectric loss) in the material. If the product has higher moisture content, the loss is greater.

Microwaves are also used as an energy source in the drying of food and agricultural products similar to their usage as a source of heat energy in thermal processes such as heating (Karabulut and Baykal, 2002), sterilization and pasteurization (Wang *et al.*, 2003), and thawing (Taher and Farid, 2001) applied to food and agricultural products. Heat generated inside the product by microwave radiation creates a vapor pressure inside the product and slowly pumps its moisture towards the surface (Turner and Jolly, 1991). With this pumping process moisture is pushed to the surface and case hardening that may occur is thus prevented. As a result of this the drying rate and product quality increase (Chua and Chou, 2005). Since the movement of water inside the product occurs from the interior towards the exterior, it brings some advantages in the drying of food and agricultural products, the qualities of which are negatively affected due to shrinkage and case hardening. This microwave radition can be thought of as an alternative drying method.

Despite some limiting factors such as high initial investment cost, low energy efficiency when compared with traditional drying technologies (Chua and Chou, 2005), and the difficulty of uniform heating control (Ramaswamy and Marcotte, 2006), it has been started to be used commercially in the food industry. This is due to its advantages, such as fast heating rate, short drying time, and advanced product quality, and has made progress. Examples of studies include asparagus (Nindo *et al.*, 2003), carrot and garlic (Baysal *et al.*, 2003), parsley (Soysal *et al.*, 2006), peach (Wang and Sheng, 2006), nettle leaves (Alibas, 2007), onion slices (Abbasi and Azari, 2009) and laurel berry (Erdem *et al.*, 2009).

When the results from microwave drying of food and agricultural products are compared with other drying methods, its primary advantages and disadvantages can be listed as follows. The advantages are:

- short drying time;
- improved heat and mass transfer;
- high drying rate;
- development of the moisture gradient without the increase of surface temperature;
- improved product quality;
- can be used in both continuous and batch processes;
- operation cost.

The disadvantages are:

- it is difficult to control the product temperature;
- irregular heating, probable textural defects;

- ecologically unfriendly due to the effects of microwaves on humans;
- it has to be operated by qualified people and microwave radiation has to be continuously controlled.

The drying of foods and agricultural products by microwaves has important effects on operation time and when compared with other drying methods it is seen that a much shorter drying time and higher drying rate are attained. Even though the increase of microwave power or microwave power intensity shortens drying time, it increases the energy consumption required for drying (Wang and Sheng, 2006; Alibas, 2007; Erdem *et al*., 2009). The increase of microwave power intensity in order to decrease energy consumption and shorten the drying time brings with it some quality problems (Varith *et al*., 2007). It has been stated that the change in microwave power affects color and rehydration capacity in carrots (Sumnu *et al*., 2005) and color properties in nettle leaves (Alibas, 2007). When microwave power increases above 500 W in a dryer with small drying gaps, arcing take place (Cohen and Yang, 1995). Since there is very low water content during the last stages of the drying process, material temperature may easily increase to a level that can cause scorching (Zhang *et al*., 2006). Also, high temperatures along the edges and corners of the product may result in negative effects such as overheating, development of off-flavors, and possible scorching (Clark, 1996; Nijhuis *et al*., 1998).

The use of convective hot-air drying, freeze drying, spouted-bed drying, infrared drying, and vacuum drying in combination with microwave drying to eliminate their negative properties (product quality, drying time, energy consumption, operational costs, etc.) is one of the most important advancements of recent years. A summary of these advancements can be seen in Table 6.1.

6.3 RADIO FREQUENCY DRYING

Radio frequency drying is used in the food industry for drying and for energy preheating, precooking, sterilization, tempering, postbaking, baking, and humidity-control processes (Nijhuis *et al*., 1998; Vega-Mercado *et al*., 2001; Chua and Chou, 2005; Marra *et al*., 2009). Radio frequency drying falls into the fourth generation category of drying technologies, along with microwave and Refractance Window drying processes (Vega-Mercado *et al*., 2001), and has similar properties to microwave drying. However, it uses a frequency range between 3 and 300 MHz (Ramaswamy and Marcotte, 2006). It uses electromagnetic energy to heat the product to be dried. When a dielectric product enters a high-frequency electric field, and an alternating electrical field is applied, one phenomenon that occurs is the movement of positive ions in the material towards negative regions of the electric field and the movement of negative ions towards positive regions of the field (Buffler, 1993). Thus, high-frequency heating is obtained via the electrical resistance created by the movement of the dissociated ions and by dipole heating (Ramaswamy and Marcotte, 2006). Continuous heating takes place inside the product. Radio frequency heating takes place inside the product and since it affects all of the product mass concurrently, wet material is heated volumetrically (Marshall and Metaxas, 1998). Therefore, radio frequency drying has the advantages of volumetric drying. Since the water inside the product moves in gas form throughout the product rather than by capillary action, the migration of solids is prevented. As a result negative effects associated with conventional drying methods, such as warping, surface discoloration, and cracking, are eliminated (Chua and Chou, 2005).

Table 6.1 Selected studies on microwave combination drying.

References	Products	Combination mode	Conclusion
Prabhanjan *et al.* (1995)	Carrot	Microwave/hot-air drying	Microwave radiation provided a 25–90% decrease in drying time and carrots had better rehydration characteristics when dried at a lower power level.
Funebo and Ohlsson (1998)	Apple and mushroom	Microwave/hot-air drying	The drying time of the products substantially decreased. Better product quality was obtained.
Lin *et al.* (1998)	Carrot	Microwave/vacuum drying	Vacuum/microwave-dried carrot slices had higher rehydration potential, higher α-carotene and vitamin C contents, lower density, and softer texture than those prepared by air drying.
Sharma and Prasad (2001)	Garlic cloves	Microwave/hot-air drying	Combined microwave/hot-air drying resulted in a reduction in the drying time to an extent of 80–90% in comparison to conventional hot-air drying and a superior-quality final product.
Nindo *et al.* (2003)	Asparagus	Microwave/spouted-bed drying	Microwave and spouted-bed drying produced asparagus particles with good rehydration and color characteristics, and was the fastest among the methods (tray, spouted-bed, combined microwave and spouted-bed, Refractance Window drying, and freeze drying) where heated air was used.
Kwok *et al.* (2004)	Saskatoon berries	Microwave/vacuum drying	The best retention of antioxidant functional properties was obtained with freeze drying followed by vacuum microwave drying, combination (air and microwave vacuum) drying, and air drying (75 °C).
Sunjka *et al.* (2004)	Cranberries	Microwave/vacuum drying, microwave/convective drying	Microwave/vacuum drying exhibited enhanced characteristics when compared to microwave/convective drying. Drying performance results (defined as mass of evaporated water per unit of supplied energy) showed that microwave/vacuum drying is more energy efficient than microwave/convective drying. Tasting panel results exhibited slight preference in all parameters for microwave/convective dried samples.
Sumnu *et al.* (2005)	Carrot	Microwave/halogen lamp	A decrease in drying time close to 98% when compared with hot-air drying. A high rehydration ratio and lower color deterioration in dried carrots.
Varith *et al.* (2007)	Peeled longan	Microwave/hot-air drying	Microwave/hot-air drying greatly reduced drying time and specific energy consumption as compared to conventional hot-air treatment up to 64.3 and 48.2%, respectively.
Abbasi and Azari (2009)	Onion slices	Microwave/freeze drying, microwave/vacuum/freeze drying	Microwave/vacuum/freeze drying is practically a rapid, simple, efficient, and economic. The quality properties of slices dried by the dryer are also completely comparable and competitive with commercial freeze drier with over 96% saving on process time and enormous amount of energy and capital investment.
Yousefey *et al.* (2009)	Papaya	Osmotic pretreatment/microwave/hot-air drying	Osmotic cabinet microwave drying produced the shortest drying time, and improved the process considerably.

Even though radio frequency is similar to microwave, it has some differences in terms of its effect. These different effects may be listed as: radio frequency generally heats more uniformly than microwave; radio frequency energy is less expensive per kilowatt than microwave; and radio frequency generator capacities range from a kilowatt to hundreds of kilowatts (Vega-Mercado *et al.*, 2001). Below 100 MHz, the penetration of radio frequencies is so large that, in comparison with microwaves, it is never a limiting factor (Ramaswamy and Marcotte, 2006). Radio frequency applicators can be extremely simple (Nijhuis *et al.*, 1998).

The most important negative results that might occur during the drying of agricultural products and food materials in this manner are structural defects and quality losses (color, nutritional value, aroma, taste, etc.) in the dried product. That is why drying to a high quality and at a high rate is very important. Nijhuis *et al.* (1998) have compared the different properties (drying characteristics, operation and investment cost, and quality) of freeze drying (high quality retention), microwave (high process operations), and radio frequency systems used for drying of fruits and vegetables. They have stated that radio frequency and microwave systems have similar properties.

6.4 INFRARED DRYING

The origin of infrared is thermal and its application directly results in a thermal effect. Infrared radiation is part of the electromagnetic spectrum. This technology has been known for a long time, but it has been continuously advancing with new materials. A large number of emitters are manufactured for various industrial applications (Ramaswamy and Marcotte, 2006). In infrared drying, heat is transferred from the hot surface of a heating element to the material. Infrared radiation is defined as electromagnetic radiation with a wavelength between 0.76 and 400 μm. Radiation at this frequency width occurs with the thermal vibration of the molecules and contrary to this the absorption of infrared radiation increases thermal vibration. (Strumillo and Kudra, 1986). It is classified in three different categories as near infrared (0.76–2 μm), middle infrared (2–4 μm), and far infrared (4–100 μm) (Fasina, 2003; Jain and Pathare, 2004). Infrared radiation can be produced by using two energy sources, either natural gas (similar to the transfer of burning energy to radiant energy with a 40–46% efficiency) or electrical energy (similar to electrically charging infrared lamps in order to produce infrared radiation with a 78–85% efficiency) (Fasina, 2003; Ramaswamy and Marcotte, 2006).

Due to some of its advantages, it has been stated by various researchers that infrared heat sources can be used for drying (Strumillo and Kudra, 1986; Lewis, 1996; Fasina, 2003; Hebbar *et al.*, 2004; Krishnamuty *et al.*, 2008). Studies in recent years have stated that infrared drying technology has a higher energy efficiency, shorter drying time, and better final product quality than convective drying methods (Fasina, 2003; Hebbar *et al.*, 2004; Kumar *et al.*, 2005; Wang and Sheng, 2006). Infrared drying can be used in rural areas with small capacities and in low-cost applications (Hebbar *et al.*, 2004), it has some advantages such as low investment cost, easy assembly, high heating and drying rate, uniform product temperature during drying, simple hardware, high process control, and clean working environment (Sandu, 1986; Sakai and Hanzawa, 1994; Chua and Chou, 2003). Infrared drying has been taken into consideration in recent years and many experimental studies have been made related to the drying of various agricultural and food materials, these are: onion (Mongpraneet *et al.*, 2002), carrot and garlic (Baysal *et al.*, 2003), apple slices (Nowak and Lewicki, 2004),

paddy (Das *et al*., 2004a, 2004b), carrot and potato (Hebbar *et al*., 2004), onion slices (Jain and Pathare, 2004), paddy (Amaratunga *et al*., 2005), pear, carrot, sweet corn (Pan *et al*., 2005), sweet potato (Lin *et al*., 2005), apple slices (Togrul, 2005), onion slices (Sharma *et al*., 2005a, 2005b, Kumar *et al*., 2005, 2006, Pathare and Sharma, 2006), peach (Wang and Sheng, 2006), banana slices (Nimmol *et al*., 2007), sweet potato slices (Lin *et al*., 2007), red pepper (Nasiroglu and Kocabiyik, 2009), and carrot (Kocabiyik and Tezer, 2009).

The main commercial applications of radiant energy are drying of foods with low moisture content (for example breadcrumbs, cocoa, flour, grains, malt, pasta products, and tea) and for baking or roasting ovens. The product passes through a tunnel on a conveyor under a radiant heater. Even so, since the penetration depth is limited, it is not commonly used as a single source of energy in the drying of large food pieces. Also, radiant energy is used in vacuum belts and cabin dryers, and to speed up freeze dryers (Fellows, 2007).

Infrared/vacuum drying (Mongpraneet *et al*., 2002), infrared/convective drying (Mongpraneet *et al*., 2002; Nimmol *et al*., 2007), infrared/freeze drying (Lin *et al*., 2005; Lin *et al*., 2007), infrared/low-pressure superheating (Nimmol *et al*., 2007) combinations have been successfully used in the drying of various agricultural products and positive results have been obtained.

Hebbar *et al.* (2004) determined that among the hot-air, infrared, and combined (infrared and hot-air) drying of potato and carrot, the drying time for both products decreased by 48% in the combined system when compared with the hot-air system, that the hot-air system had the longest drying time, and that the drying time of infrared system was between the two other systems. Similarly, Kumar *et al.* (2005) have stated that the infrared/hot-air combination results in a shorter drying time for onion slices.

In combined infrared and hot-air drying, as is the case for drying time, infrared power or infrared radiation intensity affects drying curve and drying rate (Mongpraneet *et al*., 2002; Sharma *et al*., 2005a; Wang and Sheng, 2006; Das *et al*., 2009; Kocabiyik and Tezer, 2009).

In convective hot-air drying, as a result of the low energy efficiency and long drying time, the total energy consumption required for drying increases. Sakai and Hanzawa (1994) stated that development of a continuous drying apparatus equipped with far-infrared heaters, near-infrared heaters, and hot-air blast can reduce the economic costs, drying time, and operating temperature. However, vegetable size should be restricted to no more than 5 mm in thickness to improve drying efficiency.

As a result of obtaining a short drying time by using infrared and convective hot-air combination, an advantage is obtained in the energy consumption required for the removal of a unit of water from the product. Hebbar *et al.* (2004) have stated that the specific energy consumption as a result of the combined infrared and convective hot-air drying of potato and carrot is 63% less in comparison to convective hot-air drying and that the heat utilization efficiency of the dryer is 38.5% for potato and 38.9% for carrot. In parallel to this, when compared with the hot-air drying of barley by using hot air at a temperature of 70°C, the total energy requirement of the combined infrared hot-air drying system decreased by about 245% (Afzal and Abe, 1999).

The conservation of quality of dried food and agricultural products is one of the most important indicators of the success of the drying system. Generally, the drying time is fairly long in convective hot-air drying and since the falling rate period constitutes most of the total drying time, it has an important effect on quality. One of the methods to shorten drying time and increase quality is providing heat by infrared radiation (Timoumi *et al*., 2007). For example, infrared radiation increased the quality of dried spices (Paakkonen *et al*., 1999). Discoloration can be properly prevented by significantly decreasing drying time via

intermittent infrared drying method (Chua *et al*., 2004). In corns dried with hot-air, infrared, and combined infrared and hot-air systems, total color change, protein, and caratenoid and phenolic acid content were similar for all drying systems (Yılmaz, 2008). Afzal and Abe (1999) showed that drying made by combined infrared and hot air instead of only by infrared or hot air had a higher synergic effect and that at the same time increases energy saving while improving quality. Kumar *et al.* (2005) showed that onion slices dried by a combined infrared and hot-air method had better rehydration capacity in comparison to onion slices dried by using only hot-air or infrared methods. In the same study, the combined drying system also gave good results for quality parameters of dried onion slices such as pyruvic acid content, total color change, and browning index.

The increase of infrared radiation intensity results in increased drying rate and as a result drying time decreases. However, since higher radiation intensities increase the temperature of the product, they may negatively affect product quality (Abe and Afzal, 1997). When a biological material is subject to infrared radiation for a long period of time swelling occurs and ultimately fracturing of the material occurs (Jones, 1992). Fasina *et al.* (1997, 1999, 2001) have stated that infrared heating changes the physical, mechanical, chemical, and functional properties of barley and that the heating of legume seeds up to 140°C by infrared radiation results in surface cracks. In order to prevent such negative affects, infrared heating can be used intermittently during infrared and hot-air system drying. Providing infrared heat intermittently has the potential to decrease the energy required, increase the quality of heat-sensitive product, and increase the surface temperature (Afzal, 2003). The intermittent infrared and continuous convective heating of thick porous material has resulted in good surface quality and energy efficiency (Dostie *et al*., 1989). The following results have been obtained as a result of a study performed by Afzal (2003) in which barley was dried by intermittent far infrared: the total drying time increased in comparison to the simultaneous use of infrared heating; however, the total energy input was less and higher infrared heat intensity with intermittent application was the most energy-efficient method. Discoloration was observed at higher intensity under continuous heating; however, during intermittent operation no breakage or discoloration occurred, and duration of exposure in intermittent heating had an effect on germination.

6.5 REFRACTANCE WINDOW™ DRYING

In a study by Vega-Mercado *et al.* (2001) on the historical development of drying technologies, one of the drying methods among the fourth generation was the Refractance Window™ drying system. Refractance Window drying is a new drying method developed by MCD Technologies Inc. (Tacoma, WA, USA). This method has been identified with improved product quality, short drying time, high thermal efficiency, and low energy cost (Abonyi *et al*., 2002; Nindo *et al*., 2006).

Circulating water at a temperature of 95–97°C is used to carry thermal energy to the material to be dried. The product to be dried is laid on a special transparent plastic conveyor moving on hot water (in direct contact with water). All three heat-transfer methods (convection, conduction, and radiation) are effective in this drying technology. The thermal energy of hot water initially reaches the conveyor by three heat-transfer methods. Then, the thermal energy of water that reaches the conveyor is transmitted by radiation and conduction from the conveyor to the product. It has been suggested that the water-bearing products on the transparent plastic conveyor created a "window" allowing infrared radiation passing from hot water through the conveyor to reach the food. As the product with high moisture content loses

its moisture through evaporation, the window slowly closes, leaving mainly conducted heat to finish the drying process and help prevent quality degradation. The thermal energy transfer from the product to ambient air is primarily by convection and through evaporation cooling of the food material. The conveyor then moves the dried product (almost dry) to reduce product temperature to the cooling section, before being of scrapped off the conveyor (Abonyi *et al*., 2002; Nindo and Tang, 2007; Topuz *et al*., 2009).

The unused heat in the circulation water is reused. The product on the conveyor is rapidly dried. The product stays on the conveyor for a period of about 3–5 minutes; the drying time of this method is very short when compared with dryers such as tray, tunnel (taking hours), and freezing (more than 12 h) (Tang and Yang, 2004). Refractance Window drying is similar to drum drying in that the product is dried in a thin layer on a heated surface, except that heated surface is at a much lower temperature (70–85°C as compared with 120–150°C) (Abonyi *et al*., 2002). The actual product temperature is usually below 70°C (Nindo and Tang, 2007).

Refractance Window drying is used to process products such as pureed vegetables, algae, fruit, liquid eggs (Tang and Yang, 2004); a board variety of fruits, vegetables, meat, fish, poultry, flavorings, herbs and spices, dairy, cereals, starches and grains, beverages (Vega-Mercado *et al*., 2001); avocado fruits, herbal extracts, and nutritional supplements for human use, and nutritional supplements for shrimp farming (Nindo and Tang, 2007).

The main problem in the drying of agricultural products and food materials is the potential loss of product quality. Refractance Window drying has positive effects on the preservation of product quality. Abonyi *et al.* (2002) found that ascorbic acid retention of strawberry purees (94%) after Refractance Window drying was comparable to 93.6% in freeze drying. In the same study, the color of Refractance Window-dried carrot purees was comparable to fresh puree, and the color retention of dried strawberry purees with Refractance Window drying was comparable to freeze-dried purees. Freeze-dried and Refractance Window-dried paprika showed better reflected color characteristics, and there was no significant difference in browning index between freeze-dried and Refractance Window – dried samples (Topuz *et al*., 2009).

The advantages of using Refractance Window technology in the drying of food and agricultural products can be summarized as below (Tang and Yang, 2004; Nindo and Tang, 2007):

- short drying time (3–5 min);
- relatively inexpensive;
- high thermal efficiency (77–52%);
- the energy cost to operate it is less than half that of freeze drying;
- improved dried product quality.

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7 Atmospheric Freeze Drying

Shek Mohammod Atiqure Rahman and Arun S. Mujumdar

Abstract: Vacuum freeze drying has been the benchmark technology for making high-quality dried products. This technique, however, is expensive due its high fixed and operating costs. Interest in atmospheric freeze drying (AFD) has increased in recent years as it yields high product quality while reducing the net energy consumption in comparison with vacuum freeze drying. Efforts have been made to overcome some of the limitations of AFD. For example, the required energy for sublimation can be supplied using different modes of heat transfer to enhance the heat transfer rate without compromising quality. A vortex chiller can be used as a suitable alternative to achieve required characteristics of the carrier gas inside the drying chamber. In addition to enhancing the dehydration rate during AFD by improving the external mass transfer coefficient, mixing of the frozen product with adsorbent particles in a vibro-fluidized bed is also examined as an attractive technique.

Keywords: adsorbent; atmospheric freeze drying; math model; multimode heat input; product quality; vacuum freeze drying; vibrating bed dryer; vortex tube

7.1 INTRODUCTION

With an increasing trend towards new and better-quality dried products and the added new requirements of resource conservation and sustainability, the development of appropriate drying technologies has become increasingly important. Removal of water can be achieved in a number of ways. Proper dehydration of highly heat-sensitive products requires controlled supply of heat for vaporization or sublimation and concurrent removal of the water vapor generated from the drying chamber. The structure and properties of the material to be dried are not altered much by the freeze-drying process. This technique has been widely applied industrially to drying of biological materials, pharmaceuticals, and foodstuffs (Liapis *et al*., 1996; Liapis and Bruttini, 2007). The main disadvantages of this technique are its high fixed and operational costs. The atmospheric freeze drying (AFD) technique is an outcome of efforts over the last two decades to respond to this challenge. It combines the advantages of both freeze drying (high product quality) and convective drying (low process costs), along with some of their limitations as well. In AFD, water or a solvent is removed from the wet solid or solution as a vapor by sublimation from the frozen materials in a vacuum chamber. The result is a highly porous, nonshrunken structure in the dried product that facilitates rapid and almost complete rehydration when water is added to the substance at a later time. Water removal occurs without going through the liquid phase, thus avoiding the of surface tension which can lead to collapse of the structure. Drying in a frozen solid state at low temperature

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and very low pressure maintains porous structure and retains the quality of the product. Reconstitution is faster because of the high porosity. Some of the key advantages of AFD are listed below:

- no or little thermal damage (for most products);
- good retention of volatile flavors;
- good vitamin retention;
- rapid product rehydration;
- low final moisture;
- no product shrinkage;
- good retention of biological activity (with use of cryoprotectants);
- it is a continuous process with a long drying time, meaning higher productivity and lower operating costs;
- significant reduction in energy costs due to the absence of a vacuum chamber and ancillary equipment;
- decreased energy consumption and drying time;
- product degradation is minimized by using an inert gas drying environment;
- high heat-transfer coefficient: about 20–40 times greater than in vacuum drying.

However, there are some limitations as well:

- long drying time: lower diffusivity of water vapor with increasing pressure in the chamber;
- bulky system: requires more space;
- two mechanical agents are required: not economical from the energy point of view;
- it also takes time to set up, dehumidify, and cool the drying chamber;
- the structure of the frozen product is difficult to control.

7.2 BASIC PRINCIPLES

Meryman (1959) first showed in the laboratory the possibility of freeze-drying products at atmospheric pressure. In a series of experiments he showed that the diffusion of water vapor from the drying boundary through the dried shell occurs by vapor pressure gradient, rather than by the absolute pressure on the system. Hence, it is possible to freeze-dry at atmospheric pressure. This process is accomplished by circulating cold dry air below -6 to -10°C over a frozen product to improve the heat and mass transfer from the frozen material at near atmospheric pressure. The basic principle of the process is to maintain partial water vapor pressure around the product below the triple point of water so that water removal occurs only by sublimation. The only absolute requirement is that the partial pressure of water vapor in the drying medium be kept low enough to provide a mass transfer driving force for water-vapor transfer from the frozen sample. The temperature must be low enough to maintain frozen integrity of product and should maximize the vapor pressure of products. Heldman and Hohner (1974) analyzed the kinetics of freeze drying under AFD, which is presented schematically in Figure 7.1.

The operating conditions are listed here:

- freezing temperature: -20 to -40°C;
- freeze-drying temperature: -5 to -8°C;

- operating pressure: 4.56 to 0.1 mmHg;
- fluidized bed temperature: 0 to 7.6°C;
- fluidization velocity: 10 to 50 cm/s.

A comparison between vacuum freeze drying and AFD is shown in Table 7.1.

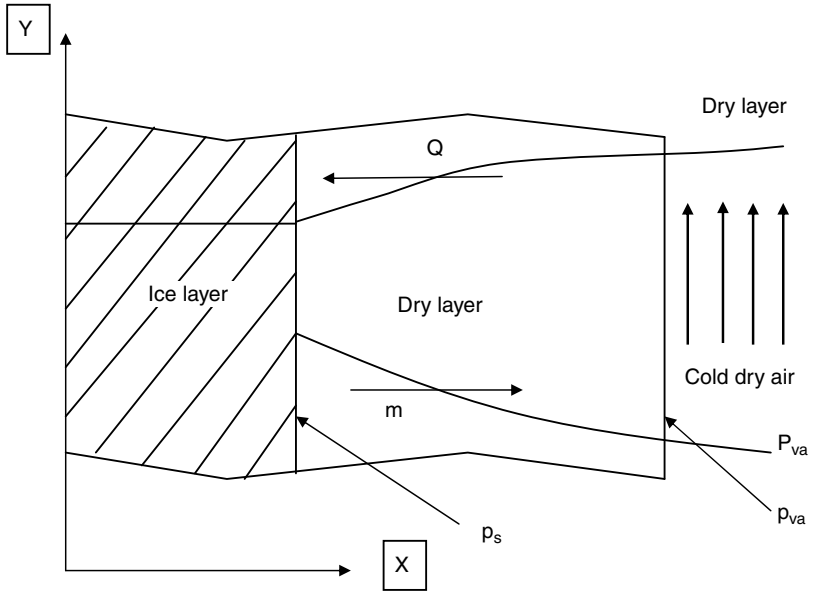

Figure 7.1 Schematic diagram of AFD. Q, Heat transfer; m, mass transfer; P_{va}, partial pressure gradient; p_s, partial pressure of water vapor around the product surface; p_{va}, partial pressure of water in the drying chamber.

Table 7.1 Comparison between VFD and AFD.

Parameter	Atmospheric freeze drying	Vacuum freeze drying
Operating pressure in drying chamber, mbar	Nearly atmospheric pressure	Rough vacuum: 10^{13} mbar–1 mbar Medium vacuum: 1 mbar–10^{-3} mbar High vacuum: 10^{-3} mbar–10^{-7} mbar At least 0.066 mbar
Operating temperature in drying chamber, °C	Just below 0°C (−6 to −8°C)	−40 to −80°C
Partial pressure of water vapor	4.56–0.1 mmHg	Vacuum
Freezing temperature, °C	−30 to −40°C	−50 to −80°C
Drying rate (potato slice)	0.09 kg/(kg h)	0.15 kg/(kg h) (1 Pa) and 0.42 kg/(kg h) (300 Pa)
Mass transfer coefficient, K	1.0 kg h^{-1} m^{-2} $torr^{-1}$	0.8 kg h^{-1} m^{-2} $torr^{-1}$
Heat transfer coefficient, h	402 kcal h^{-1} m^{-2} °C^{-1}	16.2 kcal h^{-1} m^{-2} °C^{-1}
Air velocity	5–50 cm/s	No air
Energy requirement (to sublime 1 kg of water contained in a product of 3 kg water/kg dry matter)	5690 kJ/kg approximate	7330 kJ/kg approximate

7.3 TYPES OF ATMOSPHERIC FREEZE DRYER AND APPLICATION

7.3.1 Fluid-bed freeze drying

Conventional fluid beds can be used for AFD. The heat and mass transfer is very good in a fluid-bed dryer where the drying agent passes each unit of the product. As a consequence, most of the experimental work done within AFD has been done using fluid-bed dryers. However, drawbacks of the fluid-bed dryer are that it is difficult to maintain the structure of the product and attrition of the dried product. A schematic diagram of a fluidized bed dryer is shown in Figure 7.2.

7.3.2 Tunnel freeze drying

To avoid size reduction caused by mechanical cracking, tunnel drying is a suitable alternative to fluid-bed drying. The heat and mass transfer rates, however, are not as good as in fluid-bed

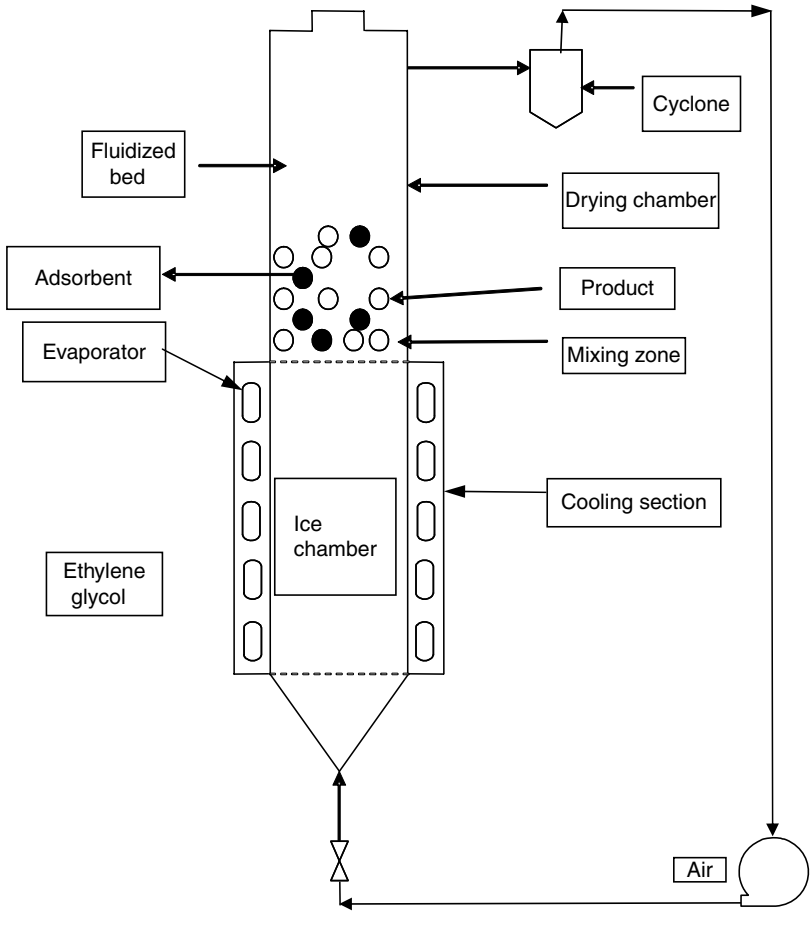

Figure 7.2 Schematic diagram of a fluidized bed dryer.

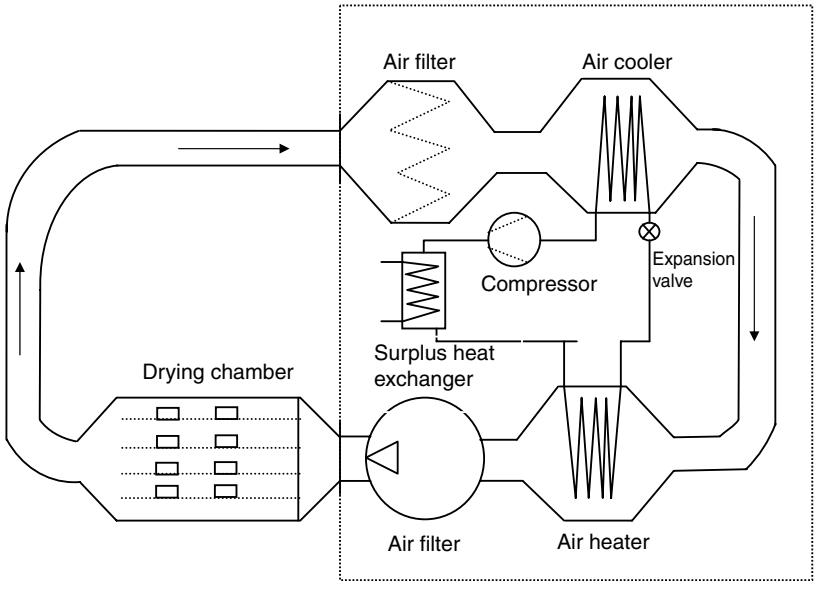

Figure 7.3 Schematic diagram of a tunnel freeze dryer.

drying. Nevertheless, many experiments on AFD of food products in a tunnel dryer with a heat-pump system have been carried out at the Norwegian University of Science and Technology (NTNU). The results from these experiments are very promising, regarding both product quality and energy consumption (Grande, 2003; Sjøvold, 2005; Claussen *et al.*, 2006). A schematic diagram of a tunnel dryer is shown in Figure 7.3.

Fish has been the main food product for product development in tunnel dryers in Norway, but other products, like apple and turnip cabbage, have also been dried (Claussen *et al.*, 2007).

Non-food products have been dried in an atmospheric tunnel freeze dryer. Rat liver has been dried in an attempt to preserve DNA and RNA for use in biobanks (Sjøvold, 2005). AFD was not found to cause any negative effects in the RNA.

7.3.3 Atmospheric spray-freeze drying

In the pharmaceutical industry, spray-freeze drying is a good alternative for producing free-flowing powder, with high surface area, a porous end product, and good instant characteristics. Enhanced solubility and a uniform, ultrafine particle size are the main advantages of this technology. Although expensive the high value of pharmaceutical products makes the process feasible.

The atmospheric spray-freeze drying process was developed for manufacturing powder as shown in Figure 7.4. The goal is to reduce drying time and operate at atmospheric conditions. In contrast with other known atmospheric spray-freeze drying technologies, this technology combines spray-freezing, deposition/collection, and convective flow drying into one step employing co-current gas flow to spray-freeze the solution, conveying the frozen powder towards an exit filter and *in situ* drying. This overcomes the difficulty of fluidizing and elutriating a cohesive frozen powder from a substrate.

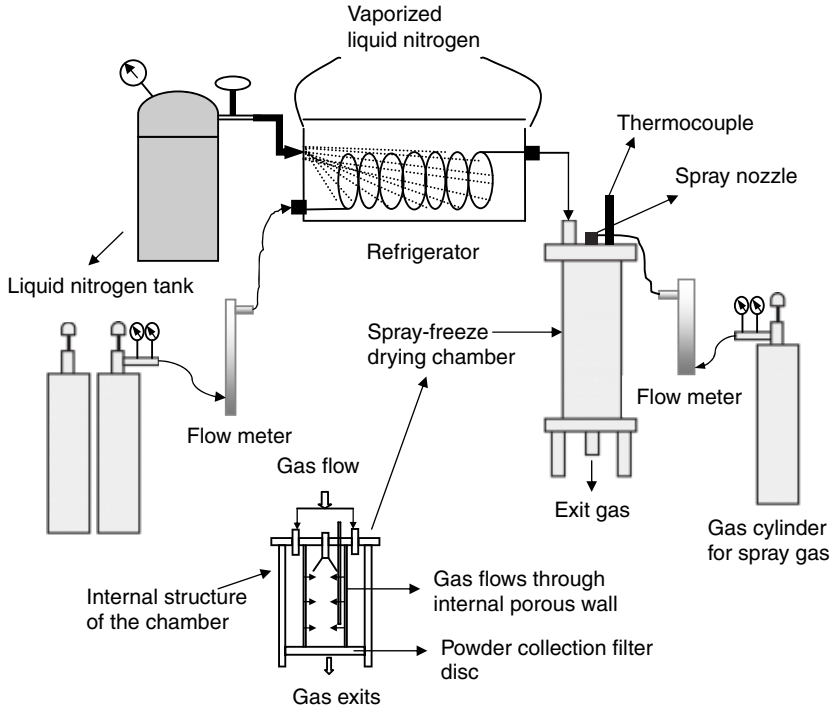

Figure 7.4 Schematic diagram of an atmospheric spray-freeze dryer.

The main advantages of spray-freeze drying into liquid (SFL)/AFD prepared drug powders are: high product yields, cryogenic temperature (which promotes drug stability), and that the drugs are molecularly dispersed in drug/excipient matrices (Evans *et al.*, 2005). Rogers *et al.* (2003) investigated it as an industrial process for producing micronized SFL powders with aqueous dissolution. The SFL powders were compared with vacuum freeze-dried SFL powders.

In later work, Leuenberger *et al.* (2006) examined the properties of a spray-freeze drying system, specifically spray-freeze drying into gases over a fluidized bed. The project encountered problems due to the electrostatic properties in the product, and the particles were not dried in a fluid bed, but were entrained in the air stream and subsequently the dried powder attracted to the matrix of a filter.

7.3.4 Heat-pump technology

There has been a significant growth in the potential market for heat-pump dryers, aided by the impact of new designs under development, or recently introduced to the market. In a review paper by Chua *et al.* (2002) new developments in heat-pump dryers are classified, together with discussion of the potential for incorporating advanced heat-pump cycles for drying applications. A schematic diagram of a heat-pump AFD system is shown is Figure 7.5.

A heat-pump dryer is basically a convective dryer, where heat is transferred by the convection of air. It is more suitable for drying solid products than liquid or semi-solid products. Thus, its application at this stage is limited mainly to solid products (Perera and

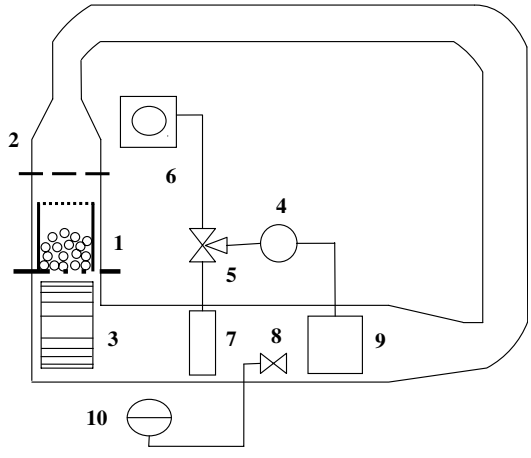

Figure 7.5 Sketch of the heat-pump dryer: 1, transparent drying chamber; 2, external drying chamber; 3, blower; 4, compressor; 5, three-way valve; 6, external condenser; 7, internal condenser; 8, throttling valve; 9, evaporator; 10, liquid receiver.

Rahman, 1997). Because the construction of continuous drying systems may require high engineering, modeling, and design costs, the benefits need to be evaluated on the basis of cost rather than on energy efficiency alone.

The main advantages of using heat-pump technology are the energy-saving potential and the ability to control drying temperature and air humidity. This creates the possibility of a wide range of drying conditions. The increased demand for ready-to-eat products and convenient foods requires well-controlled drying conditions to obtain sufficiently high-quality food products. Alves-Filho (2002) examined dried fruits and vegetables, looking at physiochemical properties such as moisture content, matrix fractions, color, water activity, and bulk density. He concluded that the results are promising with respect to producing instant fruits and vegetables by heat-pump drying technology.

Limitations of conventional atmospheric freeze dryers include that they utilize a bulky system of a mechanical heat pump to lower temperature and a condenser to reduce humidity of the air. At least two mechanical agents are required for this operation, which does not seem economical from the energy point of view. It also takes time to set up, dehumidify, and cool the drying chamber. In addition, AFD is controlled by internal resistance heat and mass transfer due to the lower vapor diffusivity at atmospheric pressure, which makes it a slower drying process.

7.4 A NOVEL APPROACH TO AFD

To overcome the abovementioned drawback Rahman (2009) carried out an experimental study of an AFD system incorporating a vortex tube coupled with multimode heat input in a fixed-bed dryer. It was reported that a vortex tube can be used as a suitable alternative to achieve the desired conditions for the carrier gas inside the drying chamber, although its application is limited to the laboratory scale. In addition, it was demonstrated that a two-stage multimode heat input enhances the drying rate along with ensuring good product quality. However, drying rate is still comparably slower than with a traditional vacuum freeze-drying system. Therefore, it is necessary to pay more attention to enhancing drying rates in AFD.

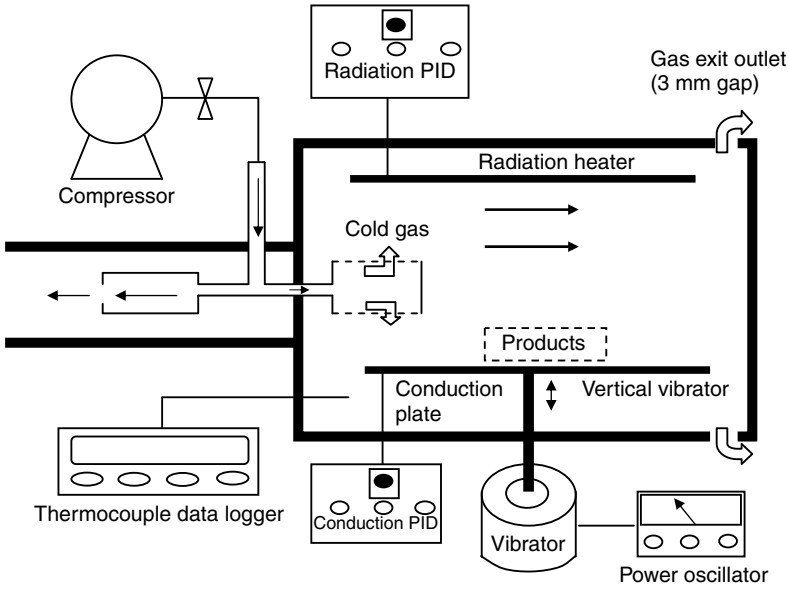

Figure 7.6 Schematic layout of the novel AFD system. PID, proportional integral derivatives controller.

The vibro-fluidized bed offers lower power needs, attrition rates, and elutriation rates than gas-fluidized beds (Gupta *et al.*, 1980; Rogelio *et al.*, 2000). Furthermore, it improves the fluidization quality of irregular and cohesive materials (Pan *et al.*, 1997; Alvarez *et al.*, 2005). In addition to enhancing the dehydration rate of AFD by improving the external mass transfer coefficient, mixing of frozen product with an adsorbent in a vibro-fluidized bed is an attractive technique. This technique presents important advantages as the adsorbent particles play a dual role as a transfer agent for both heat and mass transfer (Wolff and Gibert, 1990a). However, the drawback of the process lies in the difficulty in separation of the freeze-dried product from the adsorbent at the end of the operation.

In practice, these difficulties can be overcome either by using an adsorbent that is compatible with human consumption, or by incorporating any suitable mechanism to separate the absorbent from the dried product. The schematic diagram of the novel approach to AFD is as shown in Figure 7.6. It consists of a vibrator with variable amplitude (1–5 mm) and frequency (1–25 Hz), a screw compressor, a vortex tube cooler, a noise muffler, a ceramic radiation heater assembly, a conduction plate, an insulated dryer drum, a freezer, and an insulated dryer exhaust. Readers are referred to Rahman *et al.* (2008a, 2008b, 2008c) for details of the new system. Detailed system component specifications are listed in Table 7.2.

7.4.1 Experimental results

Injection of compressed air at room temperature circumferentially into the vortex tube at high velocity produces a vortex which spins annularly along the tube inner wall as it moves axially down the tube. Part of this air is adiabatically expanded inward to the center, according to the published explanation of the flow. Following the vortex tube, air is passed through a muffler to reduce noise and expanded suddenly into the drying chamber. As a result the temperature of the air rises somewhat inside the drying chamber, as shown in Figure 7.7. The measured temperature distribution generated by the vortex tube as well as inside the drying chamber at

Table 7.2 Component specification and characteristics of the system parameters.

Drying chamber	
Material	Acrylic sheet
Shape	Drum type
Dimension	Length 300 mm, inner diameter 200 mm, thickness 5mm
O-ring	1. Inner radius 115 mm, thickness 3 mm 2. Inner radius 125 mm, thickness 3 mm
Flange	Material: acrylic sheet, radius 145 mm, thickness 5 mm
Insulation material	Armoflax, thickness 5 mm
Tray	
Material	Aluminum sheet
Dimension	Length 300 mm, width 150 mm
Conduction heater	
Type	Silicon rubber heater
Dimension	245 mm × 147 mm
Capacity	300 W, 240 V
Radiant heater	
Type	Infrared heater
Dimension	245 mm × 60 mm
Capacity	300 W, 240 V
Vortex tube	
Model	3240
Flow rate	0.0188 m^3/s
Refrigeration capacity	706 kcal/h
Vibrator	
Type	Magnetic coil vibrator
Model	406
Capacity	1 kg
Frequency	3–30 Hz
Amplitude	5 mm (max)

inlet air pressure of 4 and 6 bar pressure (absolute) is shown in Figure 7.8. The air temperature inside the drying chamber drops from the ambient temperature to about −10°C and −3°C within 15 min of start-up of the experiment, at operating compressed air pressures of 6 and 4 bar absolute, respectively. The rate of decrease of the chamber temperature becomes slower with time and stabilizes at about −19°C and −6°C, respectively. The air temperature, immediately after the vortex tube, decreases rapidly and remains constant at −26°C and −17°C, respectively, after only about 4 min. Tangential injection of compressed air at room temperature into the vortex tube at high velocity produces a vortex, which spins annularly along the tube inner wall as it moves axially down the tube. A part of this air is adiabatically expanded inward to the center, according to the explanation of the flow in a vortex tube (Crocker *et al.*, 2003).

Figure 7.9 shows the effect of inlet air pressure on the carrier gas temperature inside the drying chamber as well as at the vortex tube exit. Initially the pressure was set at 6 bar absolute and the corresponding temperature after the vortex tube and also inside the drying chamber was found to reach about −30°C and −17°C, respectively. An inlet pressure of 4 bar was also set at an elapsed time of 14 min, which resulted in an instantaneous change in the air temperature of the cold stream to about −16°C, while the chamber air temperature reached around −11°C. Figure 7.9 shows the temperature variation at different locations inside the

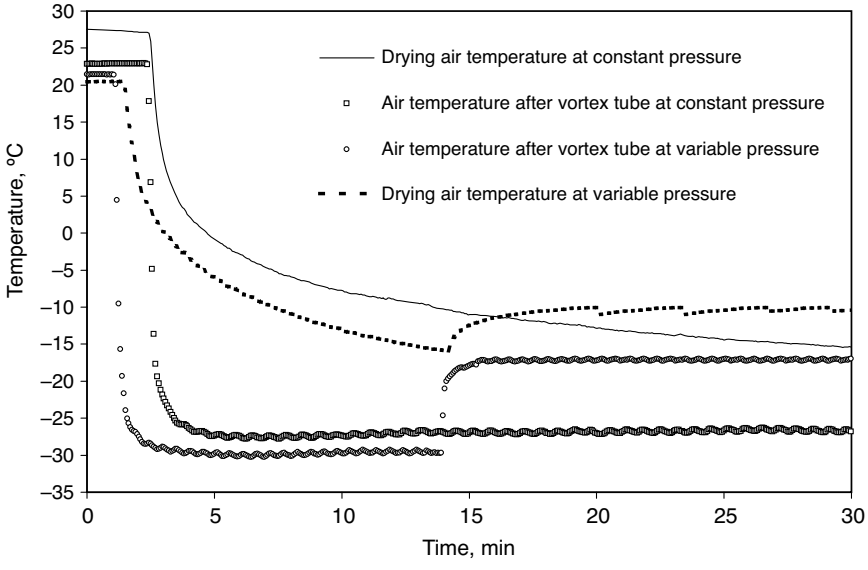

Figure 7.7 Temperature distribution inside the drying chamber at constant pressure at the inlet of the vortex tube.

drying chamber with time at a fixed operating pressure of 6 bar also. It can be seen from the figure that all points inside the chamber show a similar pattern of temperature distribution. After 40 min, temperature at all locations approaches asymptotic values between −16°C and −18°C. This indicates a relatively uniform temperature distribution at different locations within the drying chamber in the absence of heat input.

Figure 7.10 shows the product temperature of the two-stage drying process that is well below (−14°C and −9°C) the freezing point of product and maintains a temperature gradient with the drying air temperature. It ensures integrity of frozen potato during drying.

To investigate viability of the proposed AFD system (vibro-fluidized bed dryer with multimode heat input and adsorbent), a comparison was made between the proposed AFD

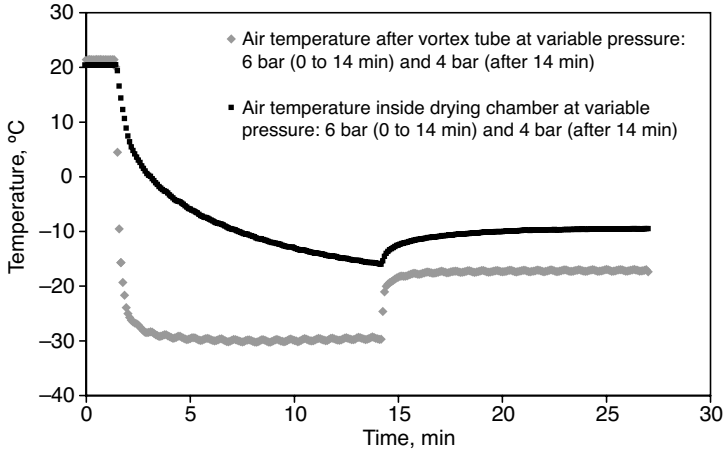

Figure 7.8 Temperature distribution inside the drying chamber at variable pressure at the inlet of the vortex tube.

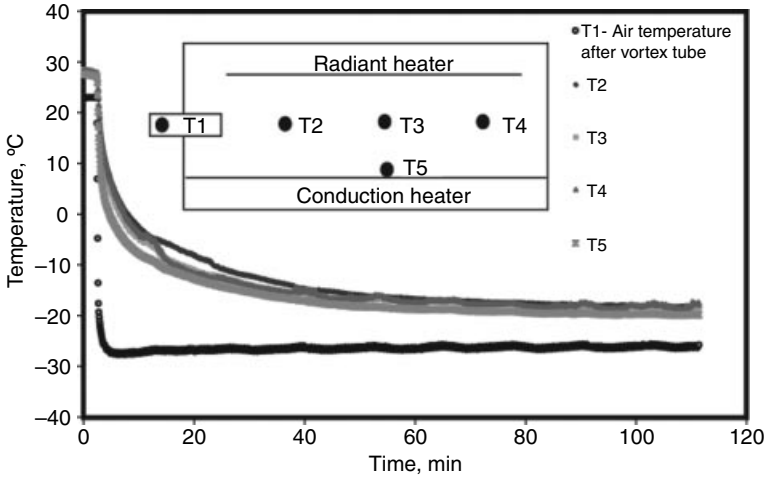

Figure 7.9 Temperature distribution inside the drying chamber with time at 6 bar absolute pressure.

process with similar sets of data available in the literature (Claussen *et al.*, 2007), for the other types of drying, i.e. of AFD using heat-pump assisted fluidized bed dryer; this is shown in Figure 7.11.

Comparison was carried out under the drying conditions for the two-stage process at $-8°C$ and $+20°C$. Cubic-shaped (5 mm) cod fish product was used for this comparison. It can be seen from Figure 7.11 that the proposed system displays better drying performance than a heat-pump based system. The final dimensionless moisture content for the heat-pump-assisted fluidized bed dryer after 8 h of drying time was about 0.38. However, for the vibro-fluidized bed dryer with multimode heat input and vibro-fluidized bed dryer with multimode heat input and adsorbent, the values were about 0.19 and 0.16, respectively, for the same drying time. Supply of the required amount of energy for sublimation through

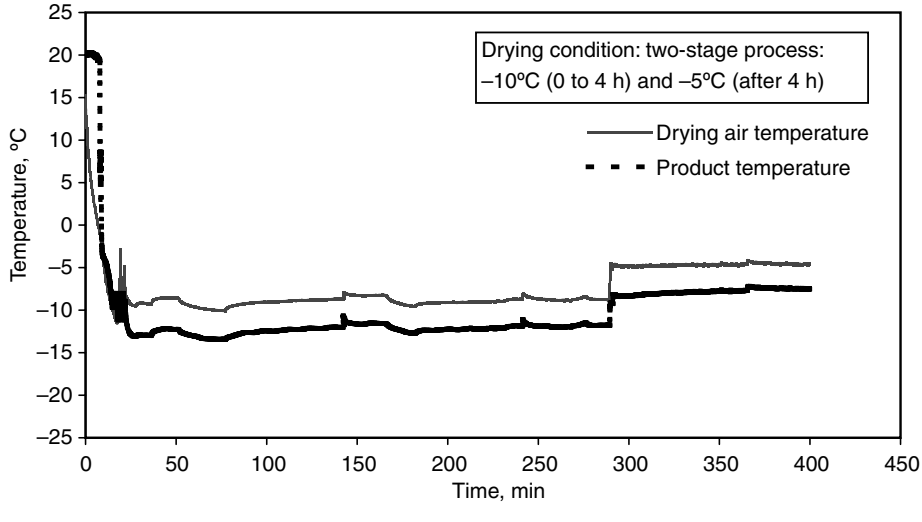

Figure 7.10 Variation of product and air temperature inside the drying chamber at different inlet pressures.

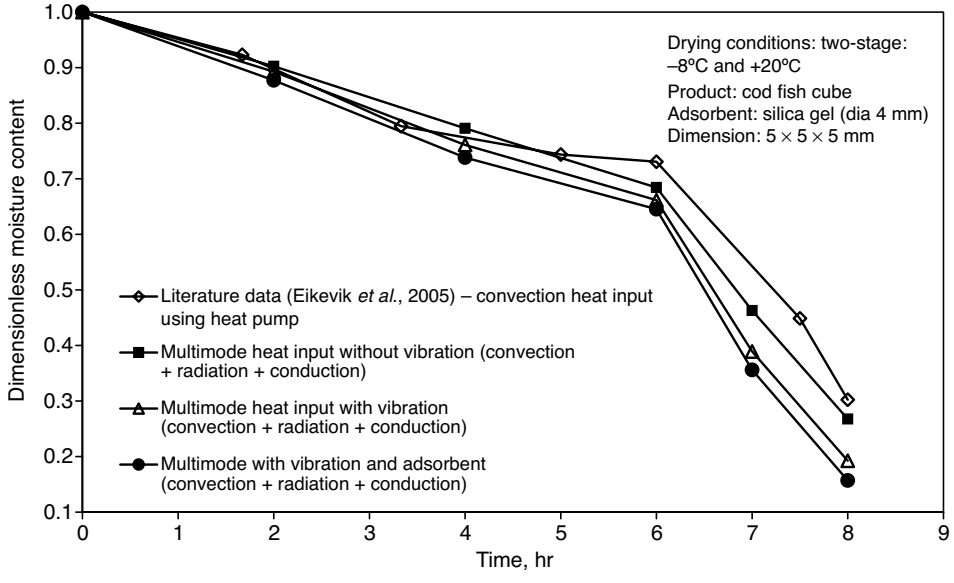

Figure 7.11 Comparison of different drying conditions on dimensionless moisture content with time.

multimode heat input (Rahman *et al.*, 2007) plays an important role in achieving this improvement. Figure 7.12 and Figure 7.13 show scanning electron microscope images of the cross-sections of potato samples subjected to vacuum freeze drying and AFD, respectively. The figures show that the AFD product gives a very similar structure to the vacuum-freeze-dried sample.

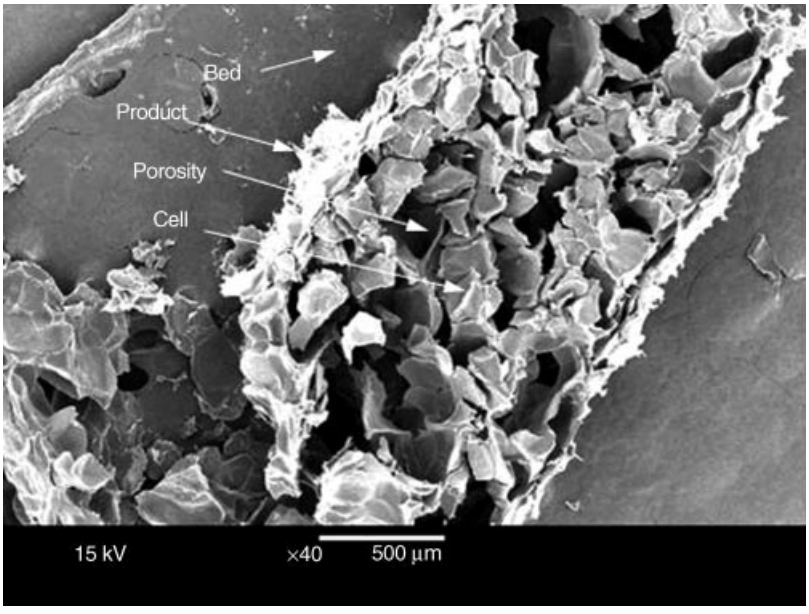

Figure 7.12 Scanning electron micrograph of cross section of dried potato under VFD (original magnification ×40, scale bar 500 µm).

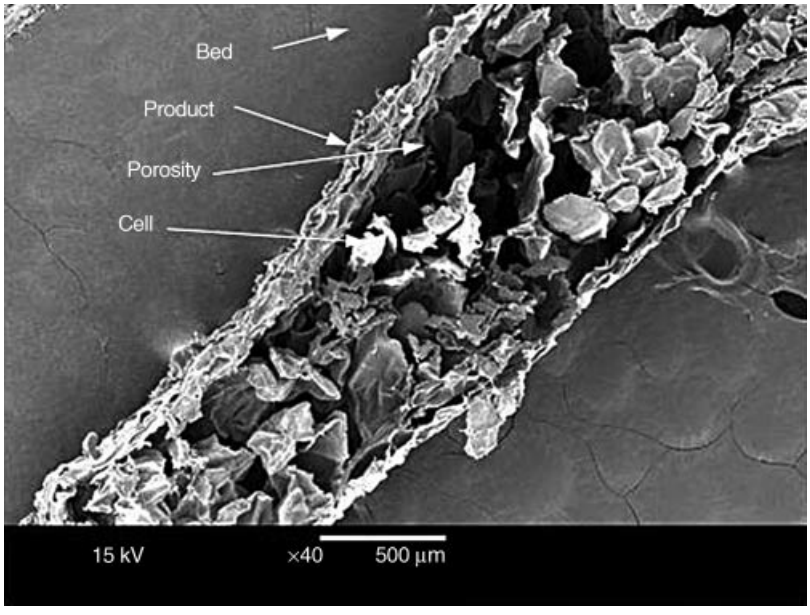

Figure 7.13 Scanning electron micrograph of cross section of dried potato under AFD (original magnification ×40, scale bar 500 µm).

The advantages of this system include the following:

- The required characteristics of the carrier gas inside the drying chamber are obtained using the vortex tube.
- Drying time is reduced by supplying the required amount of energy to provide optimal drying kinetics, as well as maintaining quality of the dried product through different modes of heat transfer.
- The vibro-fluidized bed offers lower power needs, attrition rates, and elutriation rates than gas-fluidized beds (Gupta *et al*., 1980; Rogelio *et al*., 2000). Furthermore, it improves the fluidization quality of irregular and cohesive materials (Pakowaski *et al*., 1984; Pan *et al*., 1997).
- Gentle vibration helps to keep the structure of the product intact as well as reduce the abrasion of the final dried product.
- In addition to enhancing the dehydration rate of AFD by improving the external mass-transfer coefficient, mixing of frozen product with an adsorbent in a vibro-fluidized bed is an attractive technique. This technique presents important advantages as the adsorbent particles play a dual role as a transfer agent for both heat and mass transfer (Wolff and Gibert, 1990a).

However, accompanying the above advantages are several limitations:

- The drawback of the process lies in the difficulty in separation of the freeze-dried product from the adsorbent at the end of the operation. In practice, these difficulties can be overcome either by using an adsorbent that is compatible with human consumption or by incorporating a suitable mechanism to separate the absorbent from the dried product.
- The drying rate is still lower than for vacuum freeze drying.
- Scale-up of the process is complex and yet to be attempted.

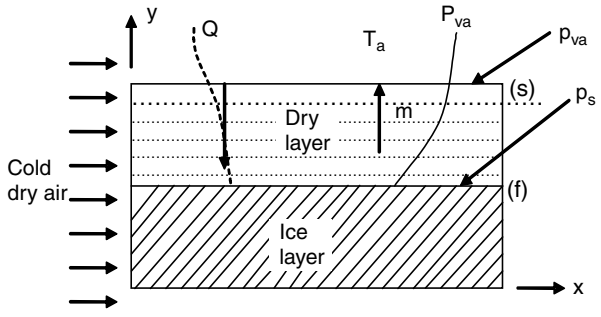

Figure 7.14 Physical model of atmospheric freeze drying. f, Interface; s, surface; Q, heat transfer; m, mass transfer; T_a, temperature gradient; P_{va}, partial pressure gradient; p_s, partial pressure of water vapor around the product surface; p_{va}, partial pressure of water in the drying chamber.

7.5 MODEL

The determination of optimal process parameters is usually obtained from experimental runs by trial and error. However, a reliable mathematical model that captures the key features of the drying process can a useful tool for design and optimization of dryers, thus decreasing the development time and associated cost. Based on the concept of a uniformly retreating ice front coupled with basic heat and mass equations as well as models of the adsorbent sorption isotherms, Wolf and Gilbert (1990b) proposed a model for AFD using a fluidized bed of particulate adsorbents (starch) of different masses. Other investigators (Tomova *et al*., 2005; Li *et al*., 2007) have reported their modeling efforts on AFD.

A mathematical model is used based on solution of the governing conservation equations of energy and mass for drying of different shapes of products subject to appropriate boundary and initial conditions. A schematic representation of the physical model of a food product is shown in Figure 7.14. Using a one-dimensional model, the ice interface (f) recedes to the center line as heat of sublimation (Q) flows from the surface (s) to the interface due to a temperature gradient (T_a) represented by the dashed curved line.

Simultaneously, water vapor flows through the dry layer in response to the water vapor pressure (P_{va}) gradient indicated by the solid curved line. The following mechanisms are considered in the model: convective heat transfer from the carrier gas to the surface of the solid mass, radiant heat transfer from the infrared radiation heater to the solid's surfaces, and conductive heat transfer within the solid mass.

7.5.1 Assumptions

- There is one-dimensional heat and mass transfer, normal to the large surfaces.
- There is equilibrium between ice and water vapor at the interface.
- Supplied energy is used to remove only ice at the sublimation front.
- The frozen region is considered to have homogeneous and uniform thermal conductivity, density, and specific heat.
- The shape of the product remains constant during the drying period considered.
- Shrinkage and deformation are negligible.

7.5.2 Governing equations

The conservation equation of energy for the dry layer is:

$$\rho_p C_P \frac{\partial T}{\partial t} = K_p \frac{\partial^2 T}{\partial x^2} - \dot{C}'' \frac{\partial T}{\partial x} \tag{7.1}$$

The conservation equation of water vapor inside the dry layer is:

$$\rho_g \frac{\partial Y}{\partial t} = \rho_g D_p \frac{\partial^2 Y}{\partial x^2} - \dot{m}'' \frac{\partial Y}{\partial x} \tag{7.2}$$

Where, $L_v < x < L$.

The boundary conditions for heat and mass transfer are:

$$h_e(T_e - T_s) = K_p \left(\frac{\partial T}{\partial x}\right)_s \tag{7.3}$$

$$h_d(Y_g - Y_s) = D_p \rho_g \left(\frac{\partial Y}{\partial x}\right)_s \tag{7.4}$$

Where effective temperature and heat transfer coefficient of the atmospheric air are:

$$T_e = (h_c T_g + h_r T_r)/(h_c + h_r) \tag{7.5}$$

$$h_r = \varepsilon\sigma(T_r + T_s)(T_r^2 + T_s^2) \tag{7.6}$$

Analytical solutions of equations (7.1) and (7.2) subject to boundary condition equations (7.3) and (7.4) are given by Jaakko and Impola (1995) as:

$$\frac{T - T_v}{T_e - T_v} = \frac{\exp[K_c(z - Z_v)] - 1}{(1 + \dot{C}''_s/h_e)\exp[K_c(1 - Z_v)] - 1} \tag{7.7}$$

$$\frac{Y - Y_v}{Y_g - Y_v} = \frac{\exp[K_d(z - Z_v)] - 1}{(1 + \dot{m}''_s/h_d)\exp[K_d(1 - Z_v)] - 1} \tag{7.8}$$

where

$$K_c = \dot{C}''_s R/K_p,\ K_d = \dot{m}''_s R/D_p \rho_g,\ z = x/L,\ Z_v = L_v/L$$

The vaporization temperature is obtained as:

$$T_v = T_e - l_v\{(1 + \dot{C}''_s/h_e)\exp[K_c(1 - Z_v)] - 1\}/c_g \tag{7.9}$$

Table 7.3 (a) Thermophysical and transport properties of subzero air (Oosthuizen and Naylor, 1999) and (b) thermodynamic and transport properties of potato and carrot.

(a) **Temperature of air**	**−11°C**	**−6°C**
Saturated vapor pressure at t_{air}	(0.000000003)*exp(0.0957*t_{air})	(0.000000003)*exp(0.0957*t_{air})
Drying air relative humidity	0.0	0.0
Diffusivity of gas	0.000019 m^2/s	0.000019 m^2/s
Total pressure	101000.0 Pa	101000.0 Pa
Density of air	1.352 kg/m^3	1.326 kg/m^3
Specific heat of moist gas	1004.713 J/kg K	1004.718 J/kg K
Viscosity of air	0.000016 kg/ms	0.000017 kg/ms
Heat conductivity of gas	0.023 W/m K	0.024 W/m K
Slip velocity of surface	2.5 m/s	2.5 m/s
Molecular mass of air	29	29

(b)	**Potato**	**Carrot**	**Reference**
Effective thermal conductivity of dry product	0.552 W/m K	0.564 W/m K	Saravacos and Maroulis (2001)
Diffusivity of product	5.2e-6 m^2/s	7.8e-6 m^2/s	Sablani *et al*. (2000)
Mean density of dry product	1.526 kg/m^3	1.253 kg/m^3	Senadeera *et al*. (2000)
Effective sp. heat of dry product	7616 J/kg K	3780 J/kg K	Oosthuizen and Naylor (1999)
Latent heat of evaporation	10^5*(2.29e-10*T_V^3–4.06e-6*T_V^2+1.9e-3*T_V+2.612)	10^5*(2.29e-10*T_V^3–4.06e-6*T_V^2+1.9e-3*T_V+2.612)	Keey (1972)

The moisture mass fraction at the receding front is given by the following expression:

$$Y_v = 1-(1-Y_g)\frac{\exp[-K_d(1-Z_v)]}{1+\dot{m}''_s/h_d} \tag{7.10}$$

The evaporation rate can be solved:

$$\dot{m}''_s = \frac{\rho_g D_g SH}{2R}\frac{\ln\left(\frac{1-Y_g}{1-Y_v}\right)}{1+(1-Z_v)\frac{ShD_g}{2D_p}} \tag{7.11}$$

Readers are referred to Keey (1972), Oosthuizen and Naylor (1999) and Mujumdar and Devahastin (2004) for the thermodynamic and transport properties of the air/water system and to Sablani *et al*. (2000) and Saravacos and Maroulis (2001) for the physical properties of different food products. The thermophysical and transport properties of the carrier gas, potato, and carrot used in the simulations are summarized in Table 7.3.

7.6 CONCLUSIONS

So far the investigation of freeze drying shows that this is a good method for drying of high-value, heat-sensitive products that cannot be dried/stabilized in any other way. It is certain that freeze drying has a future, and it is likely that this will essentially be in the fields of high-value

food, materials science, biological science, medicine, pharmaceuticals, and biotechnology. Most food products are still dried by conventional means for obvious economic reasons. In this respect AFD could represent a viable alternative. It seems AFD is a viable technology because the process produces high-quality product at a lower cost relative to vacuum freeze drying. Fluid-bed dryers can be made inexpensively, and combined with a heat-pump system; they could be competitive in the food industry and the pharmaceutical industry where vacuum freeze dryers are widely used today. Investigation revealed that with a combination of vortex tube and multimode heat input to a vibro-fluidized bed dryer AFD can be an environmentally friendly and economically favorable technology. However, this novel process has not yet been scaled up. It can be considered for small-scale production.

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8 Osmotic Dehydration: Theory, Methodologies, and Applications in Fish, Seafood, and Meat Products

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Abstract: Osmotic dehydration is an effective and viable process useful for the partial removal of water. During osmotic dehydration, cellular materials are placed in a concentrated solution of solute, and this has been widely studied as a convenient method to improve the economics of dehydration processes. The rate of diffusion of water from any material made up of such tissue depends upon various factors such as: temperature and concentration of the osmotic solution, the size and the geometry of the material, the solution-to-material mass ratio, and the level of agitation of the solution. This chapter summarizes all the methods currently applied for osmotic dehydration of foods, including advantages and disadvantages per category. Apart from the methodologies, an extensive review is carried out on the application of osmotic dehydration on fish and seafood products with emphasis on its effect on physical properties (a_w, pH, viscosity) and shelf-life prolongation.

Keywords: fish; meat products; osmotic dehydration; seafood; shelf-life prolongation

8.1 INTRODUCTION

Water is the main component of biological materials, playing a very important role in determining their appearance, structure, texture, and chemical composition (Kingduean *et al*., 2005). The drying process greatly affects the quality of food products (color, aroma, nutritional components) by both chemical and physical processes. The physical changes induced or accompanied by shrinkage affect substantially the product density, the crystallinity, and the phase behavior of the system (Bar *et al*., 2002). Prevention of undesirable changes is feasible with the addition of various sugars, amino acids, organic acids, phosphates, and functional proteins (Khan *et al*., 2003).

Drying was one of the very first food-preservation practices. Large quantities of fruits such as figs have been dried since ancient times. However, in the case of meat and fish, two other preservation methods, such as smoking or salting, which yielded a palatable product, were generally preferred. Commercial dehydration of vegetables started in the USA during the American Civil War, because of the low quality of the food products. The dehydration industry further thrived after World War II. In the beginning this industry was limited to

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production of dried foods, such as milk, soup, eggs, yeast, and powdered coffee. Nowadays, dehydration techniques focus on the application of a stream of warm air to vegetables. Protein foods are of good quality when freeze-dried. Liquid food is dehydrated usually by spraying it as fine droplets into a chamber of hot air (www.scribd.com/doc/37995229/Joseph-M-Pasag-Drying-Research).

Dehydration is the oldest method for food preservation by water removal because it retards food spoilage (Kingduean *et al*., 2005). Although microorganisms cannot grow in a water-free environment and enzymatic activity is non-existent, some kinds of deterioration still occur during the drying process, resulting in decreased water retention and protein solubility (Yaowalux *et al*., 2006). Osmotic dehydration is a viable process usually aiming at the partial or total removal of water in which cellular materials are placed in a concentrated solution of solute. This has been widely studied as a convenient method to improve the economics of dehydration processes (Corzo *et al*., 2006b). The driving force for water removal is due to the difference in osmotic pressure between the food and its surrounding solution (Corzo *et al*., 2006c). The osmotic water flow is initiated by water and solute activity gradients across a cell membrane. It is noteworthy that there is always some solute diffusion into the food (Medina-Vivanco *et al*., 2002).

Utilization of waste from the fish industry has been gaining ground over the past 10 years. Generally, these wastes have found applications mainly for the preparation of fishmeal and silage in view of their poor functional properties. Further processing techniques for seafood waste are required to turn the underutilized wastes into a more marketable, valuable, and acceptable product. Application of enzymatic hydrolysis of protein is considered a viable option because, apart from avoiding the extremes of chemical and physical treatments, it also minimizes the occurrence of undesirable reactions (Yaowalux *et al*., 2006). Addition of enzymatic protein hydrolysate to foods revealed several advantages, such as greater water-binding ability and enhanced heat stability of myofibrillar protein, better emulsifying stability, higher solubility of protein, and better nutrional quality of foods (Yaowalux *et al*., 2005).

Conversion of fishery wastes by proteolytic hydrolysis can be made into a more marketable and functional form called fish protein hydrolysate. Further, fish protein hydrolysate has the potential to meet the enhanced demand for natural protein sources, and it has been effectively used either to enrich food products or as a nutritional supplement for human consumption (Khan *et al*., 2003).

Forced ventilation drying has been commonly used as a conventional technique. A major disadvantage of forced ventilation drying is the formation of a case-hardening layer, characterized by a surface layer with an extremely low moisture content which has formed because of overdehydration, typically occurring over a continuous drying process. Complexity of the moisture-diffusion mechanism, steep distribution of moisture content, and pore structure developed in the food solid are some of the undesirable side effects of over-dehydration. A poultice-up process has conventionally been used as an empirical technique in an attempt to minimize case hardening. Poultice-up processes are regarded as reliable methods for getting good quality food (Konishi and Kobayashi, 2003).

Several drying and dehydration processes have the potential to utilize limited heating at relatively low temperatures, such as low-pressure steam to ensure that the dried material has not undergone any damage or alterations. This increased the interest in dehydration technologies with regard to using the excess heat for new industrial activities. The use of heat for drying purposes has found a wide range of products and applications but energy costs

for heating air or surface are often an important economic factor affecting drying industries (Walde *et al*., 2006).

There is an entire family of operations involving the interactions between water-containing foodstuffs and water in the surrounding medium at the prevailing temperature. Osmotic dehydration is generally used as an upstream step for the dehydration of food prior to its being subjected to further processing such as freezing, freeze drying, or air drying (Corzo and Bracho, 2005). According to Corzo and Bracho (2006b) "the rate of diffusion of water from any material made up of such tissue depends upon factors such as temperature and concentration of the osmotic solution, the size and the geometry of the material, the solution to material mass ratio, and the level of agitation of the solution."

Fick's law of diffusion has been extensively and quite successfully used to describe the moisture-diffusion process for food products. The two main parameters required in this law are sample dimensions and effective diffusion coefficient (Corzo and Bracho, 2007a). The determination of effective diffusion coefficient can be made by finding numerical or analytical solutions to experimental data, calculating the relation between the slope of theoretical diffusion curve and the slope of experimental mass-transfer ratio (Corzo and Bracho, 2007b). Several models were developed for osmotic dehydration from Fick's law of diffusion by applying equilibrium kinetics for all substances need the equilibrium kinetics data for water and solids. A model, with an exponential approach to the equilibrium value of these parameters, was proposed by Zugarramurdi and Lupin (1980) in an attempt to interpret the observed moisture and salt concentrations due to fish salting. This model applies successfully to salting procedures where the external surface of fish is exposed to concentrated brines (Corzo and Bracho, 2006a).

Corzo and Bracho (2008) also applied the probabilistic Weibull model to describe the behavior of rehydration kinetics, microbial death kinetics, pressure inactivation of bacteria, thermal resistance of bacteria, spore germination, thermal preservation, survival curves, and water-loss phenomena during osmotic dehydration. Osmotic dehydration, which is also known as "dewatering impregnation soaking," provides an adjustment of water activity (a_w) and the achievement of structural, textural, sensory, and other functional properties (Rodriguez *et al*., 2003). Final product characteristics and mass-transfer kinetics are greatly affected by uptake of solute from the solution. If these equations are applied, one has to take into account the changes in porosity and the overall shrinkage of the samples as they lose moisture. The importance of the shrinkage is two-fold: firstly it affects texture and other quality factors, and secondly its knowledge is required for mass-transfer modeling (Corzo and Bracho, 2004a). There are two common approaches for treating food shrinkage during dehydration. The first one is an empirical approach toward evaluating the changes in the boundary conditions of the heat- and mass-transfer equations or the parameters that are related to shrinkage, such as porosity and surface area. The second approach applies mechanical models to calculate the stresses and the ensuing deformations that occur within the product during dehydration (Bar *et al*., 2002).

8.1.1 Determination of physical characteristics

Texture is one of the most important textural attributes of processed foods. Texture of food products can be determined both with instrumental analysis and sensory evaluation. Instrumental analysis can be more conviently used than sensory evaluation. The determination of texture with instrumental analysis is easy to perform, simple to reproduce, and less

time-consuming (Corzo *et al.*, 2006b). Color also plays a role in appearance, processing, and acceptability of food materials. Color is perceived as a part of the total appearance, which is the visual recognition and assessment of the surface of the object. Several researchers have instrumentally investigated the color of food (Corzo *et al.*, 2006c). Among the parameters responsible for loss of color are non-ezymatic and enzymatic browning, and processing conditions such as pH, acidity, duration of storage, and temperature. Color change can be used to evaluate quality of food material during processing (Corzo *et al.*, 2006a).

The osmotic dehydration process is described by equilibrium and dynamic periods. In the dynamic period, the mass-transfer rates increase or decrease until equilibrium is reached. Equilibrium is the end of the osmotic process and its study is necessary for the modeling of the osmotic process as a unit operation and is also important for a comprehensive understanding of the mass-transfer mechanisms involved in this system (Corzo and Bracho 2004b). Moreover, knowledge of the end-point criterion can allow development of theoretical models enabling the calculation of process parameters. During the process, the solute and moisture concentrations alter, finally leading to equilibrium (Corzo and Brancho, 2006a).

Osmotic treatment of fruits, vegetables, and meats is conducted by immersion of a food sample in an aqueous solution containing at least one osmotic agent, such as a salt, sugar, phosphate, acid, or other. During immersion, treated samples can either gain or lose water; raw meat, for example, gains water (Schmidt *et al.*, 2008). The osmotic treatment of raw meat presents some particularities, because of its natural capacity to gain water under specific conditions. The osmotic treatment of raw meat with salt solutions results in salt and water transfers, either in the same direction or in counter-current, depending on the osmotic solution concentration. Thus this process can lead to food hydration or dehydration (Schmidt *et al.*, 2008).

Salting of fish is essentially an osmotic dehydration process. It involves two major mass-transfer flows: water flow out of the fish, and a simultaneous transfer of salt into the fish (Mujaffar and Sankat, 2006). Salting the fish before drying inhibits microorganism growth and retards the action of proteolytic enzymes in the fish tissue. The chemical composition of salt has an important effect on the quality of the final tissue (Zaki *et al.*, 1976). A comprehensive overview of the salting process and its optimization require the investigation of the mass-transfer change (salt uptake and water removal), as well as the application of mathematical modeling for describing and predicting the changes (Mujaffar and Sankat, 2006).

Aqueous binary solutions of sodium chloride are commonly used in osmotic dehydration or fish salting. Sodium chloride has a higher water-activity-reduction capacity and impregnates animal tissues substantially, but its capacity to improve weight loss and moisture-content reduction is restricted. On the contrary, sucrose solution has a greater capacity for reducing moisture content. Sucrose is a good dehydrating agent, because of its high molecular weight, which facilitates increased water output. An alternative might be the combination of both solutes, which may provide the advantages of each (Medina-Vivanco *et al.*, 2002).

There are several advantages of adding a solute complementary to salt, like sucrose, in the immersion solution: (i) extension of saturation limits of the solution, and (ii) the presence of the second solute provides a higher transfer potential favorable to water loss. At the same solute impregnation, salt is hindered by the presence of sucrose. This "barrier effect" of sucrose was demonstrated in meat and fish. The effect becomes more pronounced with syrup (DE21) comprising saccharides of molecular mass varying from 180 Da (glucose) to more than 1500 Da (oligo- and polysaccharides) in variable proportions (Santchurn *et al.*, 2007).

8.2 METHODS OF DRYING

Drying or dehydration involves the removal of water from the food by means of the following controlled processes:

- evaporation due to heating of the product;
- osmotic dehydration; e.g., brining of fish;
- sublimation or freeze drying; e.g., in the drying of coffee or milk.

This method of preservation is advantageous from the point of view that most flavors are retained, there is a less bulky product, and the shelf life is prolonged. However, there also disadvantages of drying related to oxidation, which results in losses of micronutrients (carotene and ascorbic acid) and minimal loss of protein due to browning reactions. The latter is usually linked with reduced consumer appeal. Further changes may be perceived in flavor and texture if drying is not properly controlled, particularly with regard to maximum temperatures.

There are several types of dryer used, like the drum dryer, cabinet dryer, tunnel dryer, rotary dryer, spray dryer, and solar dryer. The most important methods of drying are described below.

8.2.1 Sun drying/solar drying

This is an obvious alternative for a region with high natural sunlight and temperature. Although the two terms are sometimes used interchangeably, for the purpose of this chapter sun drying refers to the removal of moisture by merely exposing the food commodity in the sun. Sun drying is not suitable for the reasons of infestation by flies, growth of bacteria, and lack of proper control. Moreover, sun-dried fish tends to be easily contaminated by dirt, sand, and other impurities. The contaminated fish has short storage life, is poor quality and is often not well accepted by consumers. Artificial drying in electrical ovens is to be preferred over sun drying because it takes a short time and the product is far more acceptable for consumption (Zaki *et al*., 1976).

8.2.2 Air and contact drying under atmospheric pressure

Here the heat is transferred through the food either by heated air or heated surfaces, and the resulting water vapor is removed by means of an air current. Solar-drying, sun drying, drum and spray drying all use this technique. According to Fito *et al*. (2001) the transport rate is greatly affected by the tissue structure and composition, both defining the effective values of its transport properties (thermal properties and water-diffusion coefficient). Therefore, pretreatments may result in different drying behaviors of foods, and different final properties of the product. It was clearly shown that the effect of osmotic dehydration prior to air drying can considerably improve the quality of dehydrated products and decrease substantially the time required for the drying process (Rodriguez *et al*., 2003).

8.2.3 Freeze drying

This is a processing method that makes use of a combination of freezing and dehydration. Foods that already have been frozen are placed in a vacuum-tight enclosure and dehydrated

under vacuum conditions with careful application of heat. Normally ice melts and turns into water when heat is applied. If further heat is applied, water is converted into steam. However, in freeze drying, the ice turns directly into vapor, and the probability that microorganisms may survive is minimal. Freeze-dried foods, similarly to dehydrated ones, are light and require minimal space for storage and transportation. They do not need to be refrigerated, but they must be reconstituted with water prior to their consumption (www.scribd.com/doc/37995229/Joseph-M-Pasag-Drying-Research). Although the food structure is better conserved, the equipment and its maintenance are quite expensive.

8.2.4 Osmotic dehydration

According to Oladele and Odedeji (2008), osmotic dehydration is a common processing method to obtain several kinds of products as minimally processed or intermediate moisture products or as a pretreatment in air drying or freezing. The main advantages of osmotic dehydration are the production of high-quality products, lower energy requirements, and less flavor loss and tissue damage.

8.2.5 Vacuum osmotic dehydration

Since evaporation of water occurs more readily at lower pressures, drying under vacuum is more rapid. This method is more expensive than air drying and is reserved for specialized products. However, vacuum osmotic dehydration (VOD) can also lead to advantages compared with atmospheric osmotic dehydration. The effect of vacuum treatment is very important on the kinetics of the mass-transfer phenomena, especially concerning water loss, weight loss, and weight reduction of food during osmotic treatment. The effect of vacuum application cannot be explained solely on the basis of diffusion and osmotic transport mechanisms. Therefore, a hydrodynamic mechanism (HDM) has been put forward and experimentally analyzed by Fito (1994). The main advantage of VOD over osmotic dehydration at atmospheric pressure lies in the mass transfer due to the HDM and the increment that occurs at the solid/liquid interface. The main obstacle is basically the high cost of the equipment.

8.2.6 Vacuum impregnation

Vacuum impregnation of a porous product consists of exchanging any substance in the product (gas or liquid) for an external liquid phase, due to the action of HDMs instigated by pressure changes. Therefore, substantial changes in physicochemical and structural properties take place in the food and these affect its behavior in drying operations (osmotic dehydration and air and contact drying). The operation is carried out in two steps after immersion of the product in a tank containing the liquid phase. In the first step, vacuum pressure is applied to the system for a short time in the closed tank, thus promoting the expansion and outflow of gas from the product. When the gas is released, it takes any liquid that is in the pores of the product with it. In the second step, atmospheric pressure is restored to the tank for some time and compression leads to a great volume reduction of the remaining gas in the pores, and thereby the external liquid fills up the porous structure. Application of external pressure can also affect the pore size but this greatly depends on the mechanical resistance of the solid matrix (Fito *et al.*, 2001).

8.2.7 Pulse VOD

Pulse VOD is when, during osmotic dehydration, pretreatments are conducted with the osmotic solution at the beginning of the process. This was shown to be very effective in promoting mass-transfer kinetics (Fito *et al.*, 2001). Osmotic dehydration increases when the food is submerged in an osmotic solution and subatmospheric pressure is applied for a short interval followed by a long period of osmotic dehydration at atmospheric pressure. Osmotic liquid penetrates the pores of the food by a HDM, thereby increasing the area of mass transfer in the food and producing a more extensive solid/liquid exchange, thus revealing the impact of material structure on mass transfer (Corzo and Bracho, 2007b). Taking into account that the most important HDM effect is very rapid and occurs just when the system is placed at atmospheric pressure again, a new procedure was designed to carry out the VOD, called pulse VOD. Fito (1994) applied short periods of vacuum treatment to the product while it was immersed in the osmotic solution. After that, the products underwent classical osmotic dehydration at atmospheric pressure. Thus, the filling of the food pores with the same osmotic solution started at the very beginning of the treatment. However, the treatment was carried out for most of the time under atmospheric pressure, clearly showing the impact of material structure on mass transfer.

8.2.8 Traditional meat smoking

Traditional meat smoking involves one salting stage followed by a smoking/cooking/drying single-step stage in an enclosed space. Processing is characterized by both mass transfers and physicochemical reactions. The most important quality criteria for consumers are stability, color, and product flavor. The specific disadvantages of this process are: difficult control, product heterogeneity, and sanitary hazards (benzo(*a*)pyrene). To solve these problems, an original dehydration/impregnation/soaking (DIS) technique has been suggested (see next section). On the other hand, the main advantages of this process are its short duration, savings in terms of energy and cost, easy control of the process, low final product temperature, and relatively high mass yield (Olmos *et al.*, 2004).

8.2.9 Meat treatments by soaking

Meat treatments by soaking in concentrated solutions (DIS processes) mainly aim to dehydrate the product and impregnate it with salt in one single operation step (Santchurn *et al.*, 2007). Thus, one of the major advantages of this technique is the elimination of sequenced operations of salting and dehydration, practiced in traditional meat processing. It is advisable to minimize the extent of salting in view of sensory and nutritional inconveniences strongly related to high salt content. In this respect, the use of ternary water/salt/sugar solutions allows good dehydration of product while limiting salt gain (Santchurn *et al.*, 2007). DIS is differentiated from other conventional drying methods with regard to two major characteristics: (i) the soaking process reaches a two-fold transformation of the product by enabling both a dewatering and a formulation effect; and (ii) soaking must be used as a preprocessing step prior to the main processing step, such as drying, freezing, pasteurization, canning, frying, and the possible addition of preservative agents. The two most convincing reasons for introducing a DIS processing step into a conventional stabilizing process are quality improvement and energy saving (Raoult-Wack, 1994).

Recent osmotic treatment applications have made it necessary to develop specially designed items of equipment, particularly where a high level of dehydration is necessary. Marouze and coworkers (2001) defined the functions required by users of osmotic dehydration equipment and presented a list of 17 principles used to contact foods with a concentrated solution. Osmotic treatment processes fall into several categories:

- processes in which the solution is external to the food;
- processes in which the solution is introduced into the food;
- processes in which solid solutes are applied on the surface; and finally
- processes in which pressure is applied to facilitate the mass transfer.

The advantages and disadvantages of drying processes and different methodologies of drying are summarized in Table 8.1

8.3 SOME RESULTS

Corzo *et al.* (2006a) investigated the effects of brine concentration (0.15–0.27 g NaCl/g) and temperature (30–38°C) on the color parameters of vacuum pulse osmotically dehydrated sardine sheets. The obtained results revealed that osmotic dehydration has a significant effect on color of sardine sheets. In fact, the redness (a^*) and yellowness (b^*) decreased through dehydration time while lightness (L^*) increased. The decreases in a^* and b^* were lower with increasing temperature and brine concentration, while the increases in L^* were higher. These changes would be predicted by simple models as a function of the temperature, brine concentration, and dehydration time and are displayed in Figure 8.1.

Corzo *et al.* (2006a) also looked into the effects of the concentration and temperature of osmotic solution on the color-change kinetics of sardine sheets during vacuum-pulse osmotic dehydration. Kinetics of color a^* value, b^* value, and L^* value were measured by tristimulus colorimetry. Corzo and coworkers reported that the rate of color changes complied with first-order kinetics and an Arrhenius relationship ($R^2 > 0.90$) for temperature dependence. The L^* value increased with an increase in dehydration time and temperature while both a^* and b^* values dropped (Figure 8.1). According to the authors "the rate constant for a^*, b^*, L^*-value fell with increase in concentration and temperature, except for L^*-value that increased with temperature increase." As for a^* value ($p < 0.005$) it was found to be highly temperature-sensitive at brine concentrations below 0.21 g NaCl/g. These temperature sensitivities underwent changes at brine concentration equal to or higher than 0.21 g NaCl/g. The a^* and L^* values increased with higher brine concentration, while that for b^* value decreased.

The applicability of the Zugarramurdi and Lupin (1980) model to study the equilibrium, and the kinetics of the process, was investigated. This model describes the water and salt concentration-variation kinetics of osmotically dehydrated sardine sheets at brine concentrations between 0.15 and 0.27 g NaCl/g and temperatures of 30–38°C. At constant temperature lower than 36°C, the rate constants increased up to 18% NaCl brine concentration and then dropped, while at a constant temperature higher than 36°C they decreased with increasing brine concentration. At a constant brine concentration, an increase in temperature induced a decrease in equilibrium water content. When the brine concentration was higher than 18% NaCl, the equilibrium salt content increased with the increasing temperature. The models applied proved to be very effective since they explained more than 97% of the water and salt concentration-variation kinetics at 95% confidence level (Corzo and Bracho, 2005).

Table 8.1 Advantages and disadvantages of different methods of drying.

Method	Advantages	Disadvantages	Conditions	Bibliography
Drying	Most flavors retained Less bulky product Shelf-life extension	Oxidation Losses of micronutrients Minimal loss in protein Potential changes in flavor and texture		Morris *et al*. (2004)
Sun drying/solar drying)	Low cost	Weather unreliability Infestation by flies Bacterial growth Lack of proper control Contamination by dirt, sand, and other impurities	For 5 days Air temperature 26–45°C Relative humidity 35–65%	Zaki *et al*. (1976)
Electrical dehydration	Shorter time More acceptable product		Temperature 30–60°C Relative humidity 65–85.4% for 24 h	Zaki *et al*. (1976)
Air drying	Osmotic dehydration prior to air drying improves quality and lowers the drying time			Rodriguez *et al*. (2003)
Freeze drying	Light products Little space occupied for storage and transportation	Potential microorganism growth	Combination of freezing and dehydration	Morris *et al*. (2004)
Atmospheric osmotic dehydration	High-quality products Requires lower energy Less flavor and tissue damage		Temperature 35–40°C Atmospheric pressure As pretreatment in air drying or freezing	Fito (1994); Oladele and Odedegi (2008)
Vacuum osmotic dehydration (VOD)	Faster dehydration Greater water-loss rate	More expensive More demanding equipment for specialized products	Temperature 35–40°C Vacuum 70 mbar	Fito (1994); Morris *et al*. (2004)

(*Continued*)

Table 8.1 (*Continued*)

Method	Advantages	Disadvantages	Conditions	Bibliography
Vacuum impregnation	Changes in physicochemical and structural properties affecting its behavior in drying operation Enhanced product density		Product development	Fito *et al*. (2001)
Pulse vacuum osmotic dehydration (pulse VOD)	Values only slightly inferior to those obtained by VOD, and much higher than those obtained by osmotic dehydration Very effective in promoting mass-transfer kinetics Volume and mass recovery more rapid		Short periods (5 min) of vacuum treatment, while being in the osmotic solution and after undergoing normal osmotic dehydration at atmospheric pressure	Fito (1994); Fito *et al*. (2001)
Traditional smoking		Control difficulty Product heterogeneity Sanitary hazard (benzopyrene)		Olmos *et al*. (2004)
Dehydration/impregnation/ soaking process (DIS)	Time Energy saving Cost saving Easy control Temperature of final product is low Relatively high mass yield Quality improvement		Dehydration and impregnation with solutes in just one step	Olmos *et al*. (2004); Santchurn *et al*. (2007)

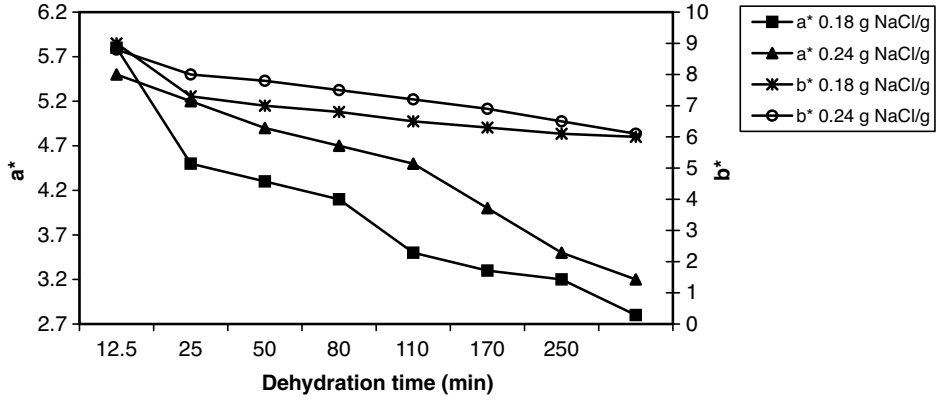

Figure 8.1 Color changes during osmotic dehydration of sardine sheets in brine at 0.18 and 0.24 g/NaCl/g (Corzo *et al.*, 2006a).

In another article Corzo and Bracho (2006a) determined experimentally the equilibrium water and salt contents and compared the prediction performance of models of Zugarramurdi and Lupin for osmotic dehydration of sardine sheets using brine at different concentrations (0.15–0.27 g NaCl/g) and temperatures (32–38°C). According to the Zugaramurdi and Lupin model, the equilibrium water and salt contents were lower. The values differed by 9–50% for equilibrium water content and by 3–44% for equilibrium salt content. The best model for to predicting equilibrium water and salt content was found to be the Zugarramurdi and Lupin model.

In another study (Corzo *et al.*, 2006b) firmness-change kinetics of vacuum-pulse osmotically dehydrated sardine sheets at temperatures between 30 and 38°C, and brine concentrations between 0.15 and 0.27 g NaCl/g, were studied using the fractional conversion technique. The equilibrium firmness increased with increasing brine concentration as well as temperature. The increases were much more rapid in the initial period of osmosis and then the rate decreased. The rate constant varied from 2.97×10^{-3} to 2.17×10^{-2} min^{-1}. It was found that the change in firmness during vacuum-pulse osmotic dehydration at selected concentrations and temperatures followed first-order reaction kinetics. The temperature dependence of the rate constant indicated an Arrhenius relationship ($R^2 > 0.90$). Higher activation energy indicated the greatest temperature sensitivity of firmness at 0.15 g NaCl/g.

The relationship between moisture content and shrinkage factor of osmotically dehydrated sardine sheets was investigated by Corzo and Bracho (2004a). Linear relationships between the shrinkage factor and moisture content and between shrinkage in volume and the volume of water lost have been determined in brines of 15–24% NaCl at temperatures of 30–38°C for dehydration times of 20–240 min. The shrinkage in volume was lower than the volume of water lost during the osmotic dehydration under the specific conditions in this experiment. The models applied explained 82.5–95.7% of the variability in the shrinkage factor as a function of moisture content and the 90–99% of variability in the shrinkage factor as a function of dimensionless moisture content.

Moreover, Corzo and Bracho (2004b) studied the effects of brine concentration (0.15–0.27 g NaCl/g) and temperatures (32–38°C) on equilibrium-distribution coefficients of sardine sheets during osmotic dehydration. The equilibrium-distribution coefficients of water and salt were determined using mass-transfer dynamics. According to these authors

"the temperature and brine concentration displayed the same trends in terms of their impact on equilibrium distribution coefficient of water but opposite trends in terms of their influence on equilibrium distribution coefficient of salt. Specifically, at a constant brine concentration, the distribution coefficient of water decreased while that one of salt increased with the increasing temperature. The distribution coefficient of water and salt ranged from 0.5008 to 0.6254 and from 0.5286 to 0.7783, respectively."

The Peleg model was applied by Corzo and Bracho (2006b) for predicting moisture loss and salt gain of sardine sheets during osmotic dehydration at brine concentrations between 0.15 and 0.27 g NaCl/g and temperatures of 30–38°C. The high regression coefficients ($R^2 > 0.92$) indicated the acceptability of the Peleg model for predicting both moisture loss and salt gain. The initial moisture-loss and salt-gain rates increased with increasing temperature but the effect of brine concentration depended heavily on the temperature. The Peleg rate constant for moisture loss and salt gain as a function of temperature followed an Arrhenius relationship irrespective of concentration. The rate constant for salt gain was found to be more temperature-sensitive than the rate constant for moisture loss. At a constant temperature, the equilibrium moisture content decreased with increasing brine concentration while the equilibrium salt content increased. When the brine concentration was kept constant the equilibrium salt content increased with increasing temperature.

More recently, Corzo and Brancho (2007a) made an attempt to apply the simplified solution of Fick's second law, considering sardine sheets as a flat plate, to calculate the water effective diffusion coefficient of sardine sheets during osmotic dehydration at various brine concentrations and temperatures. Osmotic dehydration was carried over five concentrations and temperatures between 30 and 38°C. Any change in concentration of sodium chloride resulted a change in water effective diffusion coefficient. At a constant brine concentration, water diffusion coefficient increased both with increasing temperature and increasing brine concentration below 0.24 kg NaCl/kg. This temperature sensitivity decreased at brine concentrations equal or higher than 0.24 kg NaCl/kg. The moisture content decreased with increasing dehydration time, brine concentration, and temperature.

The same authors (Corzo and Brancho, 2007b) also applied the simplified solution of Fick's second law for diffusion from a rectangular parallelepiped to determine the water effective diffusion coefficient of sardine sheets during vacuum-pulse osmotic dehydration at different brine concentrations and temperatures. According to Corzo and Brancho (2007b) "the sardine sheets were osmotic dehydrated at brine concentrations between 0.15 and 0.27 g NaCl/g, and temperature between 32 and 38°C. In general, diffusion coefficient increased with increasing concentration and temperature. The dependence of diffusion coefficient on temperature followed an Arrhenius relationship, regardless of concentration. The diffusion coefficient at 0.18 g NaCl/g was found to be the most temperature sensitive while the one at 0.15 g NaCl/g was the least temperature sensitive. The moisture content decreased with increasing dehydration time, brine concentration and temperature."

Oladele and Odejeli (2008) investigated mass-transfer kinetics during osmotic dehydration of catfish with respect to different temperature and finally time was determined. Moreover the acceptability of the dehydrated fish was investigated. The results revealed that any increase in temperature and time significantly affected the moisture content and water loss. On the contrary, weight reduction, salt gain, salt-to-water ratio, and water activity of the fish steak were not considerably affected by the increase. Only the taste of the fish was affected significantly by both temperature and time, while color, texture, flavor, and overall acceptability remained almost the same. Fish steaks dehydrated at 30°C got the highest rate in

taste while the fish dehydrated at 40°C was rated lowest. Furthermore, at 40°C, discoloration and slight shrinkage were reported.

Osmosis with a solution of sodium chloride and sucrose was assayed as a pretreatment to air drying of red seaweeds to depress the water activity (a_w) to 0.970 and the pH to 3.0. Air drying was performed at 30°C and 14% relative humidity. The determined a_w values after 2 h of drying were similar for both impregnated and non-impregnated seaweeds thereby proving that product shelf stability was not enhanced through osmosis performance. However, severe changes occurred in color. Powder flow ability evaluated through the measurement of compressibility and cohesiveness decreased with pretreatment. Therefore, osmotic dehydration could not be applied effectively as a pretreatment for air-dried seaweeds, because it did not improve product quality (Rodriguez *et al.*, 2003).

The impact of each solute (salt and sucrose) on the equilibrium, kinetics, and mass transfer in osmotic dehydration of tilapia fillets was investigated by Medina-Vivanco (2002). Limited volumes of ternary solutions (salt, sugar, water) at different initial sucrose and salt concentrations at 20°C were used. Fish-fillet processing with ternary solutions showed that the effect of sucrose on fillet water activity was minimal, contrary to sodium chloride which had a great impact. The presence of sucrose in the solution improved moisture-content reduction but negatively affected sodium chloride uptake by the tilapia fillet. Statistical analysis revealed that the effects of initial sodium chloride and sucrose solution concentrations on a_w equilibrium were negative and significant (95%). It is noteworthy that the effect of initial sodium chloride solution concentration was 16 times greater than that of sucrose solution concentration.

Zaki *et al.* (1976) studied the effect of brining, dehydration, sun drying, and storage at room temperature on the quality attributes of bolti fish. Physical, chemical and microbiological analysis (moisture, chloride, fat, total volatile bases (TVBs), thiobarbituric acid value (TBA), total bacterial count, and coliforms) were estimated in fresh bolti fish after brining, drying, and during storage at room temperature for 3 months. The moisture content decreased after the brining treatment as well as after dehydration whereas the fat content was hardly affected by these processes. Both TVBs and TBA displayed a pronounced increase after brining and drying. The rate of increase was higher for dehydrated samples than sun-dried ones. The total bacterial count was reduced. Coliform bacteria were absent both after brining and after dehydration (Figure 8.2, Table 8.2).

Mujaffar and Sankat (2006) investigated the effect of immersion time and brine temperature on weight reduction, water loss, salt gain, and a_w in shark slabs during osmotic dehydration. Salting can be successfully applied at 20 and 30°C. Dehydration at higher temperatures (above 40°C) resulted in undesirable changes such as shrinkage, discoloration, and cooking. For slabs at all temperatures, the greatest change in weight, moisture content, salt gain, and a_w occurred during the first 4 h of immersion. During this time the higher the temperature, the greater the reduction in weight and the decline in MC. Mass transfer during osmotic dehydration of shark slabs occurred in the falling rate period. Salt uptake and moisture data were analyzed applying various mathematical equations (models) based on Fick's law of diffusion.

An original immersion (DIS) process for pork meat production was successfully optimized using a coupled genetic algorithm/sequential quadratic programming method to obtain the optimal operating conditions, temperature, and soaking solution concentrations (Olmos *et al.*, 2004). Optimization criteria were mass yield maximization and operating time minimization. Optimal operating time was reduced from 32 h down to 23 h, and optimal mass yield increased up to 84.8%. The most convenient operation resulted in a two-stage immersion process. The

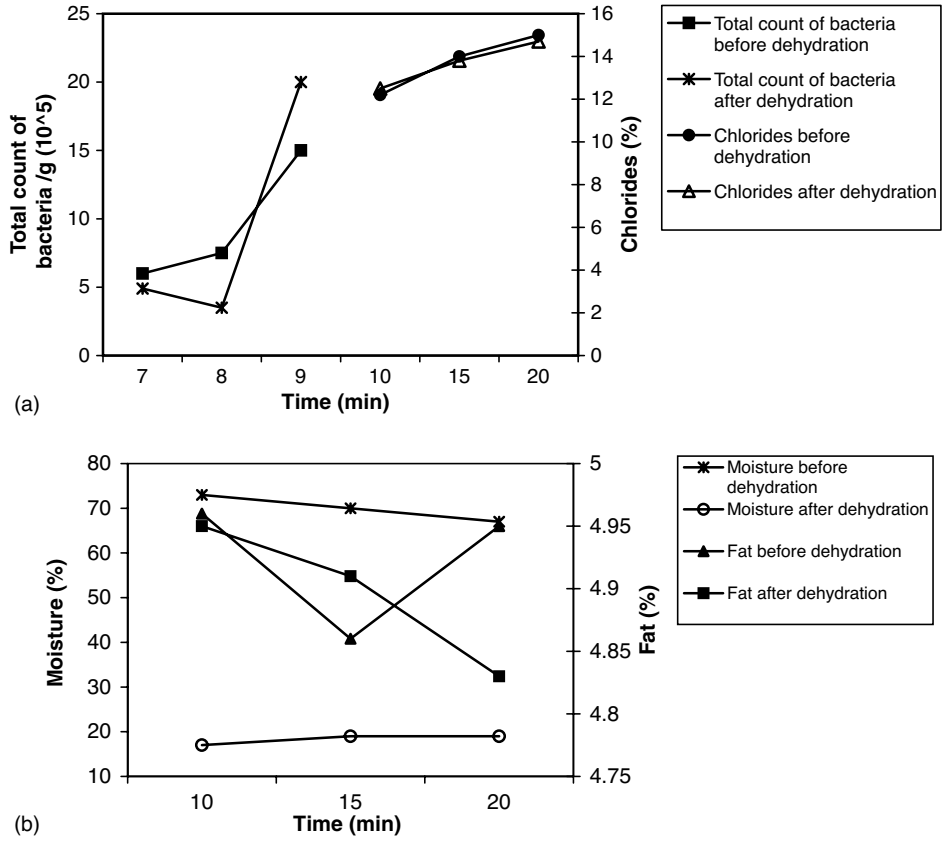

Figure 8.2 Effect of brining and dehydration on the quality attributes of bolti fish (Zaki *et al.*, 1976). (a) Total count of bacteria (per gram); chloride (%). (b) Moisture (%); fat (%).

main advantages of this process can be summarized as time, energy, and cost saving, and ease of control, low final product temperature and relatively high mass yield. Analysis of the operating conditions not only ensures the reproduction of the main characteristics of the traditional product, but can also potentially improve immersion processing.

Santchurn *et al.* (2007) investigated the effect of factors related to the composition of the solution, such as the solute molecular mass and molality, and the dynamic viscosity of the solution, on the transfer of water and solutes during the immersion in a concentrated solution. The role of corn syrup in the transfer of water and solutes, and particularly its limiting effect on salt impregnation, was studied. Moreover, immersion trials were carried out to compare corn syrup with three different combinations of polyethylene glycol (PEG) which had molecular mass distributions similar to that of corn syrup. Results revealed that water loss increased while salt and PEG gains decreased with increasing molecular mass of PEG. Moreover, an increasing molality of PEG led to increases in water loss and PEG but a decrease in salt gain. Under the applied experimental conditions, no significant effect of solution viscosity on mass transfer was established. Generally, dehydration of meat by soaking in a concentrated solution of water, salt, and corn syrup generally results in a high water loss and moderate salt and sugar gains.

Table 8.2 Application of the dehydration process on fish and meat.

Kind of food	Method	Features	Conditions	Results	References
Sardine sheets	Vacuum-pulse osmotic dehydration	Color parameters (L^*, a^*, b^*, ΔE)	T=30, 32, 34, 36, 38°C S=0.15, 0.18, 0.21, 0.24, 0.28 g NaCl/g t=20, 40, 60, 90, 120, 180, 240 min Vacuum=110 mbar	a^* values (redness) decreased with increasing dehydration time and increased with increasing temperature and brine concentration b^* values (yellowness) decreased with increasing dehydration time and increased with increasing temperature and brine concentration L^* value (lightness) increased with increasing dehydration time and brine concentration and increasing temperature ΔE (total color difference) increased with increasing brine concentration and dehydration time and increasing temperature	Corzo *et al.* (2006a)
	Vacuum-pulse osmotic dehydration	Kinetics of color (a^*, b^*, L^*)	T = 30, 32, 34, 36, 38°C S = 0.15, 0.18, 0.21, 0.24, 0.28 g NaCl/g t = 20, 40, 60, 90, 120, 180, 240 min Pressure=11 kPa	Rate constant for a^*, b^*, L^* values decreased with increase in concentration Rate constant for a^*, b^* value decreased with increase in temperature, while L^* value increased Ea>EL>Eb at 0.21 g NaCl/g Eb>Ea>EL at S>0.21 gNaCl/g Temperature sensitivity for a^*, L^* value increased with increase in brine concentration, while b^* value decreased	Corzo *et al.* (2006c)

(*Continued*)

Table 8.2 (*Continued*)

Kind of food	Method	Features	Conditions	Results	References
	Osmotic dehydration	Zugarramurdi and Lupin model (water and salt concentration)	T = 30, 32, 34, 36, 38°C S = 15%, 18%, 21%, 24%, 27% NaCl t = 20, 40, 60, 120, 180, 240 min Vacuum (0.1 mmHg) at 60°C	At a constant brine concentration, rate constants increased while equilibrium water content decreased with increasing temperature At a constant temperature lower than 36°C, rate constants increased up to 18% NaCl brine concentration and then decreased At a constant temperature higher than 36°C, decreased with increasing temperature The models explained the 97.9–99.9% of the water concentration variation kinetics and the 97.1–99.9% of the salt concentration variation kinetics at 95% level	Corzo and Bracho (2005)
	Osmotic dehydration	Equilibrium water and salt contents Comparison with the performance prediction according to the models Zugarramurdi and Lupin model and Azuara *et al.*	T=32, 34, 36, 38°C S = 0.15, 0.18, 0.21, 0.24, 0.27 g NaCl/g t = 1, 2, 3, 4 h Vacuum = 1.93 Pa	High regression coefficients (R^2>0.92) equilibrium water and salt contents according to Zugarramurdi and Lupin model were lower than Azuara values for equilibrium, water content differed by 9.4–46.9% and for salt content 3.5–44.1%, in both equilibrium water content decreased with increase of temperature Salt content increased	Corzo and Bracho (2006a)

Vacuum-pulse osmotic dehydration	Equilibrium firmness (fractional conversion technique)	T = 30, 32, 34, 36, 38°C S = 15%, 18%, 21%, 24%, 27% NaCl Time: 20, 40, 60, 90, 120, 180, 240 min Vacuum pulse 11 kPa	Firmness increased with increasing dehydration time, brine concentration, and temperature The rate constant and the equilibrium firmness increased with increasing brine concentration and temperature All rate constants, as a function of temperature, followed an Arrhenius equation The activation energy varied from 21.78–93.42 kJ/mol with increasing the brine concentration Higher activation energy indicated the greatest temperature sensitivity	Corzo *et al.* (2006b)
	Shrinkage	T= 30, 32, 34, 36, 38°C S = 15%, 18%, 21%, 24%, 27% NaCl Time: 20, 40, 60, 120, 180, 240 min Fractional design 5×5×7	Shrinkage factor diminishes when concentration, temperature, and time increased 82.54–95.71 % variability in the shrinkage factor at the 95% confidence (as a function of moisture content) 90–99% of the variability in the shrinkage factor (as a function of dimensionless moisture) the shrinkage in volume is lower than the volume of water lost	Corzo and Bracho (2004a)

(*Continued*)

Table 8.2 (*Continued*)

Kind of food	Method	Features	Conditions	Results	References
	Osmotic dehydration	Equilibrium distribution coefficients	T=32, 34, 36, 38°C S=0.15, 0.18, 0.21, 0.24, 0.27 g NaCl/g Time: 20, 40,60, 120, 180, 240 min	At a constant temperature, equilibrium water content decreased Equilibrium salt content increased with the increasing brine concentration at a constant brine concentration, equilibrium water content decreased and equilibrium salt content increased with the increasing temperature Distribution coefficients of water varied from 0.5008–0.6254 Distribution coefficient of salt varied from 0.5286–0.7783 At a constant brine concentration, distribution coefficient of water decreased Distribution coefficient of salt increased with the increasing temperature Increasing brine concentration decreased distribution coefficient of water and salt	Corzo and Bracho (2004b)
	Osmotic dehydration	Peleg model (moisture loss and salt gain)	T=30, 32, 34, 36, 38°C S=15%, 18%, 21%, 24%, 27% NaCl t=1, 2, 3, 4 h	The coefficient of determination: R^2 value varied from 0.997–0.999 at constant brine concentration moisture loss and salt gain decreased with increasing in the initial moisture loss and salt gains rates	Corzo and Bracho (2006b)

				Peleg rate and capacity content for moisture loss varied: 0.260–0.884 h (g/g db)$^{-1}$ and 0.925–1.740 (g/g db)$^{-1}$ And for salt gain: 0.897–2.770 h (g/g db)$^{-1}$ and 3.210–5.060 (g/g db)$^{-1}$ Rate constant for salt: Ea = 48.92–97.45 kJ/mol Rate constant for moisture loss: Ea=16.58–81.74 kJ/mol At a constant temperature, the equilibrium moisture content decreased with increasing brine concentration while equilibrium salt content increased ($p < 0.05$) At a constant brine concentration the equilibrium salt content increased with increasing temperature	
Vacuum osmotic dehydration	Water effective diffusion coefficient	T = 30, 32, 34, 36, 38°C S = 0.15, 0.18, 0.21, 0.27 g NaCl/g Time: 1, 2, 3, and 4 h Vacuum (1.93 Pa) at 60°C	Moisture ratio decreased with increasing dehydration time, brine concentration, and temperature Water effective diffusion coefficient: 1.46×10^{-10}–2.41×10^{-10} m^2/s Increased with increasing concentration and temperature 0.18 g NaCl/g=the most temperature sensitive (Ea=39.62 kJ/mol) 0.15 g NaCl/g=the least temperature sensitive (Ea=23.67 kJ/mol)	Corzo and Bracho (2007a)	

(*Continued*)

Table 8.2 (*Continued*)

Kind of food	Method	Features	Conditions	Results	References
	Osmotic dehydration	Water effective diffusion coefficient	T = 30, 32, 34, 36, 38°C S = 0.15, 0.18, 0.21, 0.27 kg NaCl/kg Time: 1, 2, 3, and 4 h Vacuum (1.93 Pa) at 60°C	Moisture content decreased with increasing dehydration time, brine concentration and temperature Water effective diffusion coefficient: 2.084×10^{-12}–3.015×10^{-12} m^2/s Temperature sensitivity: increased ($p < 0.05$) with increasing brine concentration below 0.24 kg NaCl/kg and decreased ($p < 0.05$) at brine concentrations equal to or higher than 0.24 kg NaCl/kg	Corzo and Bracho (2007b)
Catfish (*Hemisynodontis membranaceus*)	Osmotic dehydration	Brine temperature Time	T: 30, 35, 40°C Time: 1, 2, 4, 6, 24 h Brine solution 2.5 M	WL increased through the 24 h WR was not significantly affected MC decreased gradually throughout 24 h a_w decreased SC was not significantly affected Salt-to-water ratio (S/W) increased	Oladele and Odedeji (2008)
Air-dried red seaweeds (*Porhyra columbina*)	Osmotic dehydration (with solution of NaCl and sucrose)	Color	Air drying: 30°C, 14% relative humidity	a_w was similar for both osmosed and non-osmosed seaweeds and was higher than the monolayer a_w	Rodriguez *et al.* (2003)

		Flow pattern Rehydration ability	Osmotic dehydration: 46% sucrose, 11% NaCl, pH = 3.0, 0.2% potassium sorbate at 23°C for 72h $a_w = 0.97$	Osmosis produced changes in color parameters: luminosity (*L value) and b* value decreased whereas the loose and tapped bulk densities and compressibility increased Air-dried seaweeds have a 100% higher rehydration capacity and have a more elastic behavior	
Tilapia fillets	Osmotic dehydration with binary and ternary solution (salt, sugar, water)	Equilibrium kinetics Mass transfer	Solution/fillet ratio: 4/1 Sucrose concentration: 0–100 g of water salt concentration: 0–35.14 g in 100 g of water T: 20°C	Equilibrium a_w values very high with sucrose Moisture-content values lower using sucrose Effects on equilibrium a_w negative and significant (95%)	Medina-Vivanco *et al*. (2002)
	Dehydration Sun drying	Moisture content Chloride Fat Total volatile bases (TVB) Thiobarbituric value (TBA) Total bacterial count (TBC)	Dehydration: T = 30–60°C Humidity 65–85.4% for 24h Sun-drying: 9.0 am–6.0 pm for 5 days, humidity 35–65%	Moisture content decreased Chloride increased Fat was not affected TVB and TBA increased TBC reduced Absence of coliforms	Zaki *et al*. (1976)
Shark fillets	Osmotic drying	Immersion time Brine temperature	T: 20, 30, 40, 50°C Brine: 100%	T: 20 and 30°C → good color, odor, and texture 40°C → shrinkage, discoloration 50°C → cooking effect WL increased with an increase in WR and brine temperature a_w decreased MC decreased SC increased	Mujaffar and Sankat (2006)

(*Continued*)

Table 8.2 (*Continued*)

Kind of food	Method	Features	Conditions	Results	References
Meat (pork)	Dehydration/impregnation/soaking (DIS) Traditional smoking process	Mass yield Operating time	Time 23 h T: 10–70°C Salt, sugar, phenol	WL increase a_w decreases Salt gain increase pH decreases mass Yield increase to 84.8%	Olmos *et al.* (2004)
	Dehydration by soaking (salt, corn syrup; DE21)	Solute molecular mass Molality Dynamic viscosity	Solutions of NaCl (3.0 mol/kg water), corn syrup DE21 (950 g/kg water), or polyethylene glycol (PEG)	Water loss increased salt and PEG gain decreased, with increasing molecular mass in the range of 200–600 Da Water loss and PEG gain increased Salt gain decreased, with increasing molality from 0.6 to 1.6 mol/kg water	Santchurn *et al.* (2007)
Chicken	Osmotic treatment	Salt solution concentration Immersion time	T: 5°C Salt solution with 0%, 5%, 10%, 15%, 20% of salt	Water gain (WG) Salt gain (SG) total weight increment (WI)	Schmidt *et al.* (2008)
Shrimp	Dehydration	Effect of SHPH	Concentration of shrimp head protein hydrolysate (SHPH) 2.5–10%	Amounts of monolayer and multilayer water with SHPH were higher than those without SHPH Unfrozen water increased Ca-ATPase inactivation decreased	Ruttanapornvareesakul *et al.* (2005a, 2005b)
	Dehydration	Effect of shrimp chitin and shrimp chitin hydrolysate (SCH)	Concentration SCH and shrimp chitin 5%	a_w of myofibrillar protein (Mf) for SCH was lower than Mf with shrimp chitin Mf with SCH showed high level of inactivated Ca-ATPase activity	Somjit *et al.* (2005)

				Mf with glycose showed higher inactivated Ca-ATPase activity SCH contributed to the retardation effect on dehydration and induced denaturation of Mf	
Scrap fish	Dehydration	Effect of enzymatic fish protein hydrolysate (FPH)	Concentration of FPH 5%	a_w decreased Ca-ATPase activity was higher The amount of unfrozen water was higher FPH suppressed Dehydration	Khan *et al.* (2003)
Antarctic krill meat	Dehydration	Effect of protein hydrolysate		Stabilized the bonding of water molecules Suppressed denaturation The effect of hydrolysate was less than by glycose and sodium glutamate	Zhang *et al.* (2002)
Fish paste sausage	Air-drying	Poultice-up process (method of obtaining good-quality food)		Effective diffusion coefficient (De) Diffusion activation energy (ED) Void fraction of dried sausage (Vf) Proportion of weakly restricted water (fw) Strongly restricted water (fs)	Konishi and Kobayashi (2003)

a*, redness; b*, yellowness; L*, luminosity/lightness; Ea, activation energy for a*; Eb, activation energy for b*; EL, activation energy for L*; ΔE, total color difference; S, salt; T, temperature; db, dry basis; WL, water loss; WR, weight reduction; MC, moisture content; a_w, water activity; SC, salt content.

Table 8.3 Dehydration techniques: conditions, advantages and disadvantages.

Method	Advantages	Disadvantages	Conditions	References
Sun drying		Unreliability of weather infestation by flies Growth of bacteria Lack of proper control Contamination by dirt, sand, and other impurities	9.0 am–6.0 pm for 5 days Air temperature 26–45°C Relative humidity 35–65%	Zaki *et al*. (1976)
Artificial dehydration	Short time More acceptable product		Temperature 30–60°C Relative humidity 65–85.4% for 24 h	Zaki *et al*. (1976)
Traditional smoking		Control difficulty Product heterogeneity Sanitary danger (benzopyrene)		Olmos *et al*. (2004)
Dehydration/impregnation/ soaking process (DIS)	Time Energy Cost saving Easy control Low final product temperature Relatively high mass yield			Olmos *et al*. (2004)
Atmospheric osmotic dehydration			Temperature 35–40°C Atmospheric pressure	Fito (1994)
Vacuum osmotic dehydration (VOD)	Faster dehydration kinetics Increase water loss rate		Temperature 35–40°C Vacuum 70 mbar	Fito (1994)
Pulse-vacuum osmotic dehydration (pulse VOD)	Slightly inferior values to those achieved by VOD but very superior to those obtained by osmotic dehydration		Short periods (5 min) of vacuum treatment, while in the osmotic solution and after undergoing normal osmotic dehydration at atmospheric pressure	Fito (1994); Fito *et al*. (2001)

	Very effective in promoting mass-transfer kinetics Faster volume and mass recovery	
Vacuum impregnation	Changes in physicochemical and structural properties which affect its behavior in drying operation Product density increased	Fito *et al.* (2001)

A typical food-drying process was characteristically innovated by using a water tank model based on the physicochemical analysis of dehydration dynamics by Konishi and Kobayashi (2003). A fish paste sausage was effectively used as a model food to evaluate five typical parameters for water movement: effective diffusion coefficient (De), diffusion activation energy (ED), void fraction of dried sausage (Vf), proportion of weakly restricted water (fw), and strongly restricted water (fs).

In an attempt to utilize fishery waste products as functional food materials, shrimp head protein hydrolysate (SHPH) was produced from wastes from three species of shrimp – northern pink shrimp, endeavour shrimp, and black tiger shrimp – with enzymatic hydrolysis. The SHPH was used as a natural food preservative by being added to lizardfish myofibrils at concentrations ranging from 2.5 to 10%. Their effect on the state of water and the denaturation of myofibrils during dehydration were evaluated by Yaowalux and his coworkers (2006). The obtained results indicated that SHPH could be used as a food additive to improve functional properties and maintain food quality. Moreover, their further application could be extended to several purposes in food industry such as denaturation suppression, maintenance of structure and texture maintenance with increasing bound water in intermediate-moisture foods.

To assess the potential utilization of shrimp waste, shrimp chitin and shrimp chitin hydrolysate were isolated from black tiger, endeavor, and giant freshwater prawns by Kingduean *et al.* (2005). The effects of shrimp chitin and shrimp chitin hydrolysate on the state of water and denaturation of lizard fish myofibrillar protein (Mf) at a concentration of 5% were assessed based on a_w and changes in Mf Ca-ATPase activity on dehydration. The obtained result was compared with untreated Mf (control) and Mf containing glucose. It was found that Mf with shrimp chitin hydrolysate exhibited almost similar oligosaccharides to those isolated from crab and squid. Therefore, it may be concluded that chitin oligosaccharides from shrimp, crab, and squid reduced the a_w in Mf and also displayed a suppressive effect on the dehydration-induced denaturation of Mf. Therefore, the utility of these oligosaccharides as natural food-protective materials for dry or partially dry foods could stimulate more interest in food processing and preservation (Table 8.3).

A comparison between weight loss, salt gain, water activity, weight reduction and moisture content of catfish and shark fillets is shown in Figure 8.3.

8.4 CONCLUSIONS

Food processes consisting of making contact with food of animal or vegetable origin with concentrated salt, sugar, or mixed solutions have been around for a very long time. Brining and candying, for instance, have been in use since ancient times, the former to impregnate food with salt and the latter using sugar. More recently, studies of mass transfer during processes that fall into the category of osmotic dehydration or osmotic treatment have shown that solute impregnation was always accompanied by dehydration. More recent studies have clearly shown that if the process parameters (temperature, solution concentration, agitation of the food in the solution, and operating pressure) were effectively controlled, these processes could be oriented towards a wide range of applications, from high dehydration coupled with low impregnation to high impregnation coupled with low dehydration. In case further solutes are added to the solution, the formation of food-appropriate composition is feasible (Marouze *et al.*, 2001).

Whenever meat, fish, and aquaculture products (e.g., salted pork, smoked salmon, dried tilapia) are not consumed immediately, they will have to be processed with a range of

traditional techniques involving salting, drying, cooking, smoking, and marinating or combination of two or more operations. These techniques are often long, poorly controlled, and generate environmentally detrimental effluents. Moreover, food habits are continuously changing and consumers tend to buy milder products that are not as thoroughly processed and thus have shorter shelf life than traditional products. The already existing stability problem becomes more acute when there is inadequate control of the process because of the sudden switch from traditional small-scale and family processing conditions to high-capacity production units. These traditional processes often have a common step in which the product is placed in contact with a solute (salt, sugars, acids, seasonings, etc.). However, over the last two decades considerable progress has been made in the field of osmotic dehydration by incorporating vacuuming, pulse vacuuming, vacuuming impregnation, and freeze drying. Application of these techniques has revolutionized the shelf life of food of plant and animal origin by extending it substantially (Collignan *et al*., 2001).

Despite the considerable efforts made toward improving our understanding of mass transfer in osmotic dehydration there is still need for further research in several areas: modeling, automated control of equipment, product quality, and optimization of combined processes. The application range of the process has widened, especially since new research needs are being revealed. Thus, there is still potential for further work in the field of

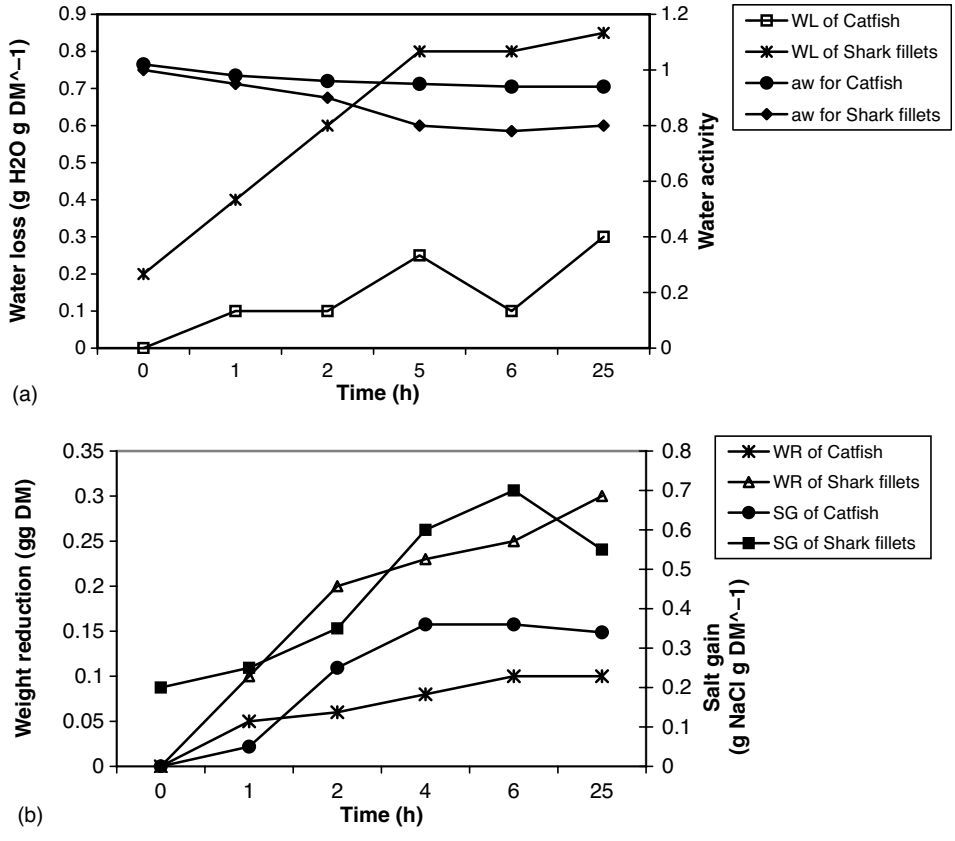

Figure 8.3 Effect of brine temperature 30°C on (a) water loss (WL), water activity (a_w); (b) weight reduction (WR), and salt gain (SG) of catfish (Oladele and Odedeji, 2008) and shark fillets (Mujaffar and Sankat, 2006).

osmotic dehydration and the issues raised should be top priorities for the future (Raoult-Wack 1994).

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9 Dehydration of Fruit and Vegetables in Tropical Regions

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Abstract: The introductory part of this chapter contains information on forms of water and its role in foods. Also merits and demerits of sun drying over dehydration will be discussed. Abundant sunshine in tropical areas can efficiently be utilized for sun drying of fruits and vegetables. In sun drying, moisture is removed by solar energy. The discussion about sun drying covers the areas of equipment, preparatory treatments, drying procedure, precautions, and post-drying care of the products. Moisture is also removed by artificial means with different dehydrators. In dehydration, drying atmosphere and factors affecting evaporation of water from food surface will be discussed. This section will include descriptions of various methods such as dehydration, freeze drying and dehydro-freezing. Types of dehydrators, including natural-draft, forced-draft, solar drier, tunnel driers, and conveyer driers, are discussed at length. Information on fluidized-bed driers for particulate or diced materials will be given. Spray driers, drum driers, special drying techniques (e.g., foam-mat drying) and use of latest techniques such as drying by application of energy from radiating microwave/dielectric sources will also be covered. Evaporating moisture, thereby concentrating the soluble solids in foods, will be dealt with under evaporation/concentration. Binding of available moisture to produce intermediate-moisture foods could be a useful technique for tropical countries. At the end of the chapter the effect of drying on the nutritional value of dried foods will be discussed.

Keywords: dehydration; dehydro-freezing; drum drier; freeze drying; forced draft; natural draft; sun drying; solar drier; tunnel drier

9.1 INTRODUCTION

Fruits and vegetables have been dried since prehistoric times. Drying has been practiced using the sun's energy and air. With the development of dehydration equipment at the beginning of 19th century, it moved into the factories. The terms drying and dehydration are used synonymously for the removal of moisture from foods. However, drying is often restricted to the process under natural conditions using solar energy. It is especially useful in tropical regions where the sun shines for most of the year. Sun drying of fruits is still practiced in these areas for drying of prunes, figs, grapes, dates, and apricots (Girdhari *et al*., 1998).

Dehydration is the term used for the process of removal of water under controlled conditions of temperature, relative humidity, and air flow. Dehydration processes are applied to apples, prunes, and several vegetables like spinach, potatoes, okra, bitter gourd, carrot, green peas, and cauliflower. Continuous processes such as tunnel, belt-trough, fluidized-bed,

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and foam-mat drying are mostly used for drying vegetables. Dehydration equipment with vacuum has enabled removal of moisture from delicate foods without loss of quality. Freeze drying has been a very helpful discovery for the preservation of delicate and heat-sensitive foods such as instant tea, coffee, and fruit juices. It involves sublimation of ice from frozen food. Spray drying is suited for fruit-juice concentrates and vacuum dehydration is helpful for low-moisture and high-sugar fruits including apricots, pears, and peaches.

9.2 FORMS OF WATER

Water is essential for chemical and biochemical reactions as well as microbial activity in foods. The microorganisms need ample free water for their survival and multiplication. Without biologically active moisture, spoilage agents would be completely inactive. It is present in varying quantities and different forms practically in all foods (Rahman, 2007). Fresh fruits and vegetables that are high in moisture spoil readily. Dry beans and cereal grains contain relatively less moisture and are safely stored for a long period at ambient temperature without a reduction in quality (Ramaswamy and Marcotte, 2006).

Water exists in foods in five different forms:

(i) free water (e.g., in tomato juice);
(ii) droplets of emulsified water (e.g., in butter);
(iii) water tied into colloidal gels (e.g., in jellies);
(iv) thin layer of adsorbed water (e.g., in powdered milk);
(v) chemically bound water of hydration (e.g., in sugar).

Moisture may be removed from the foods by sun drying and dehydration, evaporation/concentration, freeze drying, and dehydro-freezing techniques. Chemically bound water is extremely difficult to remove during drying, while free and adsorbed water is easy to manipulate in drying and dehydration. Most free and absorbed moisture is removed by suitable means. In this way, the remaining water becomes unavailable for chemical and biochemical reactions, as well as activities of microorganisms.

9.2.1 Role of water in food

Water plays one or more roles in a food system. It may serve as a solvent and participate in chemical and biochemical reactions as a prime reactant in processes involving hydrolysis. It may be a product of chemical reactions involving condensation. It may serve as a modifier of the catalytic activity of other substances in food. Reducing the water content lowers or even inhibits the chemical and biochemical reactions and activities of microorganisms. It also retards the activities of metallic catalysts associated with lipid oxidation.

9.3 ADVANTAGES OF DRIED FOODS

Dried foods are less expensive than most foods preserved by other methods. These are concentrated sources of nutrients and are high in total solids. Dehydrated foods, because of reduced weight, are less costly to transport. Due to a reduction in bulk of the product they also require less storage space. The color of dehydrated fruits and vegetables is more uniform due to controlled drying conditions. The process of dehydration should be applied in such a

manner that the nutritional value, flavor, and cooking properties of the fresh fruits and vegetables are retained.

9.4 DRYING PROCESSES

There are three fundamental types of drying processes:

(i) sun drying/solar drying;
(ii) controlled atmospheric batch-type drying with tower, cabinet, and kiln dehydrators and continuous drying with belt, belt-trough, tunnel, fluidized-bed, foam-mat, spray, explosion puff, microwave, and drum dehydrators;
(iii) sub-atmospheric drying such as vacuum shelf/belt and freeze dehydrators.

9.4.1 Sun drying/solar drying of fruit and vegetables

Sun drying refers to elimination of moisture from foods by exposing them to the direct energy from the sun's rays. Many food grains such as cereals and pulses become naturally dried in the field by solar energy and are protected from autolysis and microbial attack. Their moisture content usually varies from 8 to 16% and water activity is normally 0.75 or below. In addition to these naturally preserved foods, other perishable commodities are sun-dried in order to extend their shelf life. In tropical and sub-tropical regions with an abundance of sunshine, several fruits and vegetables are sun-dried. Grapes are sun-dried in Afghanistan, Greece, and the USA to produce raisins and dates are dried in Saudi Arabia, Iraq, Tunisia, Egypt, and Algeria. Guava, tomato, pears, and peaches are also sun-dried in several parts of the world. In Pakistan figs, plums, grapes, dates, and apricots are sun-dried since the preservation is simple and inexpensive. These are more often dried by the farmers at the farms and in producing areas when there is surplus produce and it is often difficult to transport fresh commodities to market. This method is applicable on a commercial level or at the village level subject to a hot and dry climate with no rainfall during and after the harvesting period. The crop should be fresh and of good quality. Various lots of different stages of maturity must not be mixed together; this would result in a poor dried product. Insect-, rodent-, and disease-affected parts and parts which are discolored or have a bad appearance must be removed before drying.

9.4.1.1 Equipment requirements

Equipment required for sun drying varies according to the product to be dried, local conditions, and the scale of production. However, anyone engaged in sun drying of fruits and vegetables would need wooden boxes of appropriate size for collection of raw material from the field. In the preparation shed, cutting tables, cutting knives, peeling knives, aprons, etc., are required. The prepared raw material needs to be placed in suitable-sized trays that are usually constructed of wood. Blanchers or sulfuring equipment is also needed. After drying, the raw material is brought to the sweating chamber. Packaging equipment is required to pack the dried product in suitable-sized moisture-proof containers.

9.4.1.2 Method of production

The raw material for sun drying needs preparatory treatments similar to those required for preservation by canning, freezing, or other techniques (Awan and Rehman, 2009). Before

trimming and cutting, most fruits and vegetables should be washed with potable water to remove contaminants, spray residues, and soil particles. Onions are washed after they have been peeled. After washing, trimming, and peeling, bigger-size fruits may be cut into even slices of about 3–7 mm thickness or in halves/quarters and then blanched or sulfured. It is very essential to have all slices of uniform thickness in one drying batch. Different sizes dry at different rates and result in a poor-quality product. Onions and root vegetables are sliced with a hand or vegetable slicer; apples, bananas, tomatoes, and potatoes are sliced with stainless steel knives. After peeling and slicing, apples, bananas, and potatoes turn brown very quickly when exposed to air due to an active enzyme, phenoloxidase. So, these must be kept in water until drying can be initiated. Salt or sulfite solution gives better protection.

Broadly speaking, plums, grapes, figs, and dates are dried as whole fruits without cutting and slicing. Slices are left in the open sun in trays and turned occasionally until dried to the desired degree. After sulfuring at the rate of 1.8–3.6 kg of sulfur per tonne of fruit, the trays are placed in the sun with 85 × 60 cm trays and sides about 5 cm high: the approximate load for a tray is 3.5 kg and the material should be spread in even layers. During the initial stage of the drying, the material should be turned over at least once every hour by shaking. It will facilitate quick removal of moisture, prevent sticking together and improve the quality of end product. At night, the trays should be stacked in a ventilated room or covered with canvas. The dried fruits are then sorted and stacked in boxes or bins for moisture equilibration, a process known as sweating. After drying, the products are subjected to attack by insects and rodents, so the product should now be packed or stored in insect- and rodent-proof storage rooms. Occasionally this room may be fumigated with suitable gas to kill insect pests (Saravacos and Kostaropoulos, 2002). In view of the fact that dried foods usually deteriorate at room temperature and lose both color and flavor, it is preferable to store them at lower temperatures, especially if stored in bulk bins. Most processors prefer to keep their products in cold stores at 0–4°C, which prevents damage caused by insects and rodents in addition to protecting product quality.

Some varieties of vegetables and fruits are better for sun drying; they must be able to endure natural drying without their texture becoming tough so that they are not difficult to reconstitute.

9.4.1.3 Precautionary measures

As the raw material for sun drying is directly exposed to the atmosphere, there is danger of contamination from natural sources. Microorganisms in the atmosphere will settle on the product. Birds and animals in and around the vicinity of the drying yard should be kept away. Cleanliness in all operations is absolutely essential. Great care must be taken during the drying process to prevent contamination. All equipment should properly be disinfected. Waste fruit should not be dumped near the drying area and, where this is not possible, it should be treated in a pit with calcium chloride to prevent its decay and multiplication of microorganisms and insects, especially flies.

The main problems of sun drying are dust, rain, and cloudy weather. The drying area should be free from flies and dust. In order to produce dust-free and hygienically clean products, materials should be dried well above ground level and covered with nylon mosquito net.

9.4.2 Solar driers

For commercial-scale operation, due consideration should be given to the cost of production, particularly energy consumption. The large-scale driers are more promising than small-scale

ones. However, small-scale driers should not be overlooked. The drier should be designed to maximize capital investment; i.e., multi-product and multi-use. In a broader sense, an auxiliary heat source should be provided to ensure reliability to handle peak loads and also to ensure continuous drying during periods of no sunshine. Forced-convection indirect solar driers are preferred because they offer better control and more uniform drying, and because of their high heat collection efficiency results in a smaller collector area. However, energy wastage should be minimized.

Two drier systems are identified: (i) a cabinet-type drier with natural convection for internal air circulation for the processing of fruit such as bananas, pineapples, mangoes, apricots, apples, and pears, and also for potato chips; and (ii) a greenhouse-type drier with forced-air circulation.

Barriers to commercial development include the following:

- initial investment is high, especially for poor farmers;
- misuse is possible due to lack of training and technical skills;
- durability is doubtful if low-cost building materials are used;
- dependability and reliability are reduced during the rainy season, when drying is critical, due to non-availability of solar energy;
- the lack of national policies directed to promoting the drying of produce at the production site, in order to reduce losses, improve quality, and increase farmers' earnings.

The benefits of solar driers include:

- cost of production is low;
- fuel costs are negligible;
- useful where land is abundant;
- suitable where less labor is available;
- good quality low-cost product is obtained with little care in sanitation and hygiene;
- suitable for tropical regions with ample sunshine.

9.4.3 Drying under shade

Drying in the shade is applied in those vegetables for which there is a problem of natural color loss or browning under direct sunlight. Herbs, coriander leaves, green and red sweet peppers, chillies, green beans, okra, and fenugreek have an attractive color, and hence are dried under shade. The principles for shade drying are similar to those for sun drying. Shade drying is carried out in a shed which has open sides. Air circulation is required for efficient drying. Shade drying takes little more time than is normally required for drying under the sun.

9.4.4 Osmotic drying

In this process, prepared fruits and vegetables are immersed in a strong syrup and brine, respectively, and these are then sun-dried. During immersion the material loses some of its moisture. The salt/syrup serves as a protective coating on the surface of slices. The protective effect on color, flavor, and texture remains during the drying process and results in a high-quality product.

9.5 DEHYDRATION

Dehydration is an operation in which the moisture content of food is substantially lowered under controlled conditions of temperature, relative humidity, and airflow in a chamber. Under these conditions, high-quality products are obtained that retain their natural characteristics upon reconstitution. Foods that are successfully preserved by dehydration include fruits, vegetables, milk, eggs, fish, cake mixes, soup mixes, and others. The raw material determines the handling techniques and type of equipment needed. Savings may be made in shipping costs from reduced weight, but not in terms of container size. Moreover, sometimes shipping costs are not based on weight but on volume. Another reason for dehydration is the production of convenience food items. A good example is instant mashed potato. In this case, cooking steps are completed before the product is dried. The consumer only needs to add water and mix.

9.5.1 Drying conditions

In the removal of moisture from natural foodstuffs, heat energy is applied to the food. Water, which evaporates, is removed from the vicinity of the raw material. Foods may be dried: (i) in hot air; (ii) in superheated steam; (iii) in a vacuum; (iv) in heated inert gas; or (v) by direct application of energy from a radiant microwave or dielectric source. However, air is the most common medium for elimination of moisture from foods for several reasons. Dehydrators using air are less expensive to construct and are convenient to install and operate. By using air as a drying medium, overheating, discoloration, and scorching of the product is greatly avoided. Air also permits gradual drying of foods, thereby avoiding loss of juice by dripping (Lee and Pyun, 1993; Brennan, 2006).

Primarily, there are three major roles of air in dehydration. It conveys heat energy to food, vaporizes moisture from the commodity and transfers the liberated moisture to the outside atmosphere. A larger volume of air is required to vaporize moisture from the food than to transport it out of the drying atmosphere. The volume of air required for drying is calculated from the initial temperature of air entering the drier, volume of moisture required to be evaporated, time in which the operation has to be completed, and temperature of air leaving the dehydrator. Other factors connected with this calculation are the heat losses through leakage and heat required to raise the temperature of trays and walls of the dehydrator.

9.5.2 Factors affecting evaporation of water from food surfaces

The rate of moisture evaporation from the free surface of a food material depends upon the nature of food material, particle size, bed depth (in case of pieces placed on a surface), relative humidity, temperature, and velocity of the air. Not all food raw materials react in the same manner to changes in atmospheric conditions. As moisture has to travels out of the food from inside, larger pieces take a longer time to dry than smaller ones. Thus, while it may take a few hours for okra pieces to dry in a tunnel drier, more time would be required in the case of whole okra dried under the same conditions. When tissue foods are dried in cabinet or tunnel driers, the depth of raw material on the tray surface will also determine the length of the process. Atomization of fluid foods into small particles, as observed in spray drying of fruit juices and instant beverages, cuts the drying time to a matter of seconds.

The relative humidity and velocity of air are important factors that must carefully be controlled in dehydration processes. Air, high in relative humidity, will not accommodate the

extra moisture from the food. At low temperature, air is easily saturated with moisture and so will not be efficient for the purpose. The rate of moisture evaporation from the free surface of food material is directly proportional to the velocity of air, provided other factors remain constant. It has been found that with an air velocity of about 70 m/min, drying is twice as rapid as in still air and at 140 m/min it is three times as fast.

Food materials are living units and moisture has to travel out from the inside to the outside of food pieces. At high temperature, low relative humidity, and high velocity of the drying air, the food surface can get dried, while the moisture inside the food is unable to travel towards the outer surface. This condition is known as case hardening, and results in the food being dry on the outside but wet on the inside. Therefore, air velocity is usually regulated in commercial dehydrators to between 91 and 304 m/min. Increasing the air velocity beyond this range is uneconomical. Part of the spent air may be re-circulated to avoid case hardening and to cut down on heating costs.

9.5.3 Types of dehydrator

Depending upon the mechanism of transferring heat energy from the heat exchanger to the food, all dehydrators can be grouped into one of the following four categories:

(i) hot-air driers:
  - natural-draft driers: kiln, cabinet, tower, and Oregon tunnel driers,
  - forced-draft driers: tunnel driers (concurrent, counter-current, center-exhaust, and cross-flow tunnel driers), conveyer driers, fluidized-bed driers, and spray driers;

(ii) drying by contact with a heated surface: drum driers, vacuum shelf driers;

(iii) drying by application of energy from a radiating microwave or dielectric source: radiant heating drier, continuous infrared drier, microwave and dielectric heating driers;

(iv) special drying techniques: puff drying, foam-mat drying.

9.5.3.1 Hot-air driers

Natural-draft driers

Natural-draft driers, in general, consist of a furnace room or other heating arrangement (steam pipes, electric heaters), surmounted by a drying chamber. The air is heated by contact with radiating pipes and enters the drying chamber by natural convection currents. These driers are inexpensive to build and have low fuel efficiency. Most natural-draft driers are now equipped with fans which have considerably increased their efficiency.

The kiln drier is one of the oldest types. The drier consists of an upper storey that houses the drying floor, and a ground floor that accommodates the heating system. At the top of the upper floor is an exit for escape of the gases. It is employed in the drying of hops (flowers of hop plant used in brewing to impart a bitter taste), cacao, and apples. It is unsuitable for drying soft fruits.

In the tower drier, the drying chamber can accommodate several drying trays stacked one over another in the form of a tower. The heating system is like the kiln drier. The cabinet drier is similar to a tower drier except that steam coils are placed below the trays to furnish heat. In this type, the temperature of the air can conveniently be regulated and drying made more rapid. The modern types of cabinet drier consist of compartments that hold several trays over or through which hot air is blown by special fans.

The Oregon tunnel drier consists of a series of parallel, sloping narrow chambers above the furnace room. The hot air enters at the lower end of each tunnel through an opening or throat, while trays of food material (normally fruits) enter the drier at the upper or cool end and move

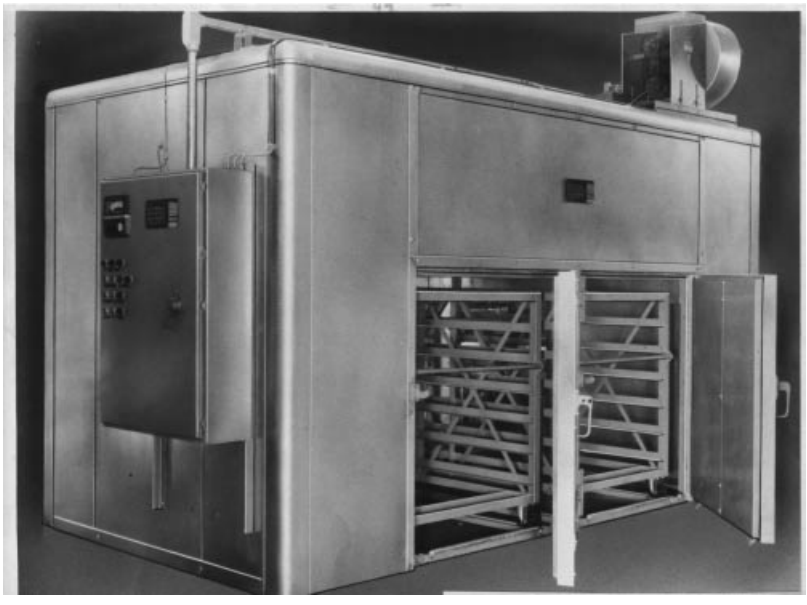

Figure 9.1 A cabinet drier (source: Awan, 2004).

towards the lower or warm end. The dry fruit is removed from the lower end of each tunnel. This type is very suitable for drying plums to produce prunes.

Forced-draft dehydrators

In principle, forced-draft dehydrators consist of a heating chamber, which may be heated by any conventional means, the drying chamber where food is placed, and a system to circulate air through the foodstuff. Normally these driers have an arrangement for re-circulation of air, hence they have increased fuel efficiency and prevent case hardening, which makes them more efficient and allows control over the product.

Tunnel driers consist of a chamber in the form of a tunnel, which is longer than its width. Depending on the direction of the movement of air over the product, a tunnel drier may be concurrent, where air and raw material move in the same direction, or counter-current (Figure 9.1), where they move in opposite directions to each other. In some driers, air enters from both sides of the tunnel and leaves from the middle. Such a drier is called a center-exhaust tunnel drier (Figure 9.2) and has the advantage of producing a better-quality product than the other driers. In another version, called a cross-flow drier, the air flows across the food.

In tunnel driers, food is normally placed in trays that are stacked on trolleys or trucks which move through the tunnel. Heating of air is done either by steam coils, electrically heated grids or hot-air furnace. The air supply is maintained by using disc, multivane, airplane propeller, axial, or paddle wheel types of fan.

The conveyer drier such as continuous draper or belt drier is similar to the tunnel drier in construction, except that the raw material moves on an endless conveyer rather than trucks or trolleys. This type is suitable for drying vegetables, starch, etc. Air is usually blown across the product in a concurrent or counter-current design.

Fluidized-bed driers are used for dehydration of particulate or diced materials, e.g., grains, cacao, coffee, onions, and diced vegetables, as well as for drying granulated materials such as sugar and salt (Potter and Hotchkiss, 1995). Hot air passes from the bed through a porous plate

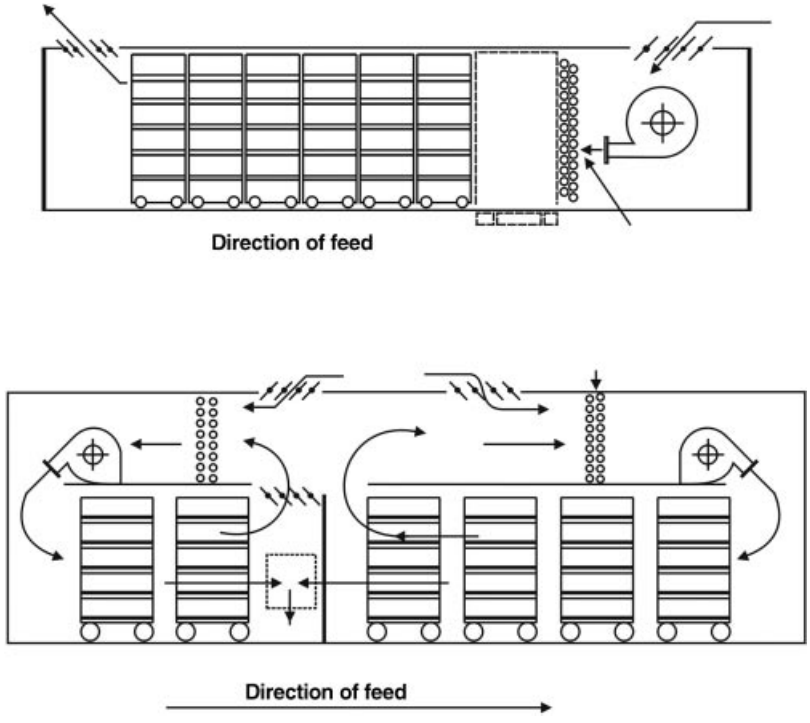

Figure 9.2 Schematic representation of a typical counter-current (top) and a center-exhaust (bottom) tunnel drier (source: Awan, 2004).

at a pressure that is enough to float the food particles in suspension. The supply of air is so regulated at different points in the bed that, in addition to removing moisture and transferring it to the outside atmosphere, it also conveys the food particles and discharges them at the exit end of the drier. The pressure of hot air through the food is just enough to suspend the particles giving an impression of a gentle boiling motion.

Spray driers are employed for liquid and other foods of low viscosity that can be atomized (Bhandari *et al.*, 1993). In a typical spray drier, liquid food is atomized into a drying chamber through a fine nozzle. Hot air is passed into the drying chamber and the food material dries instantly. As some powdered material may be lost with exhaust air, a recovery system (cyclone system) is usually attached to the air exhaust in which the suspended powdered particles are recovered and spent air is allowed to escape or re-circulate.

9.5.3.2 Drying by contact with a heated surface

Some foods – such as milk, soup mixes, baby foods, mashed potato, and others – that are either liquid or in slurry form are dried by exposing them directly to a hot surface. The drum drier is a good example in this group. It essentially consists of one or more hollow metallic cylinders or drums that are heated internally by steam, or other heating medium. Usually drum driers are classified according to the number of drums in one unit, e.g., single- or double-drum systems. The cylinder revolves on a horizontal axis. As the drum revolves, it collects a thin film of the liquid or slurry at a certain point. By the time it goes around by about 270°, the film is dried and a special scraping assembly removes the dried film and drops it onto a conveyor. Another film

of wet material then coats the drum for the next round of drying. Conventional drum driers are economical to use, but heat-sensitive products may be adversely scorched (Valentas *et al.*, 1977; Heldman and Hartel, 1997).

In the vacuum shelf drier, food is placed between two plates in a system where a vacuum can be created. The foods are dehydrated under vacuum at low temperature. The procedure is comparatively expensive, hence is used for highly priced and heat-sensitive foods.

9.5.3.3 Drying by application of energy from radiating microwave or dielectric sources

Several types of radiation in the electromagnetic spectrum are capable of producing heat in food materials and thus evaporating or boiling water from them. Infrared radiation is absorbed to a great amount by foods, resulting in heat generation. The radiation commonly employed has a wavelength of 0.8–400 nm. These are used primarily for surface heating; hence they have application in the dehydration of fruits, vegetables, grains, and tea, as well as in baking, freeze drying, and roasting of cacao beans and nut kernels (Jayaraman and Das-Gupta, 1992; Vega-Mercado *et al.*, 2001).

Some waves, especially at frequencies of 915 MHz (wavelength 3.28×108 nm, dielectric heating) and 2450 MHz (wavelength 1.22×108 nm, microwave heating), are used in the food industry for drying and other purposes. When food materials absorb waves at these frequencies, heat energy is generated. In dielectric heating, food material is heated by keeping it between parallel electrodes, while in microwave heating it is placed in a resonance cavity. In microwave heating, microwaves produced by a magnetron tube, when absorbed by the food, generate considerable heat through a series of rapidly alternating currents. The polar molecules of water are excited by the microwaves, thus generating heat. Dielectric and microwave heating have found applications in the food industry such as in concentration and drying, besides baking, cooking, blanching, pasteurization, and sterilization. These methods are also becoming popular for preliminary or finishing steps in drying potato chips, cooking chicken pieces, and baking crackers.

9.5.3.4 Special drying techniques

Special techniques are applied to produce certain effects in foods that are not possible with the normal methods of dehydration. Puff drying is employed to increase the porosity of food particles to give them a spongy look. Such products are easier to reconstitute and have a better texture than those dried by the usual techniques. Production of potato puffs represents a good example of this technique in which the escaping steam tends to puff the product.

Foam-mat drying has a similar objective, i.e., producing a product with the same or more volume than the fresh food, but having spongy texture. In this case the raw material may be whipped to trap the air, e.g., egg white. In concentrated citrus juices, fruit purees, and tomato paste an edible whipping agent is added prior to whipping. Stable foams are then cast in thin layers onto trays or belts and are dried by any appropriate system (Smith and Hui, 2004).

9.6 EVAPORATION AND CONCENTRATION

Evaporating some moisture, thereby concentrating the soluble solids, lowers the water activity of a food material. Evaporation helps to reduce the weight of food materials, thereby lowering packaging and transportation costs (Potter and Hotchkiss, 1995). Tomato paste is a

common example where evaporation is a part of the preservation technique. Initially tomato juice contains about 6% solids that are concentrated by evaporation to about 32%. Normally water activity of most evaporated foods is still high enough to encourage growth of spoilage microorganisms and, therefore, such foods require further processing. Most are given heat treatment milder than would be the case for their un-evaporated counterparts or may be concentrated by the addition of soluble constituents to further decrease their water activity.

Common salt is obtained by concentration from seawater through evaporation in artificial lagoons. In the sugar industry, sugar cane or sugar beet juice is concentrated by evaporation from approximately 15% sugar content to saturation. Sugar syrups are prepared to 70°Brix. In the production of jams, jellies and other such foods, sugar is added to fruit pulp and part of the moisture is removed by evaporation in open type or vacuum pans. Microorganisms normally do not thrive in foods that are high in sugar, especially above 65%. Hence, jams and similar products packed in hermetically sealed containers must contain at least 65% dissolved solids. If the product is packed in non-hermetically sealed containers, a minimum of 68% dissolved solids is usually desirable.

Concentration of fruit juices that must retain their very delicate chemical, textural, and sensory characteristics is carried out in special types of evaporator. These could be the natural circulation evaporators that include open-pan type, horizontal, or vertical short-tube type, natural circulation type, forced-circulation evaporator, long-tube evaporators (climbing film, falling film, and the climbing-falling film models), plate type, expanding-flow evaporator, centrifugal evaporator, low-temperature evaporator, or bubbling-type evaporator. Each of these has its own advantages and disadvantages and is suitable for concentration of specific products.

9.6.1 Freeze drying

In response to demand by consumers for preserved foods which show minimal quality differences from the fresh product, food technologists have provided various innovative solutions. Freeze drying is one processing technique that does minimal harm to a product and produces food of exceptional quality. Foods that are freeze-dried are light, porous in structure, and, when reconstituted, exhibit characteristics of the fresh commodity (Donsì *et al*., 1998). Additionally, freeze-dried products retain shape and size of the original raw material. Freeze drying is considered superior to conventional drying. The only drawback is the high cost of production (Table 9.1).

Freeze drying involves freezing of food material followed by removal of moisture by sublimation, i.e., evaporation of moisture from the solid state to vapor state without first changing into liquid. The sublimation process is carried out in a low vacuum pressure (usually at 0.1–2 mmHg). The product is often finished in an ordinary drier since some moisture may remain in the food. Some disadvantages of freeze drying include the possible damage to raw-material cell structure when freezing is poorly executed. Moreover, the product is brittle and therefore susceptible to mechanical damage. Also, the freeze-drying process is expensive compared with other conventional drying techniques. This method is suitable for coffee, fruit juices, whole shrimps, diced chicken, etc.

9.6.2 Dehydro-freezing

Dehydro-freezing is a less commonly used method of food preservation. Fundamentally, it involves partial moisture reduction by any suitable dehydration technique followed by

Table 9.1 Advantages of freeze drying over conventional drying.

Freeze drying	Conventional drying
Suitable for cooked and raw animal products	Unsuitable for meat and meat products
Adequately low temperature is applied to prevent thawing	Normal temperatures range between 37 and 93 °C are applied
Moisture loss takes place by sublimation of ice without entering the intermediate liquid stage	Evaporation of water takes place from surface of the food
Highly porous dried and hygroscopic particles produced which are readily reconstituted	Produces solid dried particles
Yields lower-density dried products than the original food	Yields higher-density dried products than the original food
Product has natural odor	Product often has an abnormal odor
Rapid and complete rehydration is possible	Product takes more time for rehydration, which is partially complete
Color of product is almost nearly natural	Color of product is typically darker than the fresh one
Product has normally natural flavor	Product has normal flavor
Carried out at pressures below 4 mmHg	Commonly carried out at atmospheric pressure
Product has excellent storage stability	The product has tendency to darken during storage
Dehydration may be completed within 12–24 h	Dehydration may be completed within a short time, commonly less than 12 h

Data from various sources.

freezing rest of the moisture present in the food. The freezing ensures an extra reduction in water activity. In practice, the process consists of removal of about 50% moisture from the foodstuff and then subjecting this partly dehydrated material to normal freezing operations. In this way, the bulk of the food is reduced, thereby lowering storage, transportation, and handling costs. The product is superior in quality to a purely dehydrated one, as it better retains the flavor and texture.

9.6.3 Intermediate-moisture food technology

The available moisture in food can be bound, making it unavailable for spoilage agents. This technique is employed in the production of intermediate-moisture foods that have moisture content usually varying from 20 to 50%. Examples of such foods are honey, jams, jellies, confectionery products, and sweetened condensed milk. The water activity of intermediate-moisture foods generally ranges from 0.70 to 0.85, which is low enough to prevent the growth of many spoilage bacteria and yeasts (Erickson, 1982). However, this water activity is relatively high for several spoilage molds and osmophilic yeasts. The moisture content in these foods is lower than in fresh commodities but higher than in conventionally dehydrated foods. The available moisture left after binding with chemicals is not high enough to support enzyme and microbial activity. Thus, the food remains semi-moist and can be kept on kitchen or retail shelves for long periods. The greatest preservation effect in these products is on account of the high concentration of solutes resulting in high osmotic pressure. Additionally, salts, acids, and other chemical substances may be used to extend storage life further. These foods also have simpler packaging requirements and can be packed in inexpensive protective wrappers.

The principle underlying the technology of intermediate-moisture foods is that water activity of the material is lowered by partly removing the moisture. This is followed by the

Table 9.2 Causes of spoilage of dried products during storage (Dauthy, 1995).

Defect	Cause	Remedies
Mold growth	Due to high moisture content of a product, above equilibrium relative humidity corresponding to water activity $a_w = 0.70$	Reduce water content down to optimum level Pack in a hermetically sealed package
Insect infestation	Presence of larvae or insects in dried product	Disinfection of storage chamber with toxic gases Fumigation of packed products and of packages Disinfection by heating (60–65 °C) of products prior to packing
Browning	Chemical reaction (Maillard, etc.)	Reduce water content down to optimum level Store in a cool place
	Enzyme-catalyzed reactions	Enzyme inactivation by heat treatment such as blanching or steaming prior to drying
Low rehydration ratio	Temperature too high in final stages of drying	Maintain final temperatures as recommended inside dehydrator

addition of such chemicals as common salt, glycerol, sorbitol, sucrose, glucose, or others that bind part of the remaining moisture. In these products, microbial growth is further retarded by employing antimicrobial agents, especially antimycotics such as propylene glycol and/or sorbic acid or its potassium salt. The technology of producing intermediate-moisture foods could particularly be useful and practicable for developing countries that lack the normal low-temperature storage facilities.

9.7 SPOILAGE OF DRIED FRUITS AND VEGETABLES

During storage, dried fruits and vegetables are attacked by insects which can be controlled by fumigation. Before storing, rooms should be sanitized to minimize the chances of infestation. Fumigation with ethylene oxide or phostoxin inside storage rooms can reduce the invasion of insects. Under damp conditions, dried fruits become musty or moldy and dried vegetables become soft and slimy. Proper packaging of dried products can minimize the chances of attack of these spoilage agents. Table 9.2 shows the possible defects, causes, and remedial measures.

9.8 MERITS OF DEHYDRATION OVER SUN DRYING

During the drying process, the relationship between the temperature of the air and moisture in food is referred to as the psychrometric relation. There are a number of factors affecting the drying process:

- size, shape, and arrangement of stacking of product;
- composition of fruits and vegetables;
- temperature, humidity, and velocity of air;
- heat transfer to the surface of raw material;
- pressure of atmosphere.

Since the dehydration is done under controlled conditions, it has many advantages over the process of sun drying:

- dehydration is quicker than sun drying;
- dehydration needs much less floor area than sun drying;
- dehydration is done in a hygienic environment;
- dehydration is not at the mercy of weather; it is possible during the rainy season;
- the color of dehydrated product is more attractive and uniform due to controlled drying temperature.

9.9 EFFECTS OF DEHYDRATION ON NUTRITIVE VALUE OF FRUITS AND VEGETABLES

Due to the loss of moisture during drying from fresh fruits and vegetables, the concentration of nutrients is enhanced. The amount of nutrients such as proteins, fats, and carbohydrates is greater in dried food than in fresh food per unit weight (Table 9.3). However, the quality of dried food is not comparable to fresh food in terms of loss of vitamins (Awan, 2007). The water-soluble vitamins are oxidized. They are also decreased during blanching, which causes enzyme inactivation. Ascorbic acid and carotene are destroyed by oxidation. Riboflavin is damaged by light. Thiamin is heat-sensitive and is also destroyed by sulfuring. More appreciable ascorbic acid is lost in sun-dried fruits than when freeze-dried. The carotene content of vegetables is decreased by 80% if processing is done without blanching. Rapid drying retains more ascorbic acid than slow drying. The major losses of carbohydrates take place in fruits. Discoloration may be due to the action of enzymes or caramelization-type reactions.

9.10 EFFECTS OF DRYING ON MICROORGANISMS

Microorganisms need moisture for their metabolic activities and multiplication. While yeasts and bacteria require higher amounts of moisture, typically above 30%, molds need much less, 12% moisture or even lower. Some can grow on food substrates even having less than 5% moisture. Fruits are dried to between 16 to 25% moisture. There are chances of mold growth if these are exposed to air and stored under high-humidity conditions. Above 2% moisture and under favorable conditions, mold growth is expected.

The most effective control is the use of high-quality vegetables with low contamination, blanching prior to drying, processing in a hygienic environment, and storage under conditions where the dried foods are protected from invasion of microbes, insects, rodents, and other animals (Gallardo-Guerrero *et al.*, 2010).

Table 9.3 Chemical composition of fresh and dried peas (Desrosier and Desrosier, 1987).

Nutrient	Fresh	Dried
Moisture (%)	74.0	5.0
Fats (%)	1.0	3.0
Carbohydrates (%)	17.0	65.0
Proteins (%)	7.0	25.0
Ash (%)	1.0	2.0

9.11 EFFECT OF DRYING ON ENZYME ACTIVITY

Enzymes need moisture for their activities. Activity is nil at moisture levels below 1%. Enzymes are more sensitive to moist heating conditions and are inactivated or destroyed near the boiling point of water. When enzymes are exposed to dry heat at the same temperature, such as applied in drying, they are markedly insensitive to the effect of the heat. Even, exposure to a temperature near 204°C for a short time has little effect on enzymes if heating and the enzyme preparation is dry. The enzymes catalase and peroxidase are used as indicators of enzyme activity.

9.12 INFLUENCE OF DRYING ON PIGMENTS

The color of fruits and vegetables are altered as a result of drying. Carotenoids are mostly changed during drying. Anthocyanins are also destroyed by drying treatments. They are bleached by sulfur treatment. On the other hand, browning and Maillard reactions occur during conventional dehydration of fruits. If fruits are treated with sulfur, enzymatic browning and Maillard reactions can be inhibited or minimized.

The natural green pigment of vegetables is a mixture of chlorophyll a and chlorophyll b. The retention of the natural green color of chlorophyll is directly related to the retention of magnesium in the pigment molecules. During moist heat treatment, it is converted to pheophytin due to loss of magnesium. The color becomes an olive green instead of grass green. However, this conversion of magnesium can be controlled by changing the medium to slightly alkaline.

9.13 RECONSTITUTION TEST

(i) Weigh out a sample of 35 g from the bulked and packed final product of the previous day's production.
(ii) Put the sample into a small container (beaker) and add 275 mL of cold water and 3.5 g of salt.
(iii) Cover the container with a watch-glass and bring the water to boil.
(iv) Boil gently for 30 min.
(v) Turn the sample out into a white dish.
(vi) At least three judges should then examine the sample for palatability, toughness, flavor, and presence or absence of bad flavors. The testers should record their results independently.
(vii) The liquid left in the container should be examined for traces of sand/soil and other foreign matter.

(Srivastava and Sanjeev, 2002; Awan and Rehman, 2009)

The test can be used also to examine dried products after they have been stored for some time. Evaluation of rehydration ratio may be performed according to the following calculations.

Rehydration ratio: if the weight of the dehydrated sample (a) used for the test is 5 g and the drained weight of the rehydrated sample (b) is 35 g, then:

$$\text{Rehydration ratio} = b/a = 35/5 = 7 : 1$$

Table 9.4 Schedule for drying fruit.

Fruit	Preparation and pretreatment	Sulfuring time	Drying temperature and time
Aonla	Wash, grate, add salt (at 40 g/kg grated material)	–	Sun dry
Apples	Sort, wash, peel, core, trim and cut slices into 5 mm thick slices	30 min or immerse in 1–2% KMS solution for 30 min and drain	Kiln dry at 60–71°C for 6–10 h or sun dry
Apricots	Wash, halve, destone, steam blanch	15–25 min	57–68°C for 10–12 h or sun dry
Banana	Wash, peel, halve cut lengthwise, or slice crosswise 12 mm thick	15–30 min	55–91°C for 18–20 h or sun dry
Dates	Wash, dip in boiling 0.5–2.5% lye solution, then rinse	–	45–50°C for 20–24 h or sun dry
Figs	Wash	1 h (only Adriatic variety)	55–60°C for 17–18 h or sun dry
Grape (Muscat and wine variety)	Dip in boiling 0.5% lye solution, then rinse	3–5 h	66–82°C for 20–30 h in a drier or sun dry
Mango	Wash, peel, cut into 12 mm-thick slices	2 h	45–50°C for 24–30 h or sun dry
Papaya	Wash, peel, halve, remove seeds, cut into 6 mm-thick slices	2 h	60–65°C for 24–26 h or sun dry
Peach	Wash, halve, remove pits, cut into halves, steam blanch	15–20 min	60–63°C for 15–20 h or sun dry
Pear	Wash, peel, halve, remove core, keep in 1–2% salt solution, steam blanch	15–30 min or immerse in 1–2% KMS solution for 30 min and drain	60–65°C for 24 to 30 h or sun dry

KMS, potassium metabisulfite; data from various sources.

Table 9.5 Schedule for drying vegetables.

Vegetable	Preparation	Pretreatment	Drying temperature and time
Beet	Wash, peel, cut into 10 mm-thick slices	Steam for 10 min	60–65°C for 12–15 h or sun dry
Bitter gourd	Wash, remove both ends, scraped, cut into 10 mm-thick slices	Blanch for 7–8 min	66–70°C for 7–9 h or sun dry
Brinjal	Wash, cut lengthwise into 10 mm-thick slices	Blanch 4–5 min, immerse for 1 h in 1% KMS solution, drain	49–52°C for 9–11 h or sun dry
Cabbage	Wash, remove outer leaves and core, cut into 10 mm-thick shreds	Blanch 5–10 min, immerse for 10 min in 0.5% KMS solution and drain or boil in sodium bicarbonate solution for 3–4 min	60–66°C for 12–14 h or sun dry
Carrot	Wash, remove stalks and tips, cut into 10 mm-thick slices	Blanch for 2–4 min in boiling 2–4% common salt solution.	68–74°C for 14–16 h or sun dry
Cauliflower	Wash, remove stalks covering leaves and stems, break flowers apart into pieces of suitable size	Blanch 4–5 min, immerse for 1 h in 1 % KMS solution, drain	60–66°C for 10–12 h or sun dry
Chillies (red)	String mature dark red pods and hang in sun	No treatment	50–60°C for 16–18 h or sun dry
Garlic	Peel clove, use as such or cut into 5 mm-thick slices	Dip for 10 min in 5% salt solution, drain	60–65°C for 15–17 h or sun dry
Green beans	Wash and remove strings, split pods lengthwise	Blanch for 3–6 min	60–65°C for 16–20 h or sun dry
Green peas	Wash, remove shell	Blanch or steam for 3–4 min, immerse in 0.5% KMS solution and drain	60–65°C for 15–18 h or sun dry
Okra	Wash, whole or cut into 10 mm disc or halve cut lengthwise	Blanch in boiling water for 4–8 min or steam blanch for 2–5 min, rinse with cold water	63–68°C for 6–8 h or sun dry
Onion	Remove outer dry scales, cut into 5 mm-thick slices	Dip for 10 min in 5% salt solution, drain	60–65°C for 11–13 h or sun dry
Potato	Wash, peel, cut into 8–10 mm-thick slices	Blanch in boiling water or steam for 3–5 min, immerse in 0.5% KMS solution	60–66°C for 9–11 h or sun dry
Pumpkin	Wash, peel, cut into 10 mm thick slices, dip in 2% common salt solution	Blanch in 2% common salt solution for 3–4 h	65–71°C for 7–8 h or sun dry
Spinach, fenugreek, other leafy green vegetables	Sort, wash, trim off rough stems and stalks, shred	Blanch for 2 min in boiling water or steam	60–65°C for 6–8 h or sun dry
Tomato	Wash	Blanch for 30–60 seconds, peel and slice 10 mm thick	60–65°C 18–20 h or sun dry
Turnip	Wash, peel, cut into 5 mm-thick slices	Blanch for 2–4 min in boiling water, immerse for 1–2 hours in 1% KMS solution	50–55°C 13–15 h or sun dry

KMS, potassium metabisulfite; data from various sources.

Rehydration coefficient: if the drained weight of 12 g of dried sample containing 4% moisture after rehydration is 65 g and the fresh sample before drying contained 90% moisture, then:

$$\text{Rehydration coefficient} = A \times (100-B)/(C-D) \times 100$$

Where A is the drained weight of the dehydrated sample, B is the moisture content of the sample before drying, C is the weight of the dried sample taken for rehydration, and D is the amount of moisture present in the dried sample taken for rehydration:

$$\text{Rehydration coefficient} = 65 \times (100-90)(12-0.4) \times 100 = 0.55$$

$$\text{Water present in the rehydrated product}\,(\%) = (a-b/a) \times 100$$

Where a is the drained weight of the rehydrated product and b is the dry matter content in the sample.

The moisture content of the rehydrated sample is $(65 - 11.8/65) \times 100 = 81.85\%$.

9.14 DRYING PARAMETERS

In drying of fruits and vegetables by conventional methods, certain operations are essential to produce good quality products. These include preparatory operations such as washing, peeling, removal of inedible constituents and size reduction (Fellows, 2000). To prevent discoloration and maintain color of the product, sulfuring by the use of burning sulfur or sulfiting by dipping in potassium metabisulfite solution in appropriate concentration are common practices prior to drying (Girdhari *et al*., 1998). Some essential requirements for drying of fruits are given in Table 9.4 and for vegetables in Table 9.5.

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10 Developments in the Thermal Processing of Food

Tareq M. Osaili

Abstract: The application of heat is an important means of preserving foods. The need to maximize process efficiency and final product safety and quality has led to a number of new developments and improvements in existing thermal processing technology. Recently, electroheating technologies such as radio frequency heating, microwave heating and ohmic heating have emerged to replace the traditional well-established preservation processes. These technologies have been used to inactivate microorganisms in different types of the food.

Keywords: electroheating techniques; high-electric-field alternating current heating; microorganisms; microwave heating; ohmic heating; radio frequency heating; thermal processing

10.1 INTRODUCTION

Recently, market and consumer demands for more convenient and varied food products have increased. This increase puts pressure on food processors to maintain quality and safety of food, particularly heat-sensitive foods. These requests together with the severity of the traditional thermal processing technologies have lead to improvements in existing technologies and the development of new food-preservation technologies. Therefore, novel food-preservation technologies have emerged to replace the traditional well-established preservation processes.

Electroheating technologies in food processing have gained increased industrial interest and have potential to replace, at least partially, traditional processes. Electroheating can be divided into either direct electroheating where electrical current is applied directly to the food (e.g. ohmic heating) or indirect electroheating (e.g. radio frequency or microwave heating) where the electrical energy is first converted to electromagnetic radiation which subsequently generates heat within a product. These novel electroheating technologies are considered as volumetric forms of heating in which thermal energy is generated directly inside the food. This common pattern of heat generation allows one to overcome excessive cooking times and consequently may have direct implications in terms of both energetic and heating efficiency. The major applications of these novel heating technologies are cooking, pasteurization, sterilization, defrosting, thawing, and drying. Some of these electroheating processes (e.g., ohmic heating and radio frequency heating) are used only in industrial situations while microwave heating can be applied commercially and is also commonly used domestically.

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The interest in electroheating has increased in recent years, as evidenced by the increasing number of publications in this area (Vicente and Castro, 2007; Marra *et al*., 2009; Pereira and Vicente, 2010).

This chapter covers the improvements in thermal processing and the use of electroheating technologies (novel technologies) including radio frequency, microwave and ohmic heating in food processing and as a means of inactivating microorganisms in foods.

10.2 THERMAL PROCESSING

Food preservation while ensuring its safety and quality has been a main goal of food processors. The use of heat for thermal processing is still a primary practice in the food industry to improve the quality and guarantee the microbiological safety of their products. These traditional heating methods (i.e., pasteurization, sterilization, drying, and evaporation) depend basically on the generation of heat outside the product to be heated, by combustion of fuels or by an electric resistive heater, and its transference into the product through conduction and convection mechanisms (Pereira and Vicente, 2010). The effectiveness of thermal processing in foods is influenced by product and pathogen characteristics, and the processing conditions. These product characteristics include the fat level, composition, pH, size and shape, levels and types of preservatives, and water activity. The pathogen's characteristics include the strain of the bacterial pathogen, its growth conditions, and resistance to stress such as acid and/or heat. The processing conditions include the heating source, heating rate, processing type, and environmental conditions while processing (Liu *et al*., 1997; Casadei *et al*., 1998; Doyle *et al*., 2001; Murphy *et al*., 2001; Chhabra *et al*., 2002).

Thermal inactivation of microorganisms in foods occurs through the irreversible denaturation of enzymes, proteins, nucleic acids, or other cellular constituents vital to cell metabolism or reproduction, resulting in cellular death. Metabolites and cofactors important to cellular function may leak through membranes damaged by heat, resulting in cellular death (Heddleson and Doores, 1994).

10.2.1 Thermal inactivation kinetics

The heat-transfer process can be used to build mathematical relationships between the heating rate of the food and the temperature of the coldest portion of the food. Models have been developed for many of the different types of food-processing system. In a similar way, mathematical relationships have been developed to describe the kinetics of thermal inactivation of microorganisms (Food and Drug Administration (FDA), 2000).

Thermal destruction of microorganisms is a time–temperature process which has long been expressed by the concept of decimal reduction time (D value) and thermal resistance constant (z value). The D value represents a heating time that results in 90% reduction of the existing microbial population. It reflects the tolerance of a microorganism to an increase in heating time at a specific temperature. D value at each temperature is calculated from the linear regression model between $\log_{10}$ of the bacterial survivors and heating time. The D value is the negative inverse slope of the survivor curve. The D value is expressed mathematically as follows:

$$D = \frac{t_2 - t_1}{\log_{10}(A) - \log_{10}(B)}$$

where A and B represent the survivor counts following heating for times t_1 and t_2 minutes (Awuah *et al.*, 2007; Griffis and Osaili, 2009).

The z value is the temperature difference required for the thermal inactivation curve to cause a 1 $\log_{10}$ reduction. It is correlated with the tolerance of a specific pathogen to the temperature changes in the product. The z value is calculated by determining the linear regression between $\log_{10}$ of D values and their corresponding temperature. The z value is the negative inverse slope of the thermal resistance curve. The z value is represented mathematically as follows:

$$z = \frac{T_2 - T_1}{\log_{10}(D_1) - \log_{10}(D_2)}$$

where D_1 and D_2 are D values at temperatures T_1 and T_2, respectively (Awuah *et al.*, 2007; Griffis and Osaili, 2009).

To calculate D and z values, microbial inactivation has traditionally been assumed to follow first-order kinetics; i.e., at a certain temperature, the $\log_{10}$ reduction of bacteria is linear over time (Heldman and Hartel, 1997). This assumption is applicable if (i) the relationship of $t\{T, N\} = g\{T\}.f\{N\}$ describes the thermal inactivation of microbes, where t is heating time at a certain temperature (T) and N is number of survivors at time t (Kormendy and Kormendy, 1997); (ii) the sub-lethality injury phenomenon is ignored; and (iii) the heating effect on the microorganisms in the food sample is homogenous (Moats, 1971). Figure 10.1 shows typical survivor and decimal reduction curves.

Efforts have been increasing to prevent foodborne illnesses and make the food supply safe. The US Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS, 1999) issued a final rule that requires each processing schedule in the meat or poultry industry to achieve a 6.5-*D* reduction in the population of a cocktail of *Salmonella* serotypes in ready-to-eat beef products and a 7-*D* reduction of a cocktail of *Salmonella* serotypes in fully cooked poultry products. The regulation applies to highly heat-resistant strains as well as to strains that have been implicated in foodborne outbreaks. The FDA (2001) set performance standards that require a minimum of 5 $\log_{10}$ reduction of *Escherichia coli* O157:H7 in processed juice. Furthermore, in the canning industry for low-acid foods, it is necessary to achieve a 12-*D* reduction in *Clostridium botulinum* spores for safety assurance from a public health standpoint.

10.2.2 Process lethality of thermal process

Estimated kinetic data (D and z values) and time/temperature history (heat-penetration data) during processing at a pre-defined location (cold spot) within the product are necessary to

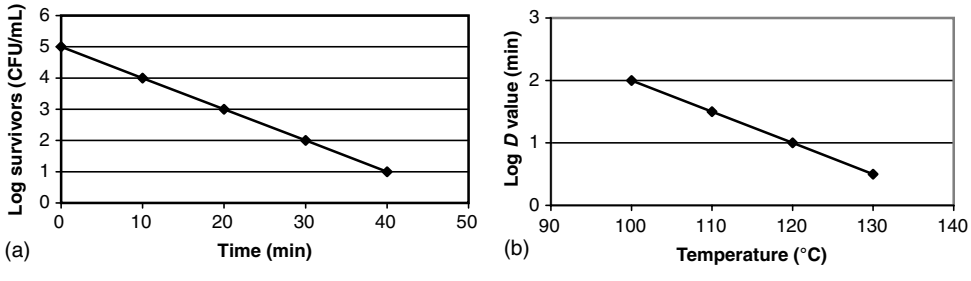

Figure 10.1 Typical (a) survivor and (b) decimal reduction curves (Awuah *et al.*, 2007).

calculate "process lethality" (F_o), which forms the basis for a sound thermal process:

$$F = \int_0^t 10\left(\frac{T(t)-T(ref)}{z}\right)dt$$

where $T(t)$ is the product temperature at a time t and $T(ref)$ is a reference temperature (Griffis and Osaili, 2009).

This equation states that thermal inactivation parameters (D and z values) for a certain microorganism should be known in order to calculate the process lethality during the thermal process. The regulation can be met if the time obtained from the process lethality is equal to or more than the performance standard.

10.2.3 Requirement of thermal process

The establishment of safe thermal processes for foods is based on two major factors: (i) knowledge of the time/temperature combination required to inactivate the most heat-resistant pathogen of concern in a particular food product; and (ii) determination of the nature of heat-penetration characteristics of the food system, generally defined by a heat-transfer rate (Awuah *et al.*, 2007). This information is necessary to establish the scheduled process, thus ensuring inactivation of pathogen(s) in that product. The validity of the established process is often confirmed using an inoculated test pack study which would be tested under actual processing conditions to reproduce the target organism and the actual process conditions. Since it is unwise to introduce viable pathogens into the production area of processing plant, surrogate organism(s) are often used in the inoculated pack study, and their inactivation is measured to validate the process. Surrogates play an important role as biological indicators that can mimic the thermal inactivation properties of a pathogen and can help to detect peculiarities or deviations in the processing procedure. Surrogates need to be non-pathogenic, non-spoilage organisms, genetically stable, have stable thermal and growth characteristics, not susceptible to injury or reversible inactivation properties, follow inactivation kinetics in a manner similar to pathogens receiving the same inactivation treatment, durable in food and processing parameters similar to target organism, easily prepared into high-density population forms, and easily differentiated from natural flora. Enumeration of surrogates in the tested food should be rapid and with inexpensive detection methods (FDA, 2000). Examples of surrogates used in validation of food processing are strains of *Listeria innocua* which have served as non-pathogenic surrogates for the pathogenic bacteria *Listeria monocytogenes*. The non-pathogenic strains of *E. coli* have served as surrogates for *E. coli* O157:H7. *Clostridium sporogenes* or *Bacillus stearothermophilus* are used as surrogates for *C. botulinum*. Novel technologies need extensive kinetic data for key pathogens of concern and appropriate surrogates in establishing their effectiveness and significance in preserving foods (FDA, 2000; Friedly *et al.*, 2008).

10.2.4 Process verification/validation

Process verification and validation is a way to guarantee the safety of thermally processed foods. It is often desirable to confirm calculated processes using inoculated pack- or count-reduction procedures. Process is verified/validated through inoculating the test product with an appropriate surrogate of known resistance and subjected to various heating times at one or a number of different processing temperatures. The validation process should be designed so

that the surrogate exhibits a predictable time/temperature process character profile that correlates to that of the target pathogen. The product is then incubated at the appropriate growth conditions (temperature, time, and aerobic, or anaerobic conditions) for survivors. An adequate process would be one with no evidence of spoilage or test organism (Awuah *et al.*, 2007). Introduction of system modifications or variables, leading to inaccurate results (e.g., thermocouple probes changing heating rates, nutrients added to the product for surrogate growth altering viscosity, food additives, etc.) should be avoided (FDA, 2000).

10.3 INNOVATIVE THERMAL PROCESSING TECHNIQUES

10.3.1 Indirect electroheating techniques: radio frequency and microwave

Radio frequency and microwaves are innovative techniques that have been used as alternatives to conventional processing methods to provide high-quality foods. Radio frequency and microwave heating refers to the use of electromagnetic waves of certain frequencies to generate heat in a material. They imply the interaction between an electromagnetic alternating field and the dipoles and ionic charges contained within a food product that enables the volumetric heating of the product. Both systems operate by the same principle, forcing polar molecules such as water and ionic species to continuously realign themselves by reversing an electric field around the food product. Radio frequency and microwaves heating are used for pasteurization and sterilization, thawing of frozen foods, meat tempering and tenderization, drying, cooking, roasting, and baking and are favored over conventional heating because they heat food rapidly and therefore require less time to come up to the desired process temperature, particularly for solid and semi-solid foods and may distribute heat relatively more uniformly than conventional heating. These techniques cause microbial inactivation mainly through thermal effects. Commercial microwave pasteurization and sterilization systems for foods have been known for decades, but commercial radio frequency heating systems for the purpose of food pasteurization or sterilization are not known to be in use (Piyasena *et al.*, 2003; Pereira and Vicente, 2010).

10.3.1.1 Radio frequency

The radio frequency band is part of the electromagnetic spectrum; it covers a broad range of high frequencies, either in the kHz range (between 3 kHz and 1 MHz) or MHz range (between 1 and 300 MHz). The radio frequency bands can interfere with communication systems; therefore, only three selected frequencies are permitted for domestic, industrial, scientific, and medical applications. These frequencies are 13.56, 27.12, and 40.68 MHz. Radio frequency is non-ionizing radiation; it has insufficient energy (less than 10 eV) to ionize atoms. It involves the heating of poor electrical conductors. It is also characterized by freedom from electrical and mechanical contact (Piyasena *et al.*, 2003; Awuah *et al.*, 2005; Pereira and Vicente, 2010).

Unlike conventional heat-transfer modes where heat energy is transferred from a hot part to a cold part resulting in temperature gradients, radio frequency thermal treatment heats foods directly through energy conversion, from electrical energy to heat (Rowley, 2001). Radio frequency heating involves the transfer of electromagnetic energy directly into the product, initiating volumetric heating due to frictional interaction between molecules.

Volumetric heating means that all parts of the food are heated together and at the same rate. This provides uniformity in heating, avoids overheating in the outer regions of the product, and could potentially increase product quality and nutritional value while reducing cooking times (Brunton *et al*., 2005). Therefore, radio frequency cooking achieves high energy efficiency, saves cooking time, and heats food uniformly (Orsat *et al*., 2001; Rowley, 2001).

The mechanism of radio frequency heating relates to the fact that the molecules within a product placed in a radio frequency environment realign themselves with the polarity of the electric field. Since the polarity changes rapidly at 27 MHz (27 million times per second), the molecules try to constantly realign themselves with the electric field by flip-flop motion. This response initiates volumetric heating within the entire product due to frictional interaction between the molecules (Piyasena *et al*., 2003; Awuah *et al*., 2005). Radio frequency heating is accomplished through a combination of dipole heating and electrical resistance heating resulting from the movement of dissolved ions present in the food (Awuah *et al*., 2005).

Radio frequency has been used in food applications since 1940s where was used to cook meat, bake bread, and dehydrate or blanch vegetables. Due to the high operating cost of radio frequency energy at that time, the process did not result in any commercial installation. Then it was used to thaw frozen products in the 1960s, to reduce microbial populations in fruit juice in the 1970s, to dry cookies in the 1980s, and to pasteurize sausage emulsion in the 1990s (Piyasena *et al*., 2003; Marra *et al*., 2009). Recently, radio frequency heating has been evaluated for pasteurization of meat, fish products, liquid milk, and apple juice thermally and non-thermally (Al-Holy *et al*., 2004; Geveke and Brunkhorst, 2004, 2008; Guo *et al*., 2006).

Radio frequency cooking can be used to reduce microbial contamination and improve food safety and quality. The effectiveness of radio frequency on the reduction of microbial contamination of fresh carrots (Orsat *et al*., 2001), meat (Houben *et al*., 1991; Brunton *et al*., 2005; Guo *et al*., 2006), apple juice (Geveke and Brunkhorst, 2004), milk (Awuah *et al*., 2005), and apple cider (Geveke and Brunkhorst, 2008) has been investigated. The effect of radio frequency on food quality has also been studied on ground beef (Laycock *et al*., 2003; Guo *et al*., 2006), caviar (Al-Holy *et al*., 2004), and pork meat products (Brunton *et al*., 2005) and the results showed that radio frequency cooking led to shorter cooking time, and acceptable color and texture.

As mentioned before, radio frequency heating offers the advantage of a reduction in processing times over conventional heating methods. Brunton *et al*. (2005) reported that cooking pork-based white pudding to an end-point temperature of 73°C took 7 min 40 s by radio frequency cooking (power 450 W) while end-point temperatures in water-bath- and steam-oven-heated products were achieved after 29 and 33 min, respectively. Similarly, Al-Holy *et al*. (2004) found that the heating times in radio frequency (6 kW, 27.12 MHz)-heated caviar (9.18–13.32 min) were significantly lower compared to equivalent water-bath-heated caviar (17.68–18.94 min) at all studied temperatures (60–65°C). Guo *et al*. (2006) compared the radio frequency cooking method at a frequency of 27.12 MHz and 1.5 kW with the water-bath cooking method on the inactivation of *E. coli* K12 (a surrogate for *E. coli* O157:H7) in meat. The samples were cooked to reach a central temperature 72°C. The time for radio frequency cooking (4.25 min) was much shorter than that for water-bath cooking (150.33 min).

Zhang *et al*. (2004) developed a cooking protocol for pasteurizing meat emulsion samples. They reported reductions of up to 79% in pasteurization times for meat products compared to equivalent steam-cooked samples. The effect of radio frequency on cooking time of two types of pork products (leg ham and shoulder ham) were compared to steam-cooked samples. Radio frequency cooking of the hams resulted in a shorter cooking time (McKenna *et al*., 2006).

Laycock *et al.* (2003) compared the heating rate, time/temperature profiles, and quality of three meat products (ground, comminuted, and non-comminuted muscle) cooked in a water bath or by a 1.5 kW radio frequency heater operated at 27.12 MHz. They found that radio frequency cooking was up to 25 times faster compared with of conventional cooking times in a water bath. Also, they found that the surface of the radio frequency-cooked products heated at a faster rate than the center, with differences in temperatures of 10–20°C at the end of the process, which the authors attributed to an uneven salt distribution in the meat.

Regarding quality, the radio frequency-cooked samples had acceptable color, texture, and sensory attributes. Laycock *et al.* (2003) found that the texture of radio frequency-cooked whole muscle was not significantly different to water bath-cooked samples. Orsat *et al.* (2004) examined moisture loss, color changes, total bacterial surface counts, and sensory quality attributes (off-odors and sliminess) of radio frequency (600W at 27.12 MHz)-pasteurized vacuum-packaged ham slices. The ham samples were packed in plastic films and were pasteurized to internal temperatures of 75 and 85°C in 5 min by radio frequency and maintained at those temperatures for an additional 5 min by adjusting the radio frequency power. The results of the study showed that radio frequency heating, coupled with appropriate packaging, could improve the storability of repacked hams by decreasing the bacterial load, reducing the moisture loss, and maintaining an overall greater product sensory and quality acceptance. Likewise, Brunton *et al.* (2005) found that radio frequency cooking did affect the quality of pork-based white pudding compared with that cooked by conventional methods. In their study, the panelists were not able to detect differences between pudding cooked by radio frequency and conventional methods at 80°C (steam processing or hot-water immersion). Radio frequency-heated puddings were not significantly different from water-bath- and steam-oven-heated products with regard to instrumental color, instrumental texture, and expressible fluid. A similar finding was reported by Al-Holy *et al.* (2004) who compared the color of two types of radio-frequency-treated caviar (salmon caviar and sturgeon caviar) at 60, 63, and 65°C with the untreated control samples; they found that the visual quality of the caviar products treated by radio frequency was comparable to the untreated control. No significant differences in color measurement (L^*, a^*, and b^*) values were detected between the control and the radio frequency-treated salmon caviar except in the case of salmon caviar treated by radio frequency at 65°C. For sturgeon caviar treated by radio frequency heating, the visual quality of the product did not change at the different temperatures. Orsat *et al.* (2001) used a parallel-plate radio frequency applicator to improve and extend the storability of vacuum-packaged carrot sticks stored at 5–6°C. They found that carrot sticks can be heated to 60°C in less than 2 min, and thus reduce the initial total microbial load. The radio frequency treatments were compared to dipping in chlorinated water, and in hot water. The quality (color, taste) of the radio frequency-treated samples was greater than for either the control samples (chlorinated water) or hot-water-treated carrot samples. Geveke *et al.* (2007) found that radio frequency treatment at 20 kV/cm and an outlet temperature of 65°C with a hold time of 2 s of orange juice at moderate temperature did not affect the ascorbic acid and did not cause enzymatic browning.

Some researchers, however, reported contrasting results. For instance, van Roon *et al.* (1994) reported that radio frequency-cooked meat doughs were much firmer than samples cooked in a water bath. In addition, Laycock *et al.* (2003) reported that radio frequency-cooked comminuted beef samples was more soggy than the conventionally cooked product. Laycock *et al.* (2003) found some textural differences between radio frequency- and water bath-cooked ground and comminuted meats, with radio frequency-cooked ground meat having higher springiness and chewiness and radio frequency-cooked comminuted meat

having lower hardness and higher springiness than water bath-cooked samples. McKenna *et al.* (2006) compared the effect of radio frequency cooking and steam cooking in terms of the cook yield, water-holding capacity, texture profile analysis, penetration test, Warner–Bratzler shear, and color and sensory evaluation of two types of pork products (leg ham and shoulder ham). Instrumental measurements indicated that radio frequency-heated samples had a higher cook yield, but a lower water-holding capacity. Texture profile analysis indicated that radio frequency-cooked samples were harder, particularly for leg hams. A sensory panel also indicated that panelists could distinguish between radio frequency- and steam-cooked samples. Many of these differences in quality parameters of radio frequency-cooked samples and their steam-cooked counterparts may be attributed to differences in the level of protein denaturation. Similarly, Zhang *et al.* (2004) studied the effect of radio frequency cooking on the texture, color, and sensory properties of a large-diameter comminuted meat product. Instrumental quality assessments showed that radio frequency-heated meat batters had a greater ability to hold water, were significantly harder, chewier, and gummier, while having less color development during cooking than their steam-cooked counterparts. Differences were also detected by sensory methods. However, the authors suggested that it is possible to eliminate the differences by adjusting the cooking protocol to produce similar cook values in radio frequency-heated samples to those in products cooked by steam.

The principle of radio frequency inactivation of microorganisms

There was a thought that microbial inactivation by radio frequency is due to heat solely (Carroll and Lopez, 1969). But, in 2008, Ukuku *et al.* (2008) proved that bacterial inactivation by radio frequency electrical field does not rely on heat application alone. They suggested that radio frequency waves have also a non-thermal effect. Radio frequency treatment induces changes on the membrane structure of the bacteria. These changes led to injury, causing efflux/leakage of intracellular ATP, protein, and/or nucleic acid of the bacteria, which affected the energy status and the enzymatic activity of the bacterial cell, leading to cell death. However, they found that an application of moderate heat to the radio frequency electrical field provides a much greater effect than when radio frequency was used alone.

Thermal inactivation of microorganisms by radio frequency

Radio frequency has been used for inactivation of microorganisms in foods. Guo *et al.* (2006) compared the effect between radio frequency (27.12 MHz and 1.5 kW) and water-bath cooking in the inactivation of *E. coli* K12 in meat and found that radio frequency caused a reduction of 7 $\log_{10}$ colony-forming units (CFU)/g, similar to water-bath heating. Al-Holy *et al.* (2004) developed a radio frequency (27 MHz) pasteurization process for sturgeon and salmon caviar products (60–65°C) inoculated with a cocktail of *L. innocua* strains. An almost 3–4 $\log_{10}$ reduction in the *L. innocua* population was observed in caviar heated by radio frequency at 65°C. Similarly, Awuah *et al.* (2005) evaluated the effectiveness of radio frequency heating (2 kW, 27.12 MHz) in inactivating surrogates of both *L. monocytogenes* and *E. coli* O157:H7 cells in milk under continuous-flow conditions. They found that radio frequency heating was capable of inactivating both *L. monocytogenes* and *E. coli* in milk, with *E. coli* being the more heat sensitive of the two. For a total residence time of 55.5 s in the applicator and holding tube, up to 5 and 7 $\log_{10}$ reductions were found in *L. monocytogenes* and *E. coli* after heating, respectively at 1200 W, and an applicator-tube exit temperature of approximately 65°C. Uemura *et al.* (2010) reported that *Bacillus subtilis* spores (5×10^7) decreased in soybean milk by 3.5 $\log_{10}$ after radio frequency heating at an exit temperature of 115°C compared with 3 $\log_{10}$ after conventional heating at 100°C for 10 min.

Non-thermal inactivation of microorganisms by radio frequency

The impact of non-thermal process of radio frequency electric fields has been used to inactivate *E. coli* K12 in orange and apple juices and in apple cider at moderately low temperatures where radio frequency treatments were applied across frequency range from a minimum of 15 kHz to a maximum of 41 kHz (Geveke and Brunkhorst 2004, 2008; Geveke *et al*., 2007). Geveke and Brunkhorst (2008) developed a process to pasteurize *E. coli* K12-inoculated apple cider in an 80 kW radio frequency pilot plant system. The juice was exposed to electric-field strengths of 20–30 kV/cm at frequencies of 21, 30, and 41 kHz and treatment times varied from 140 to 420 µs. The authors found that radio frequency processing at an outlet temperature of 60°C reduced the population of *E. coli* by 4.8 $\log_{10}$, whereas thermal processing at the same conditions had no effect. They also found no significant differences among the inactivation of *E. coli* K12 in apple cider at the frequencies 21, 30, and 41 kHz; however, increasing the treatment time, field strength, and outlet temperature enhanced inactivation. The inactivation data at 20 kV/cm and 60°C followed first-order kinetics with a calculated *D* value of 74 µs. The population of *E. coli* K12 in apple cider was reduced by 3.6 $\log_{10}$ after being exposed to a 20 kV/cm peak electric field for 280 µs with an outlet temperature of 60°C. Microbiological inactivation results of that study were similar to those obtained from employing a radio frequency electrical field to process apple juice (Geveke and Brunkhorst, 2004). The population of *E. coli* K12 in apple juice was reduced by 2.7 $\log_{10}$ after being exposed to a 20 kV/cm peak electric field for 270 µs with an outlet temperature of 60°C. Also radio frequency electrical-field processing at outlet temperatures of 60 and 65°C reduced the population of *E. coli* K12 in orange juice by 2.1 and 3.3 $\log_{10}$, respectively (Geveke *et al*., 2007). Although, there was no evidence that radio frequency energy of 18 MHz and an electric field strength of approximately 0.5 kV/cm can inactivate microorganisms in liquids without heat, Geveke *et al*. (2002) indicate that this might occur at 10 kV/cm. Those authors (Geveke *et al*., 2002) studied the non-thermal inactivation of *E. coli* K12, *L. innocua*, or yeast in apple cider, beer, deionized water, liquid whole egg, and tomato juice by radio frequency energy. Radio frequency energy was applied to the liquids while heat was simultaneously removed to control temperature. An 18 MHz radio frequency processor was applied at approximately 0.5 kV/cm electric field strength to the liquids. There were no non-thermal effects of radio frequency energy detected on *E. coli* K-12, *L. innocua*, or yeast in the tested liquids; nor were there any synergistic effects of radio frequency energy with heat. The low-temperature effects of radio frequency energy at 18 MHz and 0.5 kV/cm were due to heat.

Geveke *et al*. (2009) studied the effect of radio frequency electrical-field non-thermal processing on *Lactobacillus plantarum* (a Gram-positive bacterium) in apple cider. The juice was processed with a radio frequency electrical field with a field strength of 0.15–15 kV/cm, temperature of 45–55°C, frequency of 5–65 kHz, treatment time of 170 µs, and holding time of 5–50 s. The population of *L. plantarum* was reduced by 1.0 $\log_{10}$ at 15 kV/cm, 20 kHz, and 50°C, with a 5 s hold time. There was a synergistic effect between radio frequency electrical field and heat above 50°C. Interestingly, the authors found that the inactivation increased as frequency was decreased from 65 to 5 kHz. Inactivation increased with fields above 8 kV/cm. Their study was the first to provide evidence that non-thermal radio frequency electrical-field processing inactivates Gram-positive bacteria. Awuah *et al*. (2005) reported that Gram-positive bacteria are more resistant to radio frequency heating than Gram-negative bacteria. They found that radio frequency heating of liquid milk for a total residence time of 55.5 s (29.5 and 26 s in the applicator and holding tube, respectively) reduced the population of *L. innocua* and *E. coli* K12 up to 5 and 7 $\log_{10}$, respectively, at 1200 W, and an applicator-tube

exit temperature of approximately 65°C. The draw point of this new heating technology is process validation, as it is not possible to measure physically the temperature at the cold spot with a cheap thermocouple.

10.3.1.2 Microwave heating

Microwave energy is a form of non-ionizing radiation. To some extent, microwaves are similar to radio frequency waves in heating behavior but have a higher frequency range, between 300 MHz and 300 GHz (Ryynänen, 1995). As with radio frequency, microwaves lie in the radar range and can interfere with communication systems, thus, only selected frequencies are permitted for domestic, industrial, scientific, and medical applications. These frequencies are 915 MHz, 2450 MHz, 5.8 GHz, and 24.124 GHz. (Piyasena *et al.*, 2003). Industrial microwave food-processing applications use the two frequencies of 915 and 2450 MHz, while domestic ovens use 2450 MHz.

There are two main mechanisms by which microwaves heat foods: ionic polarization and dipole rotation. Ionic polarization occurs when ions in solution move in response to an electric field. Kinetic energy is given up to the ions by electric field. These ions collide with other ions, converting kinetic energy into heat. The other mechanism (dipole rotation) is more important. It is dependent on the existence of polar molecules; the most common polar material found in foods is water. The electric field plays the primary role in heating by promoting rotation of polar molecules (2450 MHz: 2450×10^6 times/s), resulting in heat generated by molecular friction. Molecular friction is the effect of the primary absorption of microwave energy becoming the energy of hindered rotation, and vibration of the absorbing collective molecular system. Instead of existing as internal vibrational energy, the resultant energy is transferred into vibrational energy of molecules against their surroundings and the absorbing system dissipates energy as heat (Heddleson and Doores, 1994; Awuah *et al.*, 2007).

Microwave ovens are known to provide non-uniform heating (Ramaswamy and Pilletwill, 1992; Suga *et al.*, 2007), which may produce hot and cold spots within the same food item, with temperature differences reaching up to 63.9°C (Ramaswamy and Pilletwill, 1992). Pucciarelli and Benassi (2005) reported that heating with a microwave oven produced non-uniform temperatures between outside and inside of chicken samples; the difference varied from 3 to 11°C. The uneven distribution of heat questions the possible pathogen survival in contaminated food cooked in microwave ovens. Al-Holy *et al.* (2009) justified the detection of about 0.8 $\log_{10}$ of *Cronobacter* spp. in rehydrated infant milk formula reaching 77.3°C after microwaving for 40 s because of the uneven heating pattern given by the microwave heat. For that, Levre and Valentini (1998) suggested that microbial hazards associated with microwave-cooked food may be avoided by heating the food for an adequate time to an appropriate temperature, and holding for the appropriate length of time after heating.

Microwave food applications include thawing, reheating, drying, cooking, baking, blanching, pasteurization, and sterilization (Heddleson and Doores, 1994; Awuah *et al.*, 2007). In general, the quality of microwave-treated products is better or equal to that of conventional-treated products. Ramesh *et al.* (2002) found that microwave-blanched vegetables retain nutrients better compared to conventional steam or water blanching due to the reduction of leaching losses during blanching. They found that vitamin C losses in microwave blanching were reduced by 18% for spinach, 8.5% for bell peppers, and 33.5% for carrots. Picouet *et al.* (2009) evaluated the effect of a mild microwave treatment (35 s at 652 W) on the quality parameters (vitamin C content, total polyphenol content, acidity, color, and viscosity) of an apple purée. The authors found that the treatment induced some minor quality changes

in the apple purée. From the aspect of nutrition, 100% of the vitamin C was maintained after processing. The same was observed for total polyphenol content, color, and acidity, which retained 100% of their values after treatment. Only the viscosity was altered by the microwave treatment with a reduction of 20%. Lin and Brewer (2005) compared the effect of different blanching methods (steam, water, and microwave) on the quality characteristics of frozen peas. The color of microwave-blanched peas was equal to or better than peas blanched by steam or water methods and flavor, aroma and ascorbic acid content of microwave-treated samples were similar to those of peas blanched by the other methods. Vadivambal and Jayas (2007) presented more details in their review about the changes in quality of microwave-treated agricultural products.

The principle of microwave inactivation of microorganisms

Microwaves inactivate microorganisms in foods by two mechanisms. The first mechanism depends on the traditional effects of heat. The second mechanism for inactivation by microwaves involves non-thermal effects. Kozempel *et al.* (1998) stated that there are four theories to explain the effect of non-thermal effects of microwave energy: selective heating, electroporation, cell-membrane rupture, and magnetic field coupling. The selective heating theory proposed that the microorganisms selectively absorb the electromagnetic energy and microorganisms get hotter than the surrounding fluid and reach temperatures required for pasteurization. In the electroporation theory, the electrical potential across the cell causes pores to form in the weakened membrane, resulting in leakage of cellular material and cell lysis. In the dielectric cell-membrane rupture theory, the voltage across the cell membrane is enough to rupture the cell membrane and kill the microorganisms. In the magnetic field coupling theory, the electromagnetic energy damages critical molecules in the cells (i.e., protein or DNA) and disrupts the internal components of the cells causes them to die.

Thermal inactivation of microorganisms by microwaves

Several studies have used heating by microwave oven for the control of pathogens in foods (Lund *et al.*, 1989; Sheeran *et al.*, 1989; Hollywood *et al.*, 1991; Gundavarapu *et al.*, 1995; Heddleson *et al.*, 1996; Pucciarelli and Benassi, 2005; Al-Holy *et al.*, 2009; Rodríguez-Marval *et al.*, 2009). Rodríguez-Marval *et al.* (2009) used microwave oven heating to inactivate *L. monocytogenes* on stored frankfurter sausages. Frankfurters were placed in a bowl with water (250 ml) and treated in a household microwave oven (2450 MHz) at high (1100 W) power for 30, 45, 60, or 75 s, or medium (550 W) power for 60 or 75 s. High power for 30, 45, 60, and 75 s increased the mean temperature of frankfurter surfaces to 41, 51, 62, and 62°C, respectively. High power for 75 s caused reductions of *L. monocytogenes* on frankfurters between more than 1.5 and 5.9 $\log_{10}$ CFU/cm^2. The authors concluded that frankfurters should be reheated in a microwave oven at high power for 75 s to inactivate up to 3.7 $\log_{10}$ CFU/cm^2 of *L. monocytogenes* contamination. They also recommended that reheating instructions must be designed specifically for each type of product, and consider variations in microwave appliance maximum output power, amount of food to be reheated, age of the product, and the presence of antimicrobial compounds in the formulation. Sheeran *et al.* (1989) was not satisfied with the ability of microwave heating to eliminate *L. monocytogenes* from foods. In their study, they used microwave heating to eliminate *L. monocytogenes* (10^6 CFU/g) from cook-chill food obtained from retail market. The authors found that 81% of the tested samples contained large numbers of viable *Listeria* after heating, while from five (19%) no organisms were recovered. In *Listeria*-positive samples, the mean temperature after reheating was 71°C (range 48–100°C) with a mean reduction of numbers of

only 6.5×10^1 (range 6.2×10^0–1.5×10^2). In samples where *Listeria* was not recovered, the mean temperature was 91°C (range 84–97°C). Picouet *et al.* (2009) studied the effect of a mild microwave treatment (35 s at 652 W with maximum product temperature of 75.3 ± 4.8°C) on the inactivation of *L. innocua* and *E. coli* O157:H7 in apple purée. The treatment successfully decontaminated *L. innocua* inoculated in the apple purée, but was not as efficient in reducing *E. coli* O157:H7; *L. innocua* was reduced by more than 5 $\log_{10}$ compared with a 1 $\log_{10}$ reduction of *E. coli* O157:H7. Gundavarapu *et al.* (1995) studied the effect of different microwave power levels (240, 400, 560, and 800 W) on the survival of *L. monocytogenes* in inoculated shrimp. They found that shrimp samples inoculated with approximately 5×10^5 CFU/g can be completely inactivated with 2 min holding after microwaving for 168, 84, 62, and 48 s at 240, 400, 560, and 800 W, respectively. Apostolou *et al.* (2005) exposed fresh chicken breasts (20 g) to microwave heating at full power to eliminate *E. coli* O157:H7 (10^5–10^6 CFU/g) from the samples. The chicken portions were heated for 5, 10, 15, 20, 25, 30, and 35 s. After 30 s of microwave heating the mean end-point surface temperature was 69.8°C and the mean concentration of *E. coli* O157:H7 cells were 83 CFU/g. But no *E. coli* O157:H7 cells were detected after 35 s of microwave heating at 73.7°C.

Galuska *et al.* (1988) found that microwave heating is adequate for inactivating *L. monocytogenes* in non-fat dry milk. *L. monocytogenes* Scott A or V7 suspended in reconstituted non-fat dry milk and heated in capillary tubes by microwaves to temperatures varying from 60.0 to 82.2°C had *D* values ranging from 243.6 to 0.57 s. They also reported that the survival of *L. monocytogenes* in foods heated by microwaves is strongly influenced by sodium content. The presence of salt decreased the uniformity of temperatures achieved within a food, increased temperatures at the edges of foods, and had the net effect of lowering temperatures below the surface of foods and that contributed to increased bacterial survival. Other researchers (Lentz, 1980; Ohlsson, 1983; Heddleson *et al.*, 1993) reported that salt plays a large role in affecting the rate and uniformity of microwave heating. Protein content also was found to affect microwave heating largely through binding available water and salts, thus lowering the dielectric activity. In contrast, it seems that lipid increases the thermal inactivation of microorganisms in foods when heated by microwave energy because of their rapid heating in electromagnetic fields (Heddleson *et al.*, 1996). Wang *et al.* (2003) compared among common heat-resistant spores of *Bacillus* spp., (indicator organisms for of microwave sterilization) *Bacillus cereus* CCRC14655, *Bacillus coagulans* CCRC10606, *Bacillus licheniformis* CCRC14693, and *B. subtilis* CCRC14199 heated with microwaves (2450 MHz; maximum power rating 700 W) at different power levels (60, 80, and 100%) and under different conditions in salt solutions, starch solutions, and containers. They found that *D* values decreased with increasing of power levels. The *D* values (at a power level of 60%) for *B. licheniformis* and *B. subtilis* slightly decreased after incubation at 25°C for 4 to 12 h. *B. licheniformis* spores had the highest microwave tolerance at a power level of 100% for different incubation times (4, 8, and 12 h at 25°C). Also, they found that an increase of water activity (a_w) had a synergistic effect with microwave treatment on the inactivation of spore contamination. *B. coagulans* spores showed the lowest microwave tolerance in salt solutions ($BaCl_2$, KCl, $NaNO_3$, and KCl/K_2CO_3; 1:1, wt/wt) and *B. licheniformis* spores were the most resistant in the tested salt solutions at different incubation times (4, 8, and 12 h) with a_w values of 0.6, 0.7, 0.8, and 0.9. The authors revealed that microorganisms had the lowest microwave resistance in a medium containing 0.8% starch in solution. Furthermore, they found that the microwave resistance levels of the test microorganisms were lower in glass containers than in polypropylene containers and aluminum foil-enclosed pouches. Perng *et al.* (1994) examined the heat resistance of a spore suspension of *B. stearothermophilus* and reported *D* values of

6.05 s at 130°C and 0.85 s at 140°C. Pucciarelli and Benassi (2005) used microwave ovens (800 W) at two power levels: high (level 10) and medium (level 6) power levels to determine the destruction of *Salmonella enteritidis* on chicken thighs. The temperature progress under the skin and inside the thigh increased with time in a non-smooth way, reaching maximum around 90°C for high power and 70°C for medium power at both positions in the sample. The microwave heating temperature at the high power level for 95 s resulted in 6.4 $\log_{10}$ reductions while microwave heating for 140 s at the medium power resulted in 5 $\log_{10}$ reductions. After 110 s for higher power level, no survival of *S. enteritidis* was detected in samples (100 g), but at 140 s for medium power level, these food pathogens were still present. Al-Holy *et al.* (2009) studied the effect of microwave heating on the inactivation of *Cronobacter* spp. (formerly known as *Enterobacter sakazakii*) in rehydrated infant milk formula (60 mL portions). Inoculated samples 10^5 CFU/ml were heated to different time intervals (0, 20, 30, 40, and 50 s). The authors found that as the heating time increased, inactivation level of the microorganism increased. They stated that heating by microwave for 40 s resulted in about a 4 $\log_{10}$ reduction in *Cronobacter* spp., while heating for 50 s resulted in a complete elimination of in *Cronobacter* spp. from heated rehydrated infant milk formula. A similar finding was reported by Kindle *et al.* (1996) who reported that microwaving 150 mL portions of infant formula for 85–100 s (end-point temperature 82–93°C) resulted in greater than a 4 $\log_{10}$ reduction in *Pseudomonas aeruginosa*, *E. coli*, *Cronobacter* (*Ent. sakazakii*), *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Candida albicans*, and *Mycobacterium terrae*. They concluded that microwave heating to boiling is a convenient and fast method to reduce microbial activity.

Non-thermal inactivation of microorganisms by microwaves

Contradictory results have been found in the literature concerning the non-thermal inactivation effects of microwaves on microorganisms. Kozempel *et al.* (1998) developed a non-thermal flow process using microwave energy (5.0–5.4 kW) to inactivate *Pediococcus* spp. in fluids including non-fat milk, water, 10% glucose solution, apple juice, pineapple juice, tomato juice, apple cider, and beer. The process consisted of five passes through the microwave generator. The flow rate was 0.96–1.26 kg/min and the hold time per pass was 1.1–1.5 min. The microwave energy was immediately and simultaneously applied to the system, and thermal energy was removed by a cooling tube within the process line in the microwave generator to maintain the temperature below 40°C. The authors found that there was significant reduction in microorganisms in water, 10% glucose solution, and apple juice but there was a slight decrease in microorganisms in tomato juice, pineapple juice, apple cider, and beer; and no effect in non-fat milk. The authors suggested four theories for the inactivation effect of cold pasteurization microwave energy (mentioned above). The differences in the inactivation of microorganisms in the fluids was due to the protective effect of insoluble solids present in apple cider and tomato and pineapple juice but not in water, glucose solution, and apple juice. But later, Kozempel *et al.* (2000) found that the non-thermal microwave energy had no effect on the inactivation of microorganisms in fluids. They developed a new system that was capable of isolating thermal and non-thermal effects of microwave energy of the inactivation of microorganisms. The system used a double tube that allowed input of microwave energy but removed thermal energy with cooling water. They found that the non-thermal microwave energy had no effect on the inactivation of *Enterobacter aerogenes*, *E. coli*, *L. innocua*, *Pediococcus*, or a yeast in various test fluids including water, liquid egg, apple juice, apple cider, and tomato juice at sub-lethal temperatures. They reported that, in the absence of stresses (pH or heat), microwave energy did not inactivate

microorganisms; however, they suggested that microwave energy may increase the thermal effects. Ramaswamy *et al.* (2000) found that the non-thermal effect of microwave energy at sub-lethal temperatures was ineffective to inactivate *Saccharomyces cerevisiae* and *L. plantarum* in apple juice. But at equivalent heat treatments, microwaves enhanced inactivation. They also found that *E. coli* K12 had significantly lower *D* values (12.98 s at 55°C, 6.31 s at 60°C, and 0.78 s at 65°C) using microwave energy in a continuous-flow system than equivalent heat treatments with hot water (44.7 s at 55°C, 26.8 s at 60°C, and 2.00 s at 65°C) or steam (72.71 s at 55°C, 15.61 s at 60°C, and 2.98 s at 65°C). They concluded that, while there was no non-thermal effect of microwaves, there was a significant enhancement of thermal treatments. Welt *et al.* (1994) reported that no further lethality should be expected from microwave radiation as a mode of heating away from the rapid heating offered by microwave radiation.

Recently, a non-thermal microwave treatment to the inactivation of *E. coli* and *S. aureus* in raw meats was developed (Shamis *et al.*, 2008). Non-thermal microwave treatment was achieved through the use of low power energy (16 W) for 52 s. At this combination the sample's internal temperatures did not exceed 45°C and inactivation rates were 61.2% for *E. coli* and 67.8% for *S. aureus*. Because longer exposure times would lead to sample temperatures being greater than 45°C, repeated microwave exposure with sufficient cooling in between trials was used. Three repeated exposures resulted in an increased decontamination rates reaching 98.4% for *E. coli* and 95.2% for *S. aureus*. Thus, the researchers concluded that that repeated exposure to high-frequency microwave radiation was significantly more effective in decontaminating raw meat of bacteria compared to a single exposure.

The non-thermal inactivation effect of microwave process on microbial inactivation is inadequate in degree, which should be considered during process development. Therefore, when developing methods for describing the inactivation kinetics of microwave heating, it is recommended that only thermal effects be included in the model (FDA, 2000)

10.3.2 Direct electroheating techniques: ohmic heating

Ohmic heating is a process where electric current (mainly alternating) is passed through the food product with the purpose of heating it. Heat is generated directly inside the food as a result of electrical resistance provided by the food. Heating occurs in the form of internal energy generation within the food material. The amount of heat generated is directly related to the current induced by the voltage gradient in the field and the electrical conductivity (Baysal and Icier, 2010; Knirsch *et al.*, 2010). Ohmic heating is also known as Joule heating, electrical resistance heating, direct electrical resistance heating, electroheating, and electroconductive heating (FDA, 2000; Vicente *et al.*, 2006; Knirsch *et al.*, 2010). Ohmic heating is different from other electrical heating methods by the presence of electrodes contacting the food (as opposed to microwave where electrodes are absent), frequency (unrestricted, except for the specially assigned radio or microwave frequency range), and waveform (also unrestricted, although typically sinusoidal) (FDA, 2000).

Ohmic heating can be used as a continuous in-line heating method of pumpable foods for cooking and sterilization of viscous liquids and mixtures containing particulate food products (Baysal and Icier, 2010). A large number of applications exist for ohmic heating including blanching, evaporation, dehydration, fermentation, extraction, sterilization, pasteurization, and heating of foods to serving temperature (FDA, 2000; Baysal and Icier, 2010; Knirsch *et al.*, 2010). The major advantage claimed for ohmic heating is its ability to heat materials

rapidly and uniformly, including products containing particulates. This is expected to reduce the thermal loss to the product in comparison to conventional heating.

The effect of ohmic heating on the quality of food has been studied. Piette *et al.* (2004) studied the effect of ohmic cooking on bologna sausage samples, and reported that there were no differences between the samples treated by ohmic heating in terms of appearance and textural properties by compression test and those conventionally cooked. Bozkurt and Icier (2010) found that ohmic cooking gave significantly similar cooking yield to that obtained through conventional cooking and the color was more homogeneous within the ground beef cooked by ohmic treatment while the crust layer in the surface of the ground beef could not have been achieved. Mizrahi (1996) found that blanching by ohmic heating significantly reduced the extent of solid leaching when compared to a hot water treatment and a short blanching time could be used regardless of the shape and size of the product. Sensoy and Sastry (2007) used ohmic heating to blanch mushrooms and found that ohmic treatment maintained high solids content during the process when compared to conventional blanching.

The efficiency of ohmic treatments is affected by a number of factors such as the ionic content, the moisture mobility, the field strength, soluble solids content, the melting of fats, and changes in the cell structure (de Alwis and Fryer, 1990; Halden *et al.*, 1990; Palaniappan and Sastry, 1991;Wang and Sastry, 1993, 1997).

10.3.2.1 Principles of microbial inactivation by ohmic heating

Ohmic heating provides quick death kinetics. The primary mechanisms of microbial inactivation in ohmic heating are thermal in nature. However, many researchers have reported that ohmic heating may present mild non-thermal cellular damage due to the presence of the electric field (Cho *et al.*, 1999; Pereira *et al.*, 2007; Sun *et al.*, 2008). The main reason for the additional effect of ohmic treatment may be its low frequency (usually 50–60 Hz), which allows cell walls to build up charges and form pores. This is in contrast to high-frequency methods such as radio or microwave frequency heating, where the electric field is essentially reversed before sufficient charge build-up occurs at the cell walls (FDA, 2000).

Thermal inactivation of microorganisms by ohmic process

Several studies have found that microbial inactivation rate under ohmic heating is reduced when compared to traditional heating methods. Baysal and Icier (2010) examined the effectiveness of ohmic and conventional heating for reducing spores of *Alicyclobacillus acidoterrestris* in orange juice. The *D* values of *A. acidoterrestris* in orange juice for ohmic heating were significantly lower than those for conventional heating. The effects of temperature (70, 80, and 90°C) and heating time (0, 10, 15, 20, and 30 min) on inactivation of *A. acidoterrestris* spores during ohmic heating in orange juice were significant. The *D* values at 70, 80, and 90°C for ohmic (30 V/cm) and conventional heating were 58.48, 12.24, and 5.97 min (z value of 18.8°C) and 83.33, 15.11, and 7.84 min (z value of 17.89°C), respectively. The results of their study showed significantly higher lethality for spores treated with ohmic heating than for spores treated with conventional heating. Conventional heating was ineffective for pasteurizing orange juice, whereas the maximum ohmic heating treatment applied at 30 V/cm was sufficient to inactivate 5 $\log_{10}$ units of *A. acidoterrestris* spores.

Pereira *et al.* (2007) evaluated the effect of ohmic heating and conventional heating on thermal inactivation of *E. coli* in goat's milk and *B. licheniformis* in cloudberry jam. The *D* and z values of *E. coli* using ohmic treatment ($D_{60°C} = 4.2$ min, $D_{63°C} = 1.9$ min, $D_{65°C} = 0.86$ min, $z = 8.4$°C) were lower than those obtained by conventional treatment

($D_{63^\circ C} = 3.9$ min, $D_{65^\circ C} = 3.5$, $D_{67^\circ C} = 2.8$ min, $D_{75^\circ C} = 1.5$ min, $z = 23.1^\circ$C). Lower D values means more effective treatment. Similarly, D *val*ues of *B. licheniformis* using ohmic treatment ($D_{70^\circ C} = 57.1$ min, $D_{75^\circ C} = 25.2$ min, $D_{80^\circ C} = 7.2$ min) were lower than those obtained by conventional treatment ($D_{70^\circ C} = 85.3$ min, $D_{75^\circ C} = 51.0$, $D_{80^\circ C} = 18.1$ min, $D_{85^\circ C} = 6.0$ min, $D_{90^\circ C} = 1.6$ min). Cho *et al.* (1999) evaluated the thermal inactivation of *B. subtilis* ATCC 6633 spores by continuous (single stage) or intermittent ohmic (double stage) and conventional heating. In the double-stage treatment, heating was interrupted by a 20 min of incubation at 37°C to induce a Tyndallization effect. The authors found that spore inactivation was greater with ohmic heating than with conventional heating and ohmic heating produced a higher death rate of spores during the first stage of heating and high reduction in count of viable spores immediately after the incubation period. The authors concluded that spore inactivation during ohmic heating was primarily due to the thermal effect but there was an additional killing effect caused by the electric current. Sun *et al.* (2008) compared the inactivation effects between ohmic heating and conventional heating (by hot water) on viable aerobes and *Streptococcus thermophilus* 2646 in milk under identical temperature history conditions. The authors found that ohmic heating causes higher microbial death than the conventional heating process; the viable counts and the D value of *S. thermophilus* in samples treated by ohmic heating were significantly lower than those in samples treated by conventional heating. The results suggested that ohmic heating had not only a thermal lethal effect, but also a non-thermal-lethal effect on microorganisms, due to the electric current.

The high electric-field alternating current method was used to inactivate foodborne and spoilage causing microorganisms in liquid foods (Uemura and Isobe 2002; Uemura, *et al.*, 2009). Microbial inactivation by high electric-field alternating current results from the combination of a high electric field with ohmic heating (Uemura and Isobe, 2002). Uemura and Isobe (2002) examined the effect of a continuous AC electric field (20 kHz, 14 kV/cm) on inactivation of *E. coli* in 0.1% saline water using electrical apparatus. The electric treatment caused reduction of 5 $\log_{10}$ in *E. coli* when the final process temperature was maintained at 74°C. The temperature was kept constant by alternating the concentration of saline water with different electric field intensities. When the final process temperature was set constant, an increase in the electric field resulted in a linear decrease of viable *E. coli* cells. Also, when the electric field was constant, a higher process temperature caused a larger decrease of viable *E. coli* populations. The authors concluded that high electric field combined with high temperature plays an important role in inactivation in a short treatment. Uemura *et al.* (2009) evaluated the effect of high electric-field alternating current (electric field of 2.5–2.7 kV/cm at 20 kHz for 13.8 ms) on inactivation of *A. acidoterrestris* spores inoculated in orange juice. The high electric-field alternating current heating method was compared with a conventional heating method for the inactivation of *A. acidoterrestris* spores in orange juice. The method reduced *A. acidoterrestris* spores in orange juice by 3 $\log_{10}$ at an outlet temperature of 125°C and a holding time of 0.9 s. The high electric-field alternating current method slightly inactivated *A. acidoterrestris* spores through the electrode and significantly inactivated them through the holding pipe. This method inactivated *A. acidoterrestris* spores 30 times faster than conventional heating during the holding time, and the total heating time, including the rising time and holding time, was greatly reduced. Inoue *et al.* (2008) compared the inactivation of *B. subtilis* spores in orange juice with high electric-field alternating current and ultra-high-temperature (UHT) treatment. High electric-field alternating current method at 120°C for a 0.6 s holding time and UHT at 110°C for a 10 s holding time produced equal inactivation of *B. subtilis* spores by 4 $\log_{10}$. Regarding the quality, they found that after high

electric-field alternating current treatment, 24% more linalool, 15% more limonene, 25% more β-carotene, 18% more hesperidin, and 8% more L-ascorbic acid were retained in the orange juice than after UHT treatment. Uemura and Isobe (2003) found that a pressurized electric inactivation system using the combination of high temperature and high electric field caused effective inactivation of *B. subtilis* spores in a shorter time with a smaller loss of ascorbic acid and with a less peculiar smell than a conventional heating treatment. They used a continuous AC electric field (20 kHz, 16.3 kV/cm) to inactivate *B. subtilis* spores in orange juice in electrical equipment. Electrical treatment at 100°C under atmospheric pressure has a poor ability to inactivate *B. subtilis* spores. But electrical treatment at 121°C under pressurized conditions to elevate the boiling point can reduce the viable *B. subtilis* spores in orange juice 4 $\log_{10}$ in less than 1 s of treatment. Ten percent of the original ascorbic acid in the orange juice was destroyed after electric treatment compared with 30% that was destroyed by heating in boiling water for 10 min. Furthermore, the unpalatable flavor of heat-treated mandarin juice was not developed in the electrically treated juice.

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11 Ozone in Food Preservation

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Abstract: Ozone is a triatomic form of oxygen. It is an unstable compound and decomposes to molecular oxygen spontaneously without leaving a toxic residue. Lack of toxic residues makes ozone a favorable sanitizer. Ozone has been used since the late nineteenth century to purify water. Ozone as a disinfectant is declared to be generally recognized-as-safe (GRAS) for food application in 1997. Since that time, interest in developing ozone applications in the food industry has increased. Ozone can be applied to food products as a gas or it can be dissolved in water using certain bubbling techniques. It has been used for process water sterilization and recycling, inactivation of bacterial growth, prevention of fungal decay, washing and storage of fruits and vegetables, reducing microbial populations on stainless steel surfaces, control of storage pests, destruction of pesticides and chemical residues, and control of microorganisms on poultry and meat products.

Keywords: food-processing technology; food safety; microbial inactivation; microorganism; mycotoxin degradation; ozone; ozone application; pesticide degradation; toxicity

11.1 INTRODUCTION

Ozone (O_3) is an allotrope of oxygen, and an unstable colorless gas. It has a distinctive pungent odor, from which its name is derived (from Greek *ozein*, to smell). Ozone is formed in the earth's atmosphere as a result of lightning or high-energy UV radiation. The characteristic fresh, clean smell in the air following a thunderstorm represents freshly generated ozone in nature. Ozone is also a by-product of various photochemical oxidation processes (Graham, 1997; Muthukumarappan *et al.*, 2000; Bena, 2006).

Ozone has been used in European countries for decades and has recently been approved in the USA by the Food and Drug Administration (FDA) for treatment, storage, and processing of foods, unless use is precluded by a standard of identity (Lado and Yousef, 2007). High reactivity, penetrability, and spontaneous decomposition to a nontoxic product (oxygen) make ozone a promising disinfectant for food products (Watada *et al.*, 2005).

Ozone is becoming a widely used replacement for chlorine-based chemicals for sanitation purposes in food processing, especially in the meat industry and for water-quality purposes, such as bacterial, odor, pesticide, and hazardous compound degradation (Achen and Yousef, 1999; Kim *et al.*, 1999; King and Prudente, 2005).

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11.2 HISTORY

Ozone was discovered in 1840 by German chemist Christian Schönbein (Graham 1997; Fielding and Bailey, 2005). In 1888 in the USA Fewson invented a generator to produce ozone for deodorizing sewer gases. In Germany in 1902 Siemens and Halske established the first full-scale ozone-generating plant for water treatment (Graham, 1997). Later, in 1904, the city of Nice, France, was the first to implement commercial-scale disinfection of potable water using ozone (Lebout, 1959). Ozonation has been used to purify water in Europe since 1906 (Graham, 2000). In the USA ozonation of potable water was first started in Whiting, Indiana, in 1940 (Rice, 1986). During 1953–1956, ozonated air under pressure was found to be very effective for sterilizing empty food containers and was adopted for use on glass bottles in Switzerland (Muthukumarappan *et al.*, 2000). It is approved in the USA as generally recognized-as-safe (GRAS) for treatment of bottled water and as a sanitizer for process trains in bottled-water plants (FDA, 1995). In June 1997 ozone was granted the GRAS status as a disinfectant for foods by an independent panel of experts, sponsored by the Electric Power Research Institute (EPRI, 1997). In June 2001, the US FDA officially granted GRAS status to ozone for use in food-contact applications and in December 2001 the US Department of Agriculture approved the use of ozone for contact with meat and poultry products (Cramer, 2006).

11.3 CHEMISTRY

Ozone (O_3) is formed by high energy input that splits the oxygen (O_2) molecule. Single oxygen (O) molecules rapidly combine with available O_2 to form ozone (Karaca and Velioğlu, 2007). Ozone is a bluish gas with a pungent odor and strong oxidizing properties with an oxidation potential of 2.07 mV (see Table 11.1) (Horvath *et al.*, 1985; Khadre *et al.*, 2001). It is readily detectable at concentrations 0.01–0.05 ppm (Miller *et al.*, 1978; Mehlman and Borek, 1987; Linton *et al.*, 2006).

Major physical properties of pure ozone are shown in Table 11.2. At −112°C ozone condenses to a dark blue and highly explosive liquid. Explosions may be detonated by electrical sparks or by sudden changes in temperature or pressure (Güzel-Seydim *et al.*,

Table 11.1 Oxidation potential of oxidizing agents (Legrini *et al.*, 1993). Reproduced with permission.

Oxidizing agent	Oxidation potential (mV)
Fluorine	3.03
Ozone	2.07
Permanganate	1.68
Chlorine dioxide	1.57
Hypochlorous acid	1.49
Chlorine gas	1.36

Table 11.2 Major physical properties of pure ozone. Reproduced with permission from Manley, T.C., Niegowski, S.J. (1967) Ozone. In *Encyclopedia of Chemical Technology*, vol. 14, 2nd edn. John Wiley & Sons, New York, pp. 410–432.

Boiling point	-111.9 ± 0.3°C
Melting point	-192.5 ± 0.4°C
Critical temperature	−12.1°C
Critical pressure	54.6 atm

Table 11.3 Temperature and solubility relationship of ozone in water (Roth and Sullivan, 1981). Reproduced with permission.

Temperature (°C)	Solubility (liter ozone/liter water)
0	0.641
15	0.456
27	0.270
40	0.112
60	0.000

2004). However, ozone applications mainly use concentrations of about 10%; so it is safe to handle under current industrial applications (Muthukumarappan *et al.*, 2000).

11.3.1 Solubility

Ozone is partially soluble in water and it forms a true physical solution (Horvath *et al.*, 1985). The relationship between temperature and solubility of ozone in water is shown in Table 11.3. Solubility of ozone in water is greater than oxygen (13 times) and nitrogen. However, it is less soluble than carbon dioxide and chlorine. Solubility can be affected by partial pressure, flow rate of ozone, temperature, purity of water, and contact time (Khadre *et al.*, 2001).

11.3.2 Stability

The weak bond holding ozone's third oxygen atom causes the molecule to be unstable (Lucas, 2003). Ozone is relatively unstable in aqueous solutions. It decomposes continuously to oxygen according to a pseudo first-order reaction (Tomiyasu *et al.*, 1985).

Depending on the temperature and humidity of air, ozone has a half life of 4–12 h (Lucas, 2003; Sharma, 2005). Ozone decomposes in the range between seconds and hours depending on temperature, purity, and pH of water (Rosenthal, 1974; Xu, 1999; Linton, 2006).

11.3.3 Reactivity

The ozone molecule acts as dipole with electrophilic and nucleophilic properties. Organic and inorganic compounds in aqueous solutions react with ozone in one of two pathways (Staehelin and Hoigné, 1985):

(i) direct reaction of organic compound with molecular ozone;
(ii) decomposition of ozone in water into a radical which reacts with the compound.

The direct reaction is described by the Criegee mechanism (Criegee, 1975) where ozone molecules undergo 1,3-dipolar cycloaddition with double bonds present, leading to the formation of ozonides (i.e., 1,2,4-trioxolanes) from alkenes and ozone with aldehyde or ketone oxides as decisive intermediates. This leads to electrophilic or nucleophilic reactions occurring with the aromatic compounds that are substituted with an electron donor (Tiwari *et al.*, 2009).

11.4 GENERATION

Depending on the concentration required, ozone gas can be generated using coronal discharges, and photochemical, electrolytic, and radiochemical methods (Muthukumarappan

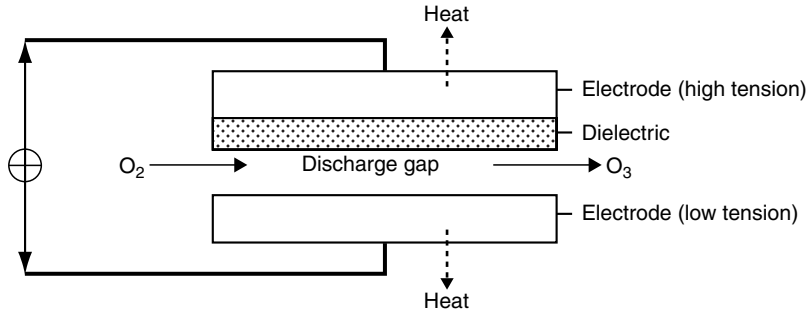

Figure 11.1 Schematic diagram of ozone generation by the coronal discharge method (Muthukumarappan *et al.*, 2000). Reproduced with permission.

et al., 2000). Ozone is commonly produced from oxygen or air by utilizing UV light or coronal discharge generators. Both these methods are inspired by naturally occurring atmospheric phenomena (Pryor and Rice, 1999). UV light systems use radiation at a wavelength of 185 nm emitted from high-transmission UV lamps. These systems are relatively low in cost and do not require dry air for ozone production (Linton *et al.*, 2006). The maximum concentration of ozone gas generated by UV light (at 185 nm) is 0.1% by weight using dry air as the feed gas. At this concentration, the maximum solubility in water at 25°C is 0.35 mg/L (Sharma, 2005). A schematic diagram of ozone generation by coronal discharge is shown in Figure 11.1.

There are two electrodes in coronal discharge, the high-tension and low-tension electrodes, separated by a dielectric insulator in a narrow discharge gap. When a high-voltage alternating current is applied across this gap, the air or oxygen passing through the gap is partially ionized, and the oxygen molecules are dissociated. The split oxygen atoms combine with other oxygen molecules to form ozone (Linton *et al.*, 2006). The concentration of ozone produced depends on many factors such as voltage, current frequency, discharge gap, dielectric material, and thickness. Oxygen or an oxygen concentrator can be used instead of air to increase the yield, but, if air is used, it should be dried to prevent the formation of nitric acid (Kim *et al.* 1999).

Considering the technical and economical factors, the coronal discharge method is preferred as it gives a higher ozone concentration of 1.5% by weight in dry air, and its power requirement is only 6–8 kWh (compared to 44 kWh for the UV method) for every 454 g of ozone generated (Pryor and Rice, 1999).

The solubility of ozone in water is a function of the partial pressure of ozone gas over the liquid surface. It follows Henry's law and is limited by the equilibrium between the gas and the saturated solution of ozone in water. For increased concentration of ozone, the exposure time required increases (Sharma, 2005). Ozonated water is produced by bubbling ozone gas into water using porous diffuser. When the bubble size becomes smaller, surface area of contact and also solubility is increased. According to Katzenelson *et al.* (1974), an optimum dissolution of ozone in water occurs when bubbles are 1–3 mm in dia.

11.5 ANTIMICROBIAL EFFECT

Ozone is a powerful broad-spectrum antimicrobial agent that is active against bacteria, fungi, viruses, protozoa, and also bacterial and fungal spores. Ozone destroys microorganisms by

oxidation of vital cellular components. In bacteria the cell wall is the primary target site for antimicrobial action. Inactivation by ozone is a complex process that attacks various cell-membrane and cell-wall constituents and intracellular constituents (Khadre *et al*., 2001).

The oxidizing mechanism of ozone may involve direct reactions of molecular ozone and reactive free radicals. However, there is no consensus on which of them is more certain. Ozone may oxidize polyunsaturated fatty acids, membrane-bound enzymes, glycoproteins, and glycolipids, and causes a weakness in cell wall and leads to leakage of cellular material (Scott and Lesher, 1963; Komanapalli and Lau, 1996; Khadre *et al*., 2001). It can also react with double bonds of unsaturated lipids and sulfhydryl groups of some enzymes, causing disruption of normal cellular activity (Fielding and Bailey, 2005).

Inactivation mechanism of ozone differs from chlorine and other disinfectants which must be transferred across the cell membrane in order to be effective.

11.5.1 Inactivation spectrum

11.5.1.1 Bacteria and bacterial spores

Ozone is generally more effective against vegetative bacterial cells than bacterial spores. Some researchers reported that Gram-negative bacteria were more sensitive than Gram-positive ones (Sobsey, 1989; Lee and Deniniger, 2000). According to Khadre *et al.* (2001), in some studies the results are inconsistent with the previous work.

Ozonated water has a higher oxidizing potential than most oxidizing agents used to kill spores (Menzel, 1971; Kim *et al*., 2003). Broadwater *et al.* (1973) found that spores of *Bacillus* spp. were up to 15 times more resistant than their vegetative cells. Ozone does not kill *Bacillus subtilis* spores by DNA damage. Spore killing by ozonated water is due to some type of damage to the spore's inner membrane (Young and Setlow, 2004).

11.5.1.2 Yeast

Farooq *et al.* (1977) observed that the degree of inactivation was extremely affected by the initial population of organisms. When the initial density of *Candida parapsilosis* was 1.4×10^5 colony-forming units (CFU)/mL, a 4 log reduction was detected. However, there were no inactivations when the initial density was 1.6×10^7 CFU/mL. Zorlugenç *et al.* (2008) reported that yeasts were completely destroyed at 15 min in ozonated water, whereas gaseous ozone (13.8 mg/L) treatment was found insufficient at 15 min.

11.5.1.3 Fungi and fungal spores

Spotts and Cervantes (1992) evaluated the effectiveness of ozonated water on control of postharvest decay caused by molds. Resistance to inactivation varied among the species of *Botrytis*, *Mucor*, and *Penicillium*. Zhao and Cranston (1995) reported that a *Penicillium* spp. was more resistant than an *Aspergillus* spp. The sensitivity of *Aspergillus flavus* and *Aspergillus parasiticus* conidia to ozone was not significantly affected at pH 5.5 and 7.0 (Beuchat *et al*., 1999). Moore *et al.* (2000) found that 2 ppm ozone significantly reduced the number of fungi attached to stainless steel coupons while Foarde *et al.* (1997) determined that levels in excess of 6 ppm were required.

11.5.1.4 Viruses

Ozone has been demonstrated to destroy a wide range of viruses including Venezuelan equine encephalomyelitis virus, hepatitis A, influenza A, vesicular stomatitis virus, and infectious bovine rhinotracheitis virus (Dosti *et al.*, 2005). Ozonated water is lethal to viruses at low concentrations (Beuchat *et al.*, 1999).

11.5.1.5 Protozoa

Wickramanayake *et al.* (1984) reported the effect of ozonated water on the inactivation of cysts of *Naegleria gruberi* and *Giardia muris*. The *N. gruberi* cysts were more resistant to ozone than *G. muris*. The intestinal parasite *Cryptosporidium parvum* was exposed to ozone that inactivated over 90% of the parasite population within 1 min at 1 mg/L ozone in ozone demand-free water (Korich *et al.*, 1990).

Effects of ozone and UV radiation treatments on the infectivity of *Toxoplasma gondii* oocysts were investigated. UV treatment was found to be an effective disinfection method to inactivate *T. gondii* oocysts in drinking water, but ozone did not show promise (Dumetre *et al.*, 2008).

11.5.2 Influencing factors

Between researchers there is disagreement about the sensitivity of different microorganisms to ozone. Susceptibility of microorganisms to ozone varies with strain of the microorganism, physiological state of the culture, density of the treated population, pH of the medium, temperature, and humidity (Khadre *et al.*, 2001; Sharma, 2005).

In addition to the factors mentioned above, efficiency of ozone is affected by ozone demand of the medium. For microbial inactivation, the effectiveness of ozone depends on residual ozone more than the amount applied. The presence of ozone-demanding substances in treatment medium greatly affects the level of residual ozone (Kim *et al.*, 2003).

Variation in methods of applying ozone, accuracy of ozone-measuring procedures and devices, and methods of measuring antimicrobial efficacy are some of the factors that make comparison of various studies impossible (Khadre *et al.*, 2001).

11.6 APPLICATIONS

Ozone has proved to be a versatile antimicrobial showing promising results both as a gas and in aqueous solution; its mode of action for inactivating the bacterial cell is the same in both forms (Cords *et al.*, 2005). Potential applications in the food-processing industry are disinfecting hatcheries and hatched eggs, poultry carcasses, and chiller water, mold control, treatment of process water for recycling and re-use, cleansing of cold storage rooms, and the extension of shelf-life of fruit, vegetables, and seafoods (Sharma, 2005).

11.6.1 Red meat

The effectiveness of ozone in reducing populations of pathogenic bacteria on the surface of carcasses is decreased compared with a low ozone-demand liquid medium (Kim *et al.*, 1999). According to Kim *et al.* (1999), rinsing beef carcasses with ozonated water at concentrations

of 0.3–2.3 ppm reduces total aerobic plate counts by 1.3 $\log_{10}$ CFU/cm^2. However, other researchers have shown that beef surfaces treated with 100 ppm gaseous ozone for 30 min had little or no effect on microbial contamination and discolored the meat (Cords *et al.*, 2005).

Ozone also has been evaluated against various sanitizing agents and water (16–74°C) for their ability to reduce bacterial contamination on beef samples in a model spray-washing cabinet (Gorman *et al.*, 1995). They concluded that hydrogen peroxide and ozonated water were more effective than other sanitizing agents. However, the effects of different treatments (74°C hot-water washing, 5% hydrogen peroxide, and 0.5% ozone) in reducing bacterial populations on beef carcasses showed that the ozone and hydrogen peroxide treatments had minor effects and were equivalent to conventional washing in reducing bacterial populations on beef (Reagan *et al.*, 1996).

11.6.2 Poultry

Ozone has been evaluated for use as a carcass wash and for use in chiller water. Kim *et al.* (1999) found that the total microbial counts on chicken carcasses treated with chiller water that contained 3.0–4.5 ppm ozone were repeatedly lower than those carcasses treated with tap water alone. Sheldon and Brown (1986) investigated the efficacy of ozone as a disinfectant for poultry carcasses. The authors indicated that poultry carcasses rinsed with ozonated water exhibited a decrease in the total bacterial load. Besides, ozonation had no adverse effect on carcass color or lipid peroxidation, and did not produce a carcass off-flavor.

Muthukumarappan *et al.* (2000) investigated the storage life of beef, veal, lamb, pork, chicken, and rabbit at ozonated atmospheres. An ozonated atmosphere increased the storage life (7 days) of all the foods studied. The growth of the surface microflora was retarded in refrigerated atmospheres and in the presence of ozone. Whistler and Sheldon (1989) evaluated ozone and formaldehyde against microorganisms that are naturally present on fertile, freshly laid, broiler hatching eggs. Misting with ozone was as equally as effective as formaldehyde fumigation in reducing microbial counts. However, ozone treatment at the concentrations tested significantly reduced hatchability when compared with results of other treatments. These findings indicated that ozone is a good disinfectant yet may adversely affect embryo development when given in the gaseous form.

A contrary result was observed by Bailey *et al.* (1996). Hatchability was not significantly reduced by sanitizing treatments (UV light, ozone, and hydrogen peroxide) when compared with untreated controls.

11.6.3 Seafood

Sensorial and microbial effects of gaseous ozone on fresh scad were investigated (Silva *et al.*, 1998). Results showed that ozone in relatively low concentrations ($<0.27 \times 10^{-3}$ g/L) was an effective bactericide against vegetative cells of five species of fish bacteria.

The influence of ozone, hydrogen peroxide and salt on microbial flora and quality attributes of the channel catfish fillets was investigated by Kim and Yousef (2000). All treatments were found to be effective in suppressing the initial number of total coliforms and psychrotrophs. These treatments increased the shelf life of fillets by 1.5–3.0 days with some changes in oxidative rancidity and color. Ozone (10 ppm) was prolonged the shelf life by more than 25%. Meunpol *et al.* (2003) determined the effects of ozone and probiotics on the survival of black tiger shrimp (*Penaeus monodon*). Postlarvae of *P. monodon* tolerated continuous ozonation for 8 h with residual ozone concentration (ROC) between 0.34 and 0.50 mg O_3/L ROC without

mortality. However, some abnormal shrimp were observed. By 10 h exposure, 23% of postlarvae were dead, which increased to 34% by 24 h. Minimal effective ROC to inhibit 3 log units of *Vibrio harveyi* D331 for 6 h and 2 log units of *Bacillus* S11 for 9 h was 0.38 mg O_3/L of ROC from 5 min ozonation.

11.6.4 Fruit and vegetables

Treatment with gaseous ozone at low concentrations extended the shelf life of some fruit and vegetables. The increase in the shelf life of some fruits may be attributed to the oxidation of ethylene and to the removal of other metabolic products by ozone (Kim *et al*., 1999).

Achen and Yousef (2001) obtained a 3.7 $\log_{10}$ CFU/g reduction in populations of *Escherichia coli* O157:H7 on apple surfaces following treatment with ozonated water. They concluded that treatments were more effective when ozone was bubbled during apple washing than by dipping in pre-ozonated water. Rodgers *et al*. (2004) compared the efficacy of ozone, chlorine solutions, and peroxyacetic acid on apples, strawberries, lettuce, and cantaloupe contaminated with *E. coli* O157:H7 and *Listeria monocytogenes*. They obtained the highest reduction in population with ozone.

A comparison of the effectiveness of sodium hypochlorite solution, acidic electrolyzed water, and ozonated water for inactivation of aerobic bacteria on lettuce showed that although ozone did not damage the surface structure of lettuce, its disinfectant effect was less than that of acidic electrolyzed water (Koseki *et al*., 2001).

The use of ozone treatment as an alternative to sulfur dioxide fumigation to reduce postharvest fungal decay of grapes showed promising results in a study by Sarig *et al*. (1996). Populations of fungi, yeast, and bacteria, naturally present on fruit surface, were considerably reduced by ozone exposure for 20 min. However, storage of strawberries under an ozone atmosphere for 3 days at 2°C did not prevent fungal decay but increased the vitamin C content (Perez *et al*., 1999).

The food value of potatoes, cabbage, and carrot was investigated after ozone treatment to prolong shelf life (Enshina and Voitik, 1989). Ozone treatment did not significantly alter the protein and starch in potatoes; sugar and carotene in cabbage or carrot. Organoleptic evaluation indicated no changes in the properties of potatoes and vegetables as compared to nontreated products. However, ozonation has decreased the ascorbic acid content by 16–25%.

Effects of ozone and storage temperature on carrots and two postharvest pathogens, *Botrytis cinerea* Pers. and *Sclerotinia sclerotiorum* de Bary were investigated (Liew and Prange, 1994). Pathogen-inoculated and uninoculated whole carrots were exposed to an ozone concentration of 0, 7.5, 15, 30, and 60 μL/L. Treatment chambers were flushed with a total flow rate of 0.5 L/min (air and ozone) for 8 h daily for 28 days. The residual ozone concentration increased with ozone supply concentration but was less at higher storage temperatures. The results indicated that ozone is a potent fungistatic system. Carrot respiration rate, electrolyte leakage, and total color differences increased with ozone concentration. Ozone-treated carrots were lighter and less intense in color than control carrots.

Berries were evaluated for fungal decay, anthocyanins, color, and peroxidase activity. While 20% of control fruits showed decay, ozone storage suppressed fungal development for 12 days. Ozone storage did not cause observable injury or defects. In all treatments, anthocyanin content of juice was found similar to initial levels at 12 days. According to

hue angle values, surface color was better retained in 0.1 ppm-stored berries by 5 days and in 0.3 ppm-stored berries by 12 days. Peroxidase was greater in controls and 0.1 ppm samples and was lowest in 0.3 ppm fruits by 12 days. Ozone storage resulted in market-quality extension (Barth *et al*., 1995).

Onions have been treated with ozone during storage. Mold and bacterial counts were greatly decreased without any change in chemical composition and sensory quality (Song *et al*., 2000).

The use of ozone as a substitute for ethylene oxide to decontaminate whole black peppercorn and ground black pepper and the effects of ozone on the volatile oil constituents of the spice were studied. A reduction of 3–4 log for black peppercorns and 3–6 log for ground black pepper was obtained. Ozone treatment of ground black pepper resulted in oxidation of certain volatile oil constituents while the treatment had no significant effect on the volatile oil of whole peppercorn (Zhao and Cranston, 1995).

Zhang *et al*. (2005) reported that the respiration rate of fresh-cut celery was inhibited by ozone treatment. Similarly, peach respiration and ethylene production were not affected by 3 weeks' exposure to 0.3 ppm ozone (Palou *et al*., 2000). Skog and Chu (2001) claimed that ozone could reduce ethylene level of air in the cold storage room, and thus prolonging the shelf life of the ethylene-sensitive and ethylene-producing commodities could be possible.

Effects of ozone and storage temperature on physicochemical properties of mushrooms were determined. Ozone treatment on mushrooms prior to packaging caused an increase in external and a reduction of the internal browning rate. Nevertheless, texture, maturity index, and weight loss of mushrooms were not significantly changed (Escriche *et al*., 2001). Watanabe *et al*. (1994) reported that ozonation showed an increase in mushroom weight, water content, proteins, calcium, potassium, zinc, riboflavin, and ascorbic acid, and a decrease in carbohydrates, iron, and thiamine.

11.6.5 Cereals

Strait (1998) established that ozone was toxic to insects. A concentration of 50 ppm for 3 days resulted in 100% mortality of adult confused flour beetles and maize weevils and greatly reduced emergence of larval Indian meal moths. The efficacy of ozone as a fumigant to disinfest stored maize was evaluated by Kells *et al*. (2001). Treatment of 8.9 tons of maize with 50 ppm ozone for 3 days resulted in 92–100% mortality of adult red flour beetle, adult maize weevil, and larval Indian meal moth and also reduced *Asp. parasiticus* Speare on the kernel surface by rate of 63%. Mendez *et al*. (2003) indicated that treatment of grains with 50 ppm ozone for 30 days had no detrimental effect on popping volume of popcorn, fatty acid and amino acid composition of soybean, wheat, and maize, milling characteristics of wheat and maize, baking characteristics of wheat, and stickiness of rice.

11.6.6 Pesticides

To improve the yield of crops and the final quality, pesticides are used in agriculture for decades. However, there is always a risk for remaining of pesticide residue on commodities at the time of sale. In particular, the misuse of pesticides causes residue accumulation on agricultural crops. Ong *et al*. (1996) examined the degradation of azinophos-methyl, captan, and formetanate from solution and on fresh apple and processed apple sauce. While captan was completely removed, maximum degradation rate of azinophos-methyl was 83% in model

systems. Dipping apples into 0.25 ppm ozanated water resulted in reduction ratios of 75, 72, and 46% for azinophos-methyl, captan and formetanate, respectively. The effectiveness of various treatments on the degradation of mancozeb and ethylene-thiourea in apples was determined by Hwang *et al.* (2001). Mancozeb residues were degreased 56–99% with chlorine, 36–87% with chlorine dioxide, 56–97% with ozone, and 44–99% with hydrogen peroxyacetic acid. All ethylene-thiourea residues were degraded at high concentrations of the oxidizing agents.

11.6.7 Mycotoxins

Mycotoxins are extremely toxic chemical substances commonly produced by the species of *Aspergillus*, *Penicillium*, and *Fusarium*. The consumption of contaminated foods could cause major health and economical problems. Therefore, the development of safer, rapid, and cost-effective degradation methods is essential. In fact, ozonation has been tested to remove mycotoxins for decades. These studies have demonstrated that toxins were removed successfully by ozonation (Dollear *et al.*, 1968; McKenzie *et al.* 1997; Prudente and King, 2002; Proctor *et al.*, 2004; Young *et al.*, 2006). However, in many countries current regulations do not allow consumption of the detoxified food products. McKenzie *et al.* (1998) investigated the ability of electrochemically produced ozone to degrade aflatoxin B_1 (AFB_1) in naturally contaminated whole kernel corn. Control and contaminated corn were treated for 92 h with ozone at 200 mg/min in 30 kg batches; greater than 95% reduction of AFB_1 in contaminated corn was achieved.

İnan (1997) used ozonation to degrade AFB_1 in paprika powder. At 1 h, 80 and 93% of the toxin was destroyed by 33 and 66 mg/L ozone treatment, respectively. Patulin was completely removed by ozone (34.56 mg/h) for 3 min. The influence of gaseous ozone and ozonated water on degradation of AFB_1 in dried figs was also investigated. Results indicated that gaseous ozone was more effective than ozonated water for reduction of AFB_1 (Zorlugenç *et al.*, 2008).

11.6.8 Food-processing equipment

In food processing plants, cleaning and sanitizing operations are essential to prevent contamination. Various sanitizing agents are used such as derivatives of chlorine, acid, iodine, and quaternary ammonium compounds (Marriott, 1994). Besides, thermal sanitation is quite effective in destroying contaminating microorganisms (Güzel-Seydim *et al.*, 2004). Since ozone is a powerful oxidant, it can be used for the disinfection of processing equipment and environments.

Greene *et al.* (1993) investigated the use of ozonated water as a sanitizer for dairy and food plants. It has been indicated that ozone was as effective as chlorine against bacteria attached to dairy surfaces. Both treatments reduced bacterial populations by 99%.

If the cleaning process is inefficient, microorganisms may form biofilms on equipment surfaces. Dosti (1998) attempted ozone treatment against bacteria which are able to form a biofilm. Both ozone and chlorine significantly reduced the biofilm bacteria compared to controls. With the exception of *Pseudomonas putida*, the difference was insignificant between ozone and chlorine. Ozone killed this microorganism more effectively than chlorine. Hampson (2000) tested ozone use in wineries for barrel cleaning, tank sanitation, and clean-in-place processes. Ozone decreased the surface flora by 3 log units.

Table 11.4 Approved levels of ozone gas (Muthukumarappan *et al.*, 2000). Reproduced with permission.

Exposure	Ozone level (ppm)
Detectable odor	0.01–0.05
OSHA 8 h limit	0.1
OSHA 1.5 min limit	0.3
Lethal in a few minutes	>1700

OSHA, Occupational Safety and Health Administration.

11.7 TOXICITY AND SAFETY OF PERSONNEL

One of the major advantages claimed for ozone is the absence of potentially toxic reaction products. However, exposure to ozone can cause some detrimental health effects. Ozone toxicity is the most important criterion for approval of ozone in the food industry. Nevertheless, sensitivity to ozone varies among individuals. Low concentrations (0.1 ppm) of ozone cause irritation to the nose, throat, and eyes. The human lung is the primary target organ of ozone gas. Pulmonary edema, capillary hemorrhage, and inflammation of respiratory tract occur initially. When exposure time is prolonged, ozone may cross the alveoli and causes damage in blood cells and also to serum proteins (Khadre *et al.*, 2001; Watada *et al.*, 2005). If exposure exceeds 24 h, the damage may be irreversible. Exposure to high concentrations (50 ppm and above) and off-gas may induce mutagenic defects and can lead to death over longer periods (Muthukumarappan *et al.*, 2000; Fielding and Bailey, 2005; Sharma, 2005).

Personnel safety is important for the practical application of ozone in food-processing plants. In addition to respirators, systems for ozone detection and destruction are essential for the safety of workers. An ozone analyzer must be installed in ozonation rooms. When the ozone concentration in the ambient air exceeds 0.1 ppm, the analyzer must trigger both a displayed and acoustic warning signal. Also, the generation system could be shut down if an ozone leak occurs (Muthukumarappan *et al.*, 2000; Khadre *et al.*, 2001). The odor of ozone is easily detected at concentrations much lower than detrimental levels. This pungent odor provides quite an advantage for the safety of personnel.

The recommended ozone levels of the Occupational Safety and Health Administration (OSHA) are shown in Table 11.4. The current permissible level for ozone exposure in the workplace is 0.1 ppm. This means that an individual may be continuously exposed to ozone (0.1 ppm) under normal working conditions for 8 h a day or 40 h a week without adverse effects (Pascal *et al.*, 2007).

11.8 CONCLUSION

Ozone seems to be an effective sanitizer for reducing microbial populations and extending the shelf life of some food products. Besides, there is growing interest in the use of ozone in dentistry and medicine. Relatively low concentrations of ozone and short contact times are sufficient to inactivate cells in pure suspensions of microorganisms. However, the presence of organic matter in foods rapidly reduces the ozone concentration in treatment solutions, thereby diminishing its antimicrobial effectiveness. The disagreement of most studies on the effectiveness of ozone treatments might be attributed to these findings.

If improperly used, ozone can cause some deleterious effects on physiology and quality of products such as losses in sensory quality. Furthermore, the effects of ozone on organic

compounds and also oxidation products should be investigated. In addition, previous contradictory results on the effects of ozone suggest that the efficacy of ozone must be assessed individually for each commodity.

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12 Application of High Hydrostatic Pressure Technology for Processing and Preservation of Foods

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Abstract: High hydrostatic pressure (HHP), also known as high-pressure processing or ultra high pressure, has been cited as one of the best breakthroughs in food science in 50 years. The benefits of HHP for food processing are manifold and include improvement in the microbiological safety of food, control of food spoilage, and extension of product shelf life. Unlike conventional thermal processing, the sensory and nutritional attributes of HHP-processed foods are not severely affected, resulting in products with fresh-like minimally processed characteristics and of higher quality. In addition, pressure transmission is uniform and quasi-instantaneous during HHP regardless of the size and geometry of food, rendering the technology more effective and energy-efficient. In light of these reasons, the use of HHP for processing food has resurged with the industry's renewed interest in its application. Within the last decade, a number of companies have introduced commercial grade high-pressure systems thus providing food processors an opportunity to preserve foods with a "cleaner" ingredient label, and offering a process of choice for applications where alternative processing methods would adversely impact product quality. This chapter opens with a general overview of HHP covering the fundamentals of this technology, and subsequently exposes the reader to the current status of HHP, highlighting its vast research scope and commercial applications for major food commodities.

Keywords: eggs; high hydrostatic pressure; meat; milk; pathogens; preservation; processing; produce; quality; spoilage

12.1 INTRODUCTION

Food preservation, *sensu stricto*, refers to a set of actions taken to retain the desirable properties of food as long as possible. Over the recent past, food processing and preservation have transitioned from being an art to a highly multi-disciplinary science (Palou *et al*., 2002) and the goals of processing and preservation have shifted. Today, ensuring a high level of food safety and a satisfactory shelf life have become two of the fundamental priorities of the food processing and preservation industry. Consumer trends and food markets are also very dynamic and are constantly undergoing changes (Gould, 1995). Minimally processed foods with higher quality and more fresh-like attributes are in increasingly greater demand and consequently less harsh processing treatments or fewer additives are desirable. Gould (1995) reported that consumers nowadays have a greater propensity to consume foods that have

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undergone minimal heat and chill damage, have greater freshness, and contain less acid, salt, sugar, and fat (Palou *et al*., 2002).

In order to satisfy these consumer demands, a lot of research has gone into identifying novel processing technologies as alternatives to conventional techniques. Till now, thermal processing for conversion or preservation of food has dominated the food industry (Tewari, 2007). However, for certain foods, thermal treatments bring about undesirable changes in food flavor, color, texture, and nutritional attributes. Non-thermal processing techniques, on the other hand, are regarded with special interest because they have minimal impact on the nutritional and sensory characteristics of foods, and extend food shelf life by inhibiting microorganisms and deteriorative enzymes. In addition, non-thermal food preservation processes are considered to be more energy-efficient and have the ability to offer value-added and novel products, opening new market opportunities with added safety margins (Tewari, 1997; Knorr *et al*., 1998)

Among non-thermal technologies, high hydrostatic pressure (HHP) processing is one emerging technology that has received a great deal of attention by virtue of its unique food processing or preservation potential as well as its ability to modify food to achieve unique functional properties (Tewari, 1997). Food deterioration or spoilage are processes that are dependent on various biological, chemical and physical factors, all of which need to be controlled or maintained at a desired level. HHP has the potential to serve as an important preservation method in: (i) directly inactivating spoilage and disease-causing microorganisms; (ii) slowing down or delaying the onset of chemical and enzymatic deteriorative processes; and (iii) retaining the important physical or physicochemical characteristics of food. Hence, in the last 30 years or so, the use of HHP has become actively explored by research institutions and adopted to some extent by the food industry (Tewari, 1997). It is anticipated that further commercialization efforts worldwide will place high-pressure-treated foods in several newer markets soon (Hoover *et al*., 1989; Farr, 1990; Pothakamury *et al*., 1995).

The aim of this chapter is to provide an overview of HHP as a non-thermal food processing and preservation technology, highlighting its ever-expanding research potential and its industrial value for various food commodities.

12.2 THE WORKING PRINCIPLES OF HIGH HYDROSTATIC PRESSURE

The operation of HHP technology is rooted in two fundamental physical principles: Le Chatelier's and the isostatic principles. According to Le Chatelier's principle, biochemical and physicochemical phenomena in equilibrium are accompanied by changes in volume and are thus influenced by pressure. Hoover *et al*. (1989) reported that pressure affects reaction systems in two possible ways: reduction in the available molecular space and enhancement of inter-chain reactions. Hence, HHP affects any reactions in food systems involving a volume change and positively influences those reactions that result in a volume decrease.

The other principle underlying the mechanism of action of HHP is the isostatic principle which relies on the instant and uniform pressure transmittance throughout food systems regardless of products' shape, size, or geometry. Essentially, during pressurization, a product undergoes reversible deformation as it is uniformly compressed in all directions before returning to its initial shape after decompression (pressure release). HHP is thought to be more energy-efficient for high-moisture foods as only a small amount of energy is

consumed to pressurize the product of interest as a result of its low compressibility, offering advantages such as reduced processing times, minimal nutrient loss, and freshness retention.

12.3 MICROBIAL INACTIVATION BY HIGH HYDROSTATIC PRESSURE

Over the past three decades, HHP technology has garnered considerable research interest, especially relating to pressure-inactivation of microorganisms in foods (Dogan and Erkmen, 2004; Donaghy *et al*., 2007) to enhance the microbial safety and quality of foods. Microorganisms vary enormously in their sensitivity to HHP treatment, and their response differs depending on the genetic make-up and physiological state of the bacterial species and strains, the medium in which they are suspended, as well as the processing parameters. Table 12.1 shows the outcomes of pressure inactivation of different pathogenic and spoilage microbes naturally present in or artificially contaminating different food systems.

Pressure treatment of microorganisms induces various changes to microbial cellular targets including changes to the cell permeability and morphology, inhibition of physiological reactions essential for cell maintenance, survival and reproduction, and genetic mechanisms (Farr, 1990). Although the intricate mechanistic details of microbial inactivation still remain uncertain, several theories have been proposed over the years in an attempt to shed light on the mechanism of microbial inactivation to optimize the high-pressure processing parameters of foods. The different cellular targets of high pressure involved in bacterial inactivation are described in the following section.

12.3.1 Effect of high pressure on bacterial cell membrane

The membrane is generally recognized as the main site of pressure damage in microorganisms as high-pressure treatment almost always leads to a perturbation of bacterial membranes (Morita, 1975; Ulmer *et al*., 2000; Casadei *et al*., 2002). Several studies have in fact demonstrated that physical damage to the membrane is marked by an increased uptake of exogenous fluorescent dyes or leakage of ATP (Smelt *et al*., 1994; Benito *et al*., 1999). In addition, it is thought that pressure resistance is correlated with the rigidity of the membrane and hence the age of cells. Hence, log-phase cells are more pressure-sensitive as a result of permanent damage induced to the cell membrane while cells in the stationary phase are more refractory as their membrane is more robust and better able to sustain the pressure stress (Casadei *et al*., 2002). HHP can also alter other membrane functions such as active transport or passive diffusion thereby offsetting cell homeostasis. Microbial inactivation is also partly due to the loss of functionality of membrane-bound enzymes. Peripheral and integral membrane proteins of pressure-treated cells were shown to detach from the surrounding lipid bilayers normally responsible for creating an environment conducive for their optimal activity (MacDonald, 1992).

12.3.2 Effect of high pressure on bacterial cell morphology

Intracellular damage has also been observed in high-pressure-exposed cells viewed by scanning electron microscopy (Patterson, 2005). Some authors observed the formation of bud scars on the surface of cells of *Listeria monocytogenes* after a 10 min pressure treatment

Table 12.1 Effect of HHP on inactivating pathogenic microorganisms in various meat, fish, dairy, egg, and produce commodities.

Substrate media	Microorganism	Treatment conditions	Log reduction	Reference
Pork slurry	*Yersinia enterocolitica*	300 MPa, 10 min	6	Shigehisa *et al*. (1991)
Pork slurry	*Campylobacter jejuni*	300 MPa, 10 min	6	Shigehisa *et al*. (1991)
Pork slurry	*Staphylococcus aureus*	600 MPa, 10 min	6	Takahashi *et al*. (1991)
Poultry meat	*Staphylococcus aureus*	600 MPa, 30 min	4	Shigehisa *et al*. (1991)
Poultry meat	*Escherichia coli* O157:H7 NCTC 12079	600 MPa, 15 min	3	Patterson *et al*. (1995)
Poultry meat	*Listeria monocytogenes*	375 MPa, 15 min	<1	Patterson *et al*. (1995)
Turkey meat	*Listeria monocytogenes*	500 MPa, 1 min	3.8	Chen (2007)
Strained chicken baby food	*Salmonella* Senftenberg 775W	340 MPa, 10 min	<2	Metrick *et al*. (1989)
Foie gras	*Staphylococcus aureus*	400 MPa, >10 min	>6	El Moueffak *et al*. (1995)
Oysters	*Vibrio parahaemolyticus* O3:K6	300 MPa, 3 min	5	Cook (2003)
Oyster meats	*Vibrio parahaemolyticus*	>350 MPa, 2 min	>5	Kural *et al*. (2008)
Oysters	*Vibrio vulnificus*	≥250 MPa, ≤4 min	>5	Kural and Chen (2008)
Canned clam juice	*Vibrio parahaemolyticus*	170 MPa, 10 min	>5	Styles *et al*. (1991)
Raw milk	*Listeria monocytogenes* Scott A	340 MPa, 60 min	6	Styles *et al*. (1991)
UHT milk	*Listeria monocytogenes* Scott A	340 MPa, 80 min	6	Styles *et al*. (1991)
Ewe's milk	*Lactobacillus helveticus*	500 MPa, 10 min	3	Gervilla *et al*. (1997b)
Ewe's milk	*Pseudomonas fluorescens*	450 MPa, 10 min	4	Gervilla *et al*. (1997b)
Model bovine milk cheese	*Yersinia enterocolitica*	300 MPa, 10 min	1.95–3.48	De Lamo-Castellvi *et al*. (2005)
Liquid whole egg	*Listeria innocua*	450 MPa, 10 min	6.6	Ponce *et al*. (1998b)
Liquid whole egg	*Escherichia coli* CECT 405	450 MPa, 10 min	7	Ponce *et al*. (1998a)
Liquid whole egg	*Salmonella enteritidis*	450 MPa, 15 min	5.1	Ponce *et al*. (1999)
Alfalfa seeds	*Escherichia coli* O157:H7	650 MPa, 15 min	>5	Neetoo *et al*. (2008)
Alfalfa seeds	*Salmonella* spp.	500 MPa, 2 min	>5	Neetoo and Chen (2010)
Apple juice	*Escherichia coli* O157:H7	615 MPa, 1 min	0.2	Teo *et al*. (2001)
Orange juice	*Escherichia coli* O157:H7	615 MPa, 1 min	1.07	Teo *et al*. (2001)
Grapefruit juice	*Escherichia coli* O157:H7	615 MPa, 1 min	2.4	Teo *et al*. (2001)
Apricot juice	*Salmonella enteritidis*	250 MPa, 10 min	4.78	Baymdirh *et al*. (2006)
Orange juice	*Salmonella enteritidis*	250 MPa, 10 min	5.53	Baymdirh *et al*. (2006)
Cherry juice	*Salmonella enteritidis*	250 MPa, 10 min	6.67	Baymdirh *et al*. (2006)
Carrot juice	*Escherichia coli* O157:H7	615 MPa, 1 min	4.51	Teo *et al*. (2001)

UHT, ultra heat-treated.

at 400 MPa (Ritz *et al*., 2001). Park *et al*. (2001) exposed cells of *Lactobacillus viridescens* to a pressure level of 400 MPa for 5 min at 25°C and showed that the ultrastructure of pressure-treated cells revealed the presence of nodes on the cell wall. Some authors also noticed that pressure injury manifested in the disruption of ribosomes (Smelt *et al*., 2001). Smelt *et al*. (2001) stated that high pressure results in the destabilization and reduction in the number functional ribosomes, preventing impaired cells from recovering and leading to eventual cell death.

12.3.3 Effect of high pressure on biochemical and enzymatic processes in microorganisms

Several authors reported that pressure affects reaction systems in two apparent ways: (i) by altering intra-molecular structures and reducing the available molecular space; and (ii) by increasing inter-chain reactions at enzyme/substrate interfaces (Hoover *et al*., 1989; Palou *et al*., 2002). High-pressure treatment favors biochemical phenomena that lead to an overall volume decrease while reactions that result in a volume increase are inhibited. A consequence of this is that electrostatic and hydrophobic interactions are negatively affected by high pressure (Palou *et al*., 2007), while hydrogen bonding, which is known to stabilize the α-helices and β-pleated sheets of proteins, is favored by pressure (Masson, 1992).

Exposure to high pressure may activate or inactivate enzymes by virtue of their inherent ability to withstand pressure stress (Patterson, 2005). Pressure effects on enzymes are also dependent on the pressure magnitude. Pressure less than 100–300 MPa results in reversible structural changes in enzymes and can induce an increased enzymatic activity. Enzyme activation can also occur following disruption of the membranes of subcellular compartments in which the enzymes are usually sequestered, thus facilitating enzyme–substrate interaction. Pressure exceeding 300 MPa causes irreversible denaturation (Hoover *et al*., 1989; Patterson, 2005). Pressurization at high levels at room temperature may result in reversible or irreversible, partial or complete enzymatic activity loss depending on the molecular structure of the enzyme (tertiary and quaternary structures), conditions affecting the microenvironment of the enzyme (e.g. pH), pressure level, temperature, and pressure exposure time (Hoover *et al*., 1989). Moreover, HHP can also negatively affect enzyme-mediated genetic processes such as DNA replication and gene transcription (Barlett, 2002) although HHP *per se* does not have any direct effect on the structure of DNA.

12.4 EFFECT OF HIGH PRESSURE ON THE PHYSICAL AND BIOCHEMICAL CHARACTERISTICS OF FOOD SYSTEMS

One of the main advantages of HHP is its minimal negative effect on the sensorial, functional, and nutritional attributes of food (Palou *et al*., 2007). The sensory (color, flavor, and texture) and nutritional properties of foods are important quality characteristics and major driving forces affecting consumer perception and acceptance of foods. HHP processing could retain the delicate sensory properties and nutritional value of food (Oey *et al*., 2008) due to its minimal effect on the covalent bonds of low-molecular-mass compounds such as color and flavor molecules and micronutrients.

High pressure, on many occasions, has the ability to even enhance the sensory properties of HHP-treated food products, stimulating investigators to capitalize on the unique effects of high pressure on foods. Hoover *et al.* (1989) described the structural modifications of macromolecules such as starch and protein by high pressure to reduce the cooking time of rice to a few minutes. Pressure-processed grapefruit juice loses the bitter taste of limonene, often persistent in thermally processed grapefruit juice. Hugas *et al.* (2002) described the benefits of HHP as an effective meat tenderizing technology for pre-rigor beef.

HHP also has limited effect on the textural characteristics of foods with a high moisture content. This is because the physical structure of such foods is unchanged after pressurization

since no shear forces are generated by pressure (Hogan *et al*., 2005). Hence, for foods with little to no air voids, HHP results in no drastic textural alterations. On the contrary, foods characterized by a large number of voids or air spaces, such as plant foods, may undergo a permanent deformation due to gas displacement and liquid infiltration (Hogan *et al*., 2005) or compression and subsequent expansion of gas during pressurization and pressure release respectively (Michel and Autio, 2001).

HHP in general also has a less severe impact on food nutritional properties compared to other food-processing methods. For instance, Farr (1990) showed that HHP of citrus juices conferred a fresh-like flavor with no loss of vitamin C while Elgasim and Kennick (1980) reported that pressure treatment of meat at 103 MPa for 2 min improved "apparent digestibility" and did not negatively impact on the nutritive value and protein efficiency ratio. There are few studies regarding HHP effects on the nutritional properties of pressure-processed foods; however, more research is needed before conclusive statements can be made (Earnshaw, 1996).

High-pressure treatment, to a large extent, also preserves the fresh color in food products. There are many examples in the literature illustrating the ability of high pressure to retain the color parameters of pressure-treated fruit and vegetable products. Watanabe *et al*. (1991) showed that pressure-processed fruit jams were superior with respect to brightness (L value) and redness (a value) compared to their untreated counterparts (Watanabe *et al*., 1991). Pressure-treatment of strawberries resulted in a higher retention of red color (Smelt *et al*., 2001). High pressure was also shown to enhance the color of tomato juice when compared to conventional thermal treatments (Poretta *et al*., 1995). Color parameters of pressure-treated milk are also not affected significantly. Mussa and Ramaswamy (1997) observed that pressure-treated milk underwent minor changes in its color when subjected to pressures required to inactivate pathogenic and spoilage microorganisms in milk. Similar observations were made by Johnston *et al*. (1992) who reported an increase in the translucence of pressure-processed milk with little impact on the a and b parameters. With regard to raw red meat, high pressure has been shown to bring about drastic color changes resulting from a whitening effect (increase in L value) and a loss of red color (decrease in a value) (Cheftel and Culioli, 1997). In contrast, HHP appears to be more feasible for pressure-processed cured or white meats, which undergo little color change.

Although food-quality characteristics such as flavor, color, texture, and nutritional value are unaffected or only minimally altered by HHP, enzymes related to food quality can be significantly affected by pressure. The effect of HHP on enzyme activity falls into two regimes: (i) enzyme activation at low pressures in monomeric enzymes, and (ii) enzyme inactivation at high pressures stimulated by the loss of tertiary and quaternary structures (Curl and Jansen, 1950; Asaka *et al*., 1993) in oligomeric enzymes. Hendrickx *et al*. (1998) explained that there is a pressure threshold above which enzyme inactivation starts occurring. When the pressure level reaches or exceeds this value, enzyme inactivation ensues and the rate and extent of inactivation depends on the pressure level and exposure time. Hence, effects of HHP on food enzymes and the reactions they mediate are often complex and, depend on the nature of the enzyme and substrate and HHP process conditions resulting in either increased or enzymatic activity. Recent findings on the effect of HHP on various food-quality enzymes in different food matrices are summarized in Table 12.2.

Taken together, high pressure has been shown to cause a wide variety of effects on the physical and biochemical properties of foods and those changes are unique to the type of food and its specific composition and matrix.

Table 12.2 Effect of HHP on inactivating endogenous enzymes in meat, milk, and produce commodities.

Source/ medium	Enzymes	Pressure level	Activity	Reference
Meat	Proteasome	<150 MPa	Increase	Otsuka *et al*. (1998)
Meat	Cathepsin D	500–600 MPa	Increase	Jung *et al*. (2000)
Meat	Calpain	300 MPa	Decrease	Homma *et al*. (1995)
Meat	Calpastatin	200 MPa	Decrease	Homma *et al*. (1995)
Cold smoked salmon	Cathepsin B, cathepsin L, calpain	300 MPa	Decrease	Lakshmanan *et al*. (2003)
Raw milk	Alkaline phosphatase	>800 MPa	Decrease	Rademacher and Kessler (1997)
Bovine milk	Phosphohexoisomerase	>500 MPa	Decrease	Rademacher and Kessler (1997)
Caprine milk	Lipoprotein lipase	500 MPa	Decrease	Trujillo *et al*. (1999)
Milk	Alcalase	<200 MPa	Increase	Penas *et al*. (2006)
Milk	Plasmin	>300 MPa	Decrease	Huppertz *et al*. (2004)
Cheddar cheese	Milk enzymes	50 MPa	Increase	O'Reilly *et al*. (2000)
Barley and wheat	α-Amylase, β-amylase	300–600 MPa	Increase	Gomes *et al*. (1998)
Strawberry	β-Glucosidase	200–400 MPa	Increase	Zabetakis *et al*. (2000)
Raspberry	β-Glucosidase	600–800 MPa	Decrease	Garcia-Palazon *et al*. (2004)
Grape juice	Polyphenoloxidase	600 MPa	Decrease	Jolibert *et al*. (1994)
Pear pieces	Polyphenoloxidase	400 MPa	Increase	Asaka *et al*. (1993)
Citrus juice	Pectin methyl esterase	600 MPa	Decrease	Ogawa *et al*. (1990)
Grapefruit juice	Pectin methyl esterase	>600 MPa	Decrease	Goodner *et al*. (1998)
Orange juice	Pectin methyl esterase	>700 MPa	Decrease	Goodner *et al*. (1998)
Orange juice	Pectin methyl esterase	>200 MPa	Increase	Cano *et al*. (1997)
Kiwifruit juice	Actinidin	>500 MPa	Decrease	Katsaros *et al*. (2009a)
Papaya juice	Papain	>500 MPa	Decrease	Katsaros *et al*. (2009b)
Figs juice	Ficin	>500 MPa	Stable	Katsaros *et al*. (2009b)
Tomato puree	Polyphenoloxidase	<200 MPa	Increase	Cano *et al*. (1997)
Tomato puree	Pectin methyl esterase	>800 MPa	Decrease	Stoforos *et al*. (2002)
Mushroom tissue	Polyphenoloxidase	600 MPa	Increase	Matser *et al*. (1998)
Mushroom	Polyphenoloxidase	800 MPa	Decrease	Matser *et al*. (1998)
Green bean slurry	Lipoxygenase	>500 MPa	Decrease	Indrawati *et al*. (2000)
Peas slurry	Lipoxygenase	>400 MPa	Decrease	Indrawati *et al*. (2001)
Peas	Peroxidase	>600 MPa	Decrease	Quaglia *et al*. (2006)
Soybean	Lipoxygenase	>400 MPa	Decrease	Heinisch *et al*. (1995)
Onion	Polyphenoloxidase	<700 MPa	Increase	Butz *et al*. (1994)
Broccoli	Myrosinase	200–500 MPa	Increase	Ludikhuyze and Hendrickx (2001)

12.5 APPLICATIONS OF HIGH HYDROSTATIC PRESSURE TO SPECIFIC FOOD COMMODITIES

Over the last 30 years, we have observed a rapid expansion in the investigation of HHP technology to process and preserve a wide variety of foodstuffs. To date, research and industrial scientists continue to seek for yet newer applications of HHP in an attempt to develop it as a powerful tool in various sectors of the food industry. The following section does not purport to be an all-inclusive review of high-pressure-related research; rather it seeks to provide the reader with an appreciation of the important concepts underlying the applications

of HHP as illustrated by numerous examples and its value as a novel processing and preservation technology across a fairly wide spectrum of food commodities and products.

12.5.1 Effect of high hydrostatic pressure on muscle foods

HHP has been shown to be a very promising technology for inactivating pathogenic and spoilage microorganisms in meat, processing cooked and cured meat products to prevent recontamination, tenderizing raw meat, as well as developing new meat product lines.

12.5.1.1 Use of high hydrostatic pressure to improve the microbiological safety of fresh and cooked meat products

Meat is mainly composed of water, protein, fat, micronutrients, and vitamins (Hugas *et al.*, 2002) and is thus a very rich matrix supportive of microorganism growth. The extent of pressure inactivation of microorganisms present in meat depends on several intrinsic parameters (food composition, pH, water activity, type of microorganism) as well as extrinsic parameters (pressure magnitude, treatment time, temperature) (Hugas *et al.*, 2002).

Several challenge studies have investigated the effectiveness of HHP against various pathogenic and spoilage microorganisms naturally present in or artificially contaminating fresh meat. Significant variation in the pressure sensitivity of different species and strains of pathogenic microorganisms (*L. monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium*) in meat systems has been reported (Simpson and Gilmour, 1997; Alpas *et al.*, 1999). Shigehisa *et al.* (1991) showed that the application of high pressure to a pork slurry (pH 6–7) at 400 MPa and 25°C for 10 min achieved at least ≈6 log reduction in the populations of several pathogenic or spoilage organisms including *E. coli*, *Campylobacter jejuni*, *Pseudomonas aeruginosa*, *S. typhimurium*, *Yersinia enterocolitica*, *Saccharomyces cerevisiae*, and *Candida utilis* inoculated at a level of 10^6–10^7 colony-forming units (CFU)/g. Schilling *et al.* (2009) determined the effects of pressure on the inactivation of *E. coli* O157:H7 as well as the consumer acceptability of frozen ground beef patties. Pressure treatment at 300 MPa for 5 min at 4°C was not able to inactivate this pathogen and it brought about a decreased sensory acceptability ($P < 0.05$) when compared to other treatments.

The use of HHP to enhance the microbial safety of poultry products has also been extensively studied. Escriu and Mor-Mur (2009) demonstrated that the fat content of chicken meat could impact on the pressure inactivation of *Listeria innocua* and *S. typhimurium* during subsequent long-term post-processing refrigerated storage. Morales *et al.* (2009) also studied the pressure inactivation of *Salmonella enteritidis* in poultry meat (chicken breast fillets) and showed that multiple cycles of HHP at 400 MPa for 5 min sufficed in eliminating the pathogen. El Moueffak *et al.* (1995) studied the effect of high pressure to "pasteurize" fatty goose or duck liver (foie gras) using a mild heat treatment of 50°C (El Moueffak *et al.*, 1995). High pressure at 400 MPa and 50°C for at least 10 min was shown to reduce the microbial burden to a similar extent as conventional thermal pasteurization with a concomitant reduction of ≈6 log of mesophilic flora, ≈5 log of psycrophilic bacteria, and complete elimination of *S. aureus*. Thus, the reported treatment conditions or pressure treatment at 400 MPa, 60°C, and 5 min would be useful to enhance the microbiological safety and even extend the refrigerated shelf life of foie gras with minimal adverse effect on its sensory properties.

In addition, research on the application of HHP on cooked, cured, or fermented ready-to-eat meat products to reduce post-process recontamination have also been reported. Jofré *et al.*

(2008) showed that the application of a 600 MPa treatment to sliced cooked and dry cured ham effectively inactivated the foodborne pathogens *L. monocytogenes*, *Salmonella enterica*, *S. aureus*, *Y. enterocolitica*, and *C. jejuni*. Garriga *et al.* (2005) showed that the addition of starter cultures consisting of selected strains of *Lactobacillus sakei* in combination with HHP treatments were necessary to ensure the absence of *L. monocytogenes*, Enterobacteriaceae, *Enterococcus*, and *Salmonella* on slightly fermented sausages.

Various experiments have shown that the application of high pressure alone may not always constitute a safe enough process but, in combination with other hurdles such as low pH, low temperatures, mild heat, or the addition of antimicrobials, the efficacy of HHP may be increased greatly. Garriga *et al.* (2002b) studied the inhibitory effect of bacteriocins (enterocin A and B, sakacin K, pediocin AcH, or nisin) and pressure at 400 MPa for 10 min at 17°C on several foodborne bacteria inoculated into a meat model system during chilled storage. The potential of combined HHP and mild heat "pasteurization" treatments could also be extended to various value-added meat or meat-based products (Zuber, 1993). Carlez *et al.* (1993) investigated the effect of high pressure to inactivate *Pseudomonas fluorescens*, *Citrobacter freundii*, or *L. innocua* (non-pathogenic models for *Salmonella* spp. *or L. monocytogenes*) on minced beef meat at refrigerated, ambient, and slightly elevated temperatures. At 20°C, the degree of inactivation was highest for *P. fluorescens* followed by *C. freundii* and *L. innocua*. Elevated (35 or 50°C) or lower (4°C) temperatures enhanced pressure inactivation of the organisms. In all, it was shown that the refrigerated shelf life was extended by 10–15 days during chilled storage at 3°C. Along the same line, Chen (2007) showed that low (<10°C) or elevated (>30°C) temperatures enhanced *L. monocytogenes* inactivation in ready-to-eat turkey breast meat.

12.5.1.2 Use of high hydrostatic pressure to improve the microbiological quality of fresh and processed meat products

Marinated beef loin, a raw meat product typified by a high water activity and low salt and nitrite contents, harbors a mixed flora of spoilage microorganisms acquired from slaughterhouse processing. Garriga *et al.* (2002a) treated sliced, skin vacuum-packaged marinated beef loin by high pressure at 600 MPa for 6 min at 31°C. The author observed that aerobic, psychrotrophic, and lactic acid bacteria (LAB) counts underwent ≈4 log reductions after treatment. In addition, the population was constantly undetectable during the refrigerated storage period of 120 days, helping to prevent the development of sour tastes and off-flavors symptomatic of spoilage. Murano *et al.* (1999) tested the efficacy of HHP in conjunction with mild heat treatment at 50°C in ground pork patties and demonstrated a shelf-life extension of 23 days at no expense of the sensory characteristics of treated patties. Sliced vacuum-packaged cooked ham is also very susceptible to spoilage by virtue of its intrinsic characteristics such as its pH, composition, water activity, and the lack of a background flora competing with spoilage microorganisms. The physicochemical and microbiological characteristics of cooked ham do not constitute any preservation hurdles to retard bacterial growth. The keeping time is thus highly dependent on the hygienic condition of the final product in the post-processing phase, as well as on the packaging methods where cross-contamination is more likely to take place. Garriga *et al.* (2002a) demonstrated that pressure treatment of cooked ham at 600 MPa for 6 min significantly delayed spoilage bacterial growth by 60 days, thus helping to retain the sensory characteristics for at least 60 days post-treatment. The same authors also showed that HHP can be a viable technology to extend the shelf life of dry cured ham, displaying at least a 2 log reduction of spoilage microbes after

treatment at 600 MPa for 6 min. O'Brien and Marshall (1996) showed that the refrigerated shelf life of fresh raw chicken mince in sealed pouches could be also significantly extended to more than 94 days by HHP depending on the pressure magnitude applied.

12.5.1.3 Effect of high hydrostatic pressure on the texture and appearance of meat products

Ludikhuyze and Hendrickx (2001) reported that when muscle foods are subjected to pressure they become firmer and more contracted. However, once cooked, pressurized meat is thought to be more moist and tender with lesser contraction and drip loss compared to unpressurized meat. Many investigators have demonstrated that the application of pressure ranging from 100 to 150 MPa can be used to tenderize pre-rigor meat (Elgasim and Kennick, 1980; Ohmori *et al.*, 1991). It is surmised that this is due to the activation of proteases such as cathepsins and/or inactivation of calpains (two enzymes involved in the tenderization of aged meat) by pressure or the disruption of connective muscle fibers. Elgasim and Kennick (1982) also studied the contribution of pressure in delaying the shrinkage of beef tissue during rigor mortis under chilled storage conditions. The investigators reported that when the product was processed at a pressure level of 103.5 MPa for 2 min at 37°C, a pH reduction occurred accompanied by a concomitant tenderizing effect on the muscle fiber. Other authors such as Bouton *et al.* (1977) and Ohmori *et al.* (1991) showed that the application of high pressure in conjunction with mild heat (55–60°C) was highly effective in preventing the development of tissue toughening. Suzuki *et al.* (1994) also reported that the application of high pressure at 500 MPa at ambient temperature promoted meat tenderization but at the expense of color loss and a change in appearance. Farr (1990) and Elgasim and Kinneck (1982) also made similar color observations on pressure-treated meat while Carlez *et al.* (1993) reported that application of pressures exceeding 150 MPa on raw meat for a duration of 10 min led to a color change from red to gray. Hence, it can be concluded that pressure offers an evident advantage with regard to meat tenderization possibly for cured and white meats but not for raw red meats.

12.5.1.4 Effect of high hydrostatic pressure on the sensorial attributes of meat

Relatively limited information is available about the sensory aspects of pressurized meat although it has been reported that pressurization confers a fairly sweet taste relative to untreated meat (Cheftel and Culioli, 1997). Suzuki *et al.* (1994) followed the effect of pressure on water-soluble compounds responsible for the characteristic meaty flavor, showing an increase in the amount of peptides and amino acids released post-pressure treatment. The authors attributed this profile change to a combination of pressure-induced denaturation of proteins from the muscle tissue as well as release of proteolytic enzymes from lysozomes (Cheftel and Culioli, 1997). Cheah and Ledward (1995) studied the effect of high pressure to retard lipid oxidation in meat and reported that pressurization of pork meat at 800 MPa for a short duration of 20 min stabilized lipids, thus prolonging its keeping time.

12.5.1.5 Commercial relevance of high hydrostatic pressure on meat commodities

Currently, there are more than 50 national or international companies that employ HHP technology to process and market a wide variety of foodstuff for added benefits of increased safety, improved quality, and enhanced sensory quality. Hormel® Foods, is a pioneer processor in the USA that has adopted large-scale HHP to pasteurize their ready-to-eat

meats branded as "Breaded ready sliced meats." Perdue® Farms is also currently employing HHP at a level of 600 MPa for in-package pasteurization of its line of ready-to-eat seasoned chicken strips. Similarly, other meat processors in the USA such as Kraft Foods, Foster Farms, and Wellshire Farms have also employed HHP to process several lines of ready-to-eat meat products (Balasubramaniam *et al.*, 2008). Esteban Epuna, a Spanish company, produces sliced cooked ham that has been pressurized at 400 MPa for 10 min, offering a shelf-life extension of 60 days and a significant reduction of the risks associated with the pathogens *Salmonella*, *L. monocytogenes*, and *S. aureus*. The same company has also carried out optimization studies on the use of HHP for meat products with lower water activity such as dry cured ham to obtain higher pathogen-reduction levels. Similarly, several Japanese companies have also developed a type of cured pork meat pressure processed at 250 MPa for ≈3 h, with improved sensory, microbiological, and textural quality. Fuji Ciku Mutterham, a Japanese company, has been utilizing HHP for both beef tenderization (Henry, 1997) and for processing various ready-to-eat meat products (Garriga and Aymerich, 2009).

12.5.2 Effect of high hydrostatic pressure processing on fishery products

12.5.2.1 Effect of high hydrostatic pressure on the microbiological status of seafood

Fish and shellfish are generally more susceptible to spoilage by Gram-negative bacteria which tend to be relatively more pressure-sensitive (Murchie *et al.*, 2005). Gram-positive bacteria such as LAB tend to be more barotolerant and are thus more difficult to inactivate by high pressure. Although LAB may not be completely inactivated by high pressure, their numbers in seafood can still be reduced (Hurtado *et al.*, 2001) and their growth delayed (López-Caballero *et al.*, 2000; Paarup *et al.*, 2002). Pressure-treated fishery product may also have a longer acceptable shelf life than their untreated counterparts as a result of reduced enzymatic activity implicated in seafood deterioration. Hurtado *et al.* (2001) reported the plausibility of quality enhancement of seafood as a result of a two-pronged action of high pressure to inhibit the growth of spoilage bacteria as well as the activity of endogenous enzymes involved in seafood quality loss.

Miyao *et al.* (1993) studied the effects of HHP on the microbiota of surimi paste. Application of pressure at 300–400 MPa completely eliminated the background flora of surimi paste with fungi presenting themselves as the most pressure-sensitive. They reported that certain spoilage bacteria prevalent in seafood such as *Moraxella* spp., *Acinetobacter calcoaceticus*, *Streptococcus faecalis*, and *Corynebacterium* spp. were noticeably barotolerant and exhibited an extended lag phase as compared to untreated bacteria. Fuji *et al.* (1994) studied the effects of high pressure to inactivate the background flora in minced mackerel meat and showed complete elimination of *Bacillus*, *Moraxella*, *Pseudomonas*, and *Flavobacterium* spp. and surviving cells of *Staphylococcus* and *Micrococcus* spp.

Carpi *et al.* (1995) spiked smoked salmon cream samples with a variety of pathogenic and spoilage microorganisms including *S.* Typhimurium, *L. monocytogenes*, *S. aureus*, *Penicillium expansum*, *Clostridium sporogenes*, *Lactobacillus casei*, and Enterobacteriaceae prior to HHP. Their findings showed that pressure-treated samples displayed significantly reduced microbial counts with a shelf-life extension of 120 days during storage at refrigerated (3°C) with minimal adverse effects on the chemical, microbiological, or the sensorial quality of the product.

12.5.2.2 Effect of high hydrostatic pressure on the sensorial quality of seafood

HHP can potentially affect the color of fish, which is dependent on various intrinsic factors such as the pH of the tissue, the pigment present and its chemical state as well as the degree of denaturation undergone by muscle proteins. Generally speaking, HHP can be more promising in treating white fish compared to dark or red fish since white flesh undergoes minimal color change during pressure treatment with a slight increase in the opacity of the tissue. Ko and Hsu (2002) examined the effect of high-pressure/normal-temperature storage on the processing quality of tilapia fillets subjected to pressures ranging from 50 to 300 MPa at 25°C for up to 12 h. The authors observed that low pressure was greatly beneficial in tilapia fish storage.

On the other hand, other researchers have reported that high pressure can have a negative impact on the organoleptic characteristics of pressure-processed seafood. Several authors also demonstrated that HHP of fish can lead to the development of off- or "warmed-over" flavors due to accelerated lipid oxidative processes, with a greater extent of lipid oxidation in the case of mackerel muscle lipids (Ohshima *et al*., 1993). Angsupanich and Ledward (1998) demonstrated that when cod muscle was subjected to pressures greater than 400 MPa for 20 min at 20°C prior to refrigerated storage at 4°C there was a higher rate of lipid oxidation indicated by elevated levels of 2-thiobarbituric acid compared to samples treated at a lower pressure level of 200 MPa. Ohshima *et al*. (1993) reported that lipids in fish muscle underwent lipolysis under high pressures. The author also treated cod muscles at pressures ranging from 200 to 600 MPa for 15–30 min and demonstrated increased lipid oxidation. Hayashi *et al*. (1990) showed that high pressure negatively impacted on the sensorial quality of fish as marked by a decrease in the levels of IMP (an indicator of fish freshness) during pressurization at 200, 350, and 500 MPa.

Hence, taken together, these studies demonstrate that the application of low pressure (generally less than 400 MPa) to seafood products at ambient temperature can be a more promising intervention to inhibit microbial growth and extend the shelf life of fishery products as well as possibly generate novel products.

12.5.2.3 Effect of high hydrostatic pressure on the textural and functional properties of HHP-treated seafood

According to Angsupanich and Ledward (1998), changes in the physical characteristics of seafood after pressure treatment differ from those that result from thermal treatments and the degree of those pressure-induced changes vary as a function of the pressure level applied and the nature of the fishery product of interest. Many investigators have reported that high pressure results in a "tougher texture" or "higher shear strength" in treated seafood than in their untreated counterparts although a few authors have observed softening of treated bluefish (Ashie and Simpson, 1996) or carp (Yoshioka and Yamamoto, 1998) flesh after pressure processing. Ohshima *et al*. (1993) studied the effect of high pressures on fish muscle proteins and showed that pressurizing myofibrils at 150 MPa for 30 min led to a disruption of fish muscle fibers.

HHP also has the potential to induce the gelation of fish proteins and modification of protein functionality, thus achieving products with unique characteristics that distinguish them from those processed by conventional heat treatment (Cheftel and Culioli, 1997; Messens *et al*., 1997; Perez-Mateos and Montero, 1997). Pressure-induced gels display superior sensory qualities compared to heat-induced gels (Cheftel and Culioli, 1997; Messens *et al*., 1997), thus opening new avenues for creating potentially novel fish products with

interesting textural properties. Gelation can also be used for binding applications, meat restructuring, and molding of surimi gels into imitation seafood.

12.5.2.4 Commercial relevance of high hydrostatic pressure on seafood commodities

Oysters are high-value products and are generally eaten raw from the shell. HHP has been used for the commercial processing of oysters (Voisin, 2001, 2002). Pressurized oysters tend to have an increased moisture content, are juicier and plump, and their adductor muscles are easily released from the shell allowing them to be readily shucked. Currently, Motivatit Seafood Inc. employs low-pressure treatment of 260 MPa for 3 min at ambient temperature to inactivate *Vibrio vulnificus* in raw oysters marketed as Gold Band™ fresh oysters. Other nationally recognized firms which also utilize HHP to produce high-quality safe oysters include Nisbet Oyster Co., Inc and Joey Oysters (Raghubeer, 2008).

In Japan, HHP at a level of 400 MPa is used to induce gelation of fish proteins of pollock, sardine, and skipjack tuna in the making of specialty products such as surimi (Okazaki and Fukuda, 1996). Various other fishery products such as fish sausages, terrines, and puddings also manufactured using high pressure are already available commercially in Japan (Cheftel, 1995). The scope for manufacturing gelled seafood at low temperature and elevated pressures is wide and presents great practical interest to the seafood and more specifically to the surimi industry.

Pressure-processed kipper products have also been commercialized in Japan since 2000. HHP not only reduces initial counts of spoilage (coliforms) and pathogenic (*Staphylococcus*, *Salmonella*, and *Vibrio*) microorganisms, but also brings about an extended refrigerated shelf life of up to 1 year. Moreover, pressure-processed pre-marinated kipper is widely appreciated due to their improved texture profiles (Suzuki, 2002). Similarly, HHP-treated smoked salmon has also been marketed in Japan with reduced microbial counts and improved flavor and texture (Suzuki, 2002).

12.5.3 Effect of high hydrostatic pressure processing on milk and dairy products

Many studies have been conducted on the use of HHP on milk and other dairy products. Studies conducted encompass the application of HHP to: (i) increase the safety and keeping quality of milk and dairy products; (ii) process milk for cheese and yogurt production; as well as (iii) manufacture innovative dairy products with novel properties.

12.5.3.1 Effect of high hydrostatic pressure on the microbiological safety of milk and dairy products

In recent years, many studies have been carried out on the pressure inactivation of pathogenic microorganisms (naturally present in or artificially contaminating) in milk and dairy products. These analyses have generally showed that HHP at a pressure level of 400–600 MPa can enhance the microbiological safety to a similar extent as heat pasteurization conditions (72°C for 15 s) (Kolakowski *et al*., 1997; Mussa and Ramasawmy, 1997; Buffa *et al*., 2001). To that effect, several studies have compared the degree of pressure tolerance of various pathogenic and spoilage organisms present in milk. Gervilla *et al*. (1997a, 1997b, 1999a, 1999b) studied the effect of high pressure on five different organisms: *E. coli* CECT 405 (a good indicator of direct or indirect contamination of fecal origin), *P. fluorescens* CECT 378 (major spoilage microflora of refrigerated milk), *L. innocua* CECT 910 (indicator for human pathogen *L. monocytogenes*), *S. aureus* CECT 534 (major spoilage microorganism in

mastitic milk), and *Lactobacillus helveticus* CECT 414 (a non-pathogenic lactic microflora) (Gervilla *et al*., 1997a, 1997b, 1999a, 1999b). Results from this study have shown that high-pressure inactivation was greater for Gram-negative than Gram-positive organisms in the overall order of *P. fluorescens*>*E. coli*>*L. innocua*>*L. helveticus*>*S. aureus*.

High pressure has also been shown to enhance the microbiological safety and sensorial quality of milk to produce high-quality cheeses. Linton *et al*. (2008) showed that pressure-treatment of raw bovine milk inoculated with 2 or 4 log CFU/ml *L. monocytogenes* completely inactivated the pathogen. The authors showed that Camembert cheese made from such pressure-treated milk retained the desirable sensorial characteristics such as the appearance and aroma of raw milk. Gallot-Lavallée (1998) studied the listericidal efficacy of high pressure for processing goat cheese made from raw milk and was able to report more than 5.6 log reduction with minimal adverse effects on the sensory characteristics of cheese. Szczawinski *et al*. (1997) showed that the application of high pressure at 500 MPa for 15 min brought about a ≈6 log reduction of *L. monocytogenes* and cheese background microflora in ripened cheese. Reps *et al*. (1998) also observed a significant decrease in total microbial counts when Gouda and Camembert cheeses were pressurized above 400 MPa although spore counts were unchanged even after exposure to pressure of 1000 MPa.

Several investigators have also studied the effect of high pressure in combination with additional hurdles such as mild heat (30–50°C) and/or bacteriocins (nisin, pediocin, and lacticin) for the inhibition of foodborne bacteria and spores and their findings have showed that additional hurdles enhance the efficacy of high pressure either additively or synergistically (Garcia Graells *et al*., 1999; Alpas and Bozoglu, 2000; Morgan *et al*., 2000). Capellas *et al*. (1996) reported a reduction of ≈7 log units of *E. coli* populations in inoculated fresh goat milk pressurized in the range of 400–500 MPa for 5–15 min at refrigeration and room temperatures thus helping to extend the refrigerated storage lives of cheese. These authors also studied the resistance of cocci (*Staphylococcus carnosus*) and spores (*Bacillus subtilis*) in fresh cheese, as these groups of microorganisms are known to be resistant to pressure. They found that when high pressure was applied in conjunction with bacteriocins or in multiple cycles (oscillatory pressurization), higher inactivation was achieved. Multiple cycle treatment of 500 MPa and nisin was the most effective treatment in inactivating indigenous cheese microflora. Lopez-Pedemonte *et al*. (2003) similarly showed that high pressure and mild temperatures with the addition of nisin may be useful for improving the safety and keeping time of soft curd cheeses made from raw milk.

These results collectively point to the promising application of HHP to enhance the safety of milk and milk-derived products.

12.5.3.2 Effect of high hydrostatic pressure on the microbiological quality of dairy products

The investigation of the efficacy of HHP to improve the microbiological quality of milk has already been mentioned in the previous section. Various researchers have demonstrated the potential of using HHP to considerably reduce the microbial burden of other dairy products, thereby extending their shelf life. Raffalli *et al*. (1994) showed that the application of HHP considerably reduced the microbial flora of dairy cream (35% fat) after pressurizing at 450 MPa and 25°C for 10–30 min with minimal adverse effects on the rheological properties of dairy creams, the average size of fat globules, or the pH of the samples (Dumay *et al*., 1996). Hence the authors pointed to the possibility of applying high pressure to extend the chilled storage life of dairy creams.

Tanaka and Hatanaka (1992) studied the effect of high pressure (200–300 MPa at 10–20°C for 10 min) on LAB populations and sensorial characteristics of packaged yogurt. HHP did not appear to have any impact on the sensorial properties immediately after treatment, although over-acidification was observed during subsequent storage. HHP has also been investigated by other authors for its ability to inactivate yogurt microflora (especially LAB) in order to improve its storability (Krompkamp *et al.*, 1995; Reps *et al.*, 1999, 2001).

In addition to yogurt, Reps *et al.* (2000) also studied the effects of HHP on microbial populations and acidity development of kefir when processed at 200–800 MPa for 15 min and subsequently stored for 3 weeks. Increasing the pressure level from 200 to 800 MPa brought about an increased reduction of bacterial counts with complete yeast inactivation occurring at 400 MPa. Kefir pressurized at 600 and 800 MPa underwent a slight reduction in pH during storage. Mainville *et al.* (2001) also studied the inactivation of bacteria and yeasts in kefir using both thermal and non-thermal processing technologies including high pressure. Thermal technologies including conventional and ohmic heating were detrimental to the kefir physicochemical properties. On the contrary, high pressure at 400 MPa for 5 or 30 min successfully inactivated the microorganisms while maintaining the intact lipid and protein structure of the product.

12.5.3.3 Use of high hydrostatic pressure to inactivate enzymes in milk and dairy products

Raw milk contains a wide variety of enzymes that can promote biochemically deteriorative processes thereby limiting its shelf life. Trujillo *et al.* (1999) treated goat's milk at 500 MPa for 15 min to inactivate the enzyme lipase and observed significantly higher residual enzymatic activity than in thermally treated milk. Seyderhelm *et al.* (1996) on the other hand was able to report complete inactivation of the enzyme milk lipase during pressurization at a level of 700 MPa at 45°C. Scollard *et al.* (2000) studied the effect of pressure on the plasminogen/plasmin system (a major milk proteinase system) and showed the enzymes were very piezotolerant and pressures above 400 MPa was required to bring about any appreciable reduction in plasmin. Collectively, these studies indicate that enzymes that dictate the keeping quality of milk are quite baroresistant and require the application of moderate to high pressure levels and mild heat to ensure their destruction.

12.5.3.4 Effect of high hydrostatic pressure on the sensory quality of milk and dairy products

Milk color is affected to a certain extent by high pressure. Gervilla *et al.* (2001) reported that high-pressure-treated milk underwent a decrease in brightness marked by a lower L value and an increase in a and b color parameters. It is surmised that the decrease in the L value visually observed as an increase in the translucence in HHP-treated milk is due to the degradation of the casein micelles (Johnston, 1995). Mussa and Ramasawmy (1997) on the contrary did not observe any adverse color changes in high-pressure-treated milk, thus pointing to the plausibility of the application of high pressure to process milk.

One of the fundamental principles of high pressure is that it does not affect covalent bonds (unlike thermal treatments) and thus has little adverse impact on the structure of micro-nutrients such as vitamins, amino acids, simple sugars, and flavor compounds (Cheftel, 1992). As a result, minimal loss or degradation of B vitamins was observed during HHP of milk (Sierra *et al.*, 2000). Garcia-Risco *et al.* (2000) found that HHP at 400 MPa at a mild temperature (25–60°C) resulted in the retention or improvement of the sensory characteristics

of milk, thus demonstrating the great scope of high pressure to extend the keeping quality of milk with minimal adverse impact on its organoleptic characteristics.

There are numerous studies that have also highlighted the important contribution of high pressure to accelerate cheese-ripening following the pioneering study of Yokoyama *et al.* (1992). They showed that pressurizing cheese at 50 MPa at 25°C imparted a flavor and taste comparable to aged cheese. Kolakowski *et al.* (1995) studied the effect of high pressure on Gouda and Camembert cheese and similarly found an increase in peptide and free amino acid content upon a 50 MPa pressure application. Saldo *et al.* (2002, 2003) compared the effect of an extended low-pressure (50 MPa) treatment and a brief high-pressure (400–600 MPa) level on the extent of cheese ripening. The authors observed a higher increase in free amino acid content in cheese subjected to a pressure treatment at 400 MPa for 5 min. Clearly, the application of high pressure has great prospects for accelerated cheese ripening although further research is needed to elucidate the mechanisms by which it promotes increased proteolysis in cheese.

12.5.3.5 Commercial relevance of high hydrostatic pressure for processing milk

Recently, a new type of soft cheese spread pressure-treated to inactivate microorganisms and to extend its shelf life was launched in Spain (Clark, 2007). Moreover, Fonterra, a large New Zealand dairy cooperative, has proposed the pressure treatment of yogurt to preserve its probiotic cultures and extend its shelf life (Clark, 2007).

12.5.4 Effect of high hydrostatic pressure on eggs and egg products

Eggs, either whole or as segregated yolk and albumen, are widely used in human nutrition. They are either consumed directly, or indirectly added as ingredients in food preparations and processes. Egg contains a wide spectrum of proteins and egg proteins have excellent nutritional and functional characteristics and therefore are amenable for use in a variety of food products. Besides its excellent nutritional profile, liquid whole egg contributes unique physicochemical properties to foods such as foaming, coagulating, and emulsifying properties. Application of high pressure on liquid egg presents several advantages over conventionally heat-treated egg. With high-pressure processing, the risk of post-process contamination is reduced since the process is carried out in the packaged form of the product. The product can also be prepared into a ready-to-eat form without any further processing step.

12.5.4.1 Effect of high hydrostatic pressure to improve the microbiological safety of eggs

Although eggs have an excellent nutritional profile, consumers, food preparers, food scientists, and technologists are always concerned about the microbiological safety of eggs. Egg pasteurization in the USA has been set in place to reduce the risks of contracting salmonellosis with a minimum requirement of 3.5 min at 60°C. Because egg proteins are known to coagulate and denature very easily, thereby diminishing their nutritional value, there is little leeway to modify the standard pasteurization conditions. Hence, the use of HHP as a non-thermal technology has been widely studied for the processing of eggs and egg products. Ponce *et al.* (1998b) studied the pressure tolerance of *L. innocua* CECT 910, a model organism for *L. monocytogenes*, in liquid whole egg at different pressures (300, 350, 400, and 450 MPa), temperatures (−15, 2, and 20°C) and treatment times (5, 10, or 15 min). *L. innocua* populations were only partially reduced with any of the treatments. Pressure

inactivation was temperature dependent with a higher degree of reduction at 2 and −15°C than at 20°C at lower pressures although the greatest inactivation was observed after exposure to 450 MPa for 15 min at 20°C. The pressure inactivation of *S. enteritidis* in liquid egg has also been studied by the same author. Ponce *et al.* (1999) showed that complete inactivation of ≈8 log CFU/ml could be achieved when the treatment temperature is set to 50°C. Treatment in three successive cycles of 5 min showed a greater degree of inactivation than continuous treatment for an overall exposure time of 15 min. The presence of coliforms in pasteurized liquid egg is usually symptomatic of inadequate processing or post-process contamination. Ponce *et al.* (1998a) also studied the pressure inactivation of *E. coli* CECT 406 inoculated into liquid whole egg at levels ranging from 300–450 MPa, temperatures (−15, 2, 20, and 50°C) and exposure times of 5, 10, and 15 min in continuous or oscillatory pressurization modes. The highest reductions (≈7 log CFU/ml) were observed at 50°C. At 300 MPa, cyclic pressurization treatments brought about greater inactivation than continuous treatment. This finding is in close agreement with that of Honma and Haga (1990) who also showed that egg white pressurized at 300 MPa at 20°C for three successive cycles of 2 min was adequate to completely kill *E. coli* while a continuous treatment exceeding 10 min was needed to achieve a comparable reduction. It is surmised that the greater degree of inactivation observed in oscillatory pressurization is due to the repeated pressurization/decompression cycles that lead to far more severe cellular damage.

12.5.4.2 Effect of high hydrostatic pressure to improve the microbiological quality of eggs

The shelf life of liquid egg is known to vary as a function of the initial microbial burden, the pasteurization process, and the storage-temperature history. Kalchayanand *et al.* (1992) showed that high pressure in conjunction with nisin, a bacteriocin produced by *Lactococcus lactis* ssp. *lactis* has the ability to sensitize Gram-negative and Gram-positive bacteria to HHP inactivation. Ponce *et al.* (1998c) reported ≈5–6 log CFU/ml reduction of *E. coli* and *L. innocua* in liquid whole egg during the concerted application of high pressure at a level of 450 MPa for 10 min at 20°C in the presence of nisin. The two microorganisms were undetectable after 30 days' storage at refrigeration temperature. Overall, application of nisin in conjunction with high pressure enhanced the bactericidal effect of high pressure, lowering the pressure requirement and helping to retain the desirable physicochemical and biological properties of egg constituents.

Anton *et al.* (2001) pressure-treated egg yolk emulsions at low and neutral pH. His findings showed that pressure treatment at low pH at a level of 200 MPa for 10 min at 10°C was adequate to sterilize egg yolks whereas, at neutral pH, comparable reduction could only be achieved at 500 MPa for 10 min. Hence, the application of high pressure was able to completely sterilize egg yolk emulsions by capitalizing on the bactericidal effect of acidic pH.

12.5.4.3 Effect of high hydrostatic pressure on the functional and nutritional properties of eggs or egg-derived products

Various researchers have reported the application of HHP in various aspects of egg processing in addition to inactivation of pathogenic or spoilage microorganisms. The various egg proteins can be denatured, gelled, or coagulated and the degree of structural alteration varies as a function of factors such as the pH, type of protein, temperature, pressure level, and salt concentration (Hayashi *et al.*, 1989; Honma and Haga, 1990; Okamoto *et al.*, 1990). Several

investigators have studied the specific effects of high pressure on the structure and functions of egg proteins as well as on the physical characteristics of the pressure-treated eggs.

The ability of high pressure to unfold and denature globular proteins has been widely documented in the past by several authors (Cheftel, 1992; Masson, 1992). High-pressure-induced egg gelation was first studied by Bridgman (1914) who demonstrated protein coagulation at pressures of 500–700 MPa. Lee *et al.* (1999) subsequently studied the pressure-induced egg protein coagulation using steady shear analysis and showed that a threshold level exceeding 250 MPa was required to cause complete structure breakdown at ambient temperature while a lower pressure range (100–150 MPa) assisted by mild heat (45°C) could induce coagulation. Ahmed *et al.* (2003) studied the effects of HHP on the rheological characteristics of pressure-denatured whole liquid egg, albumen, and yolk at pressures ranging from 100 and 400 MPa for an exposure of 30 min. The authors observed enhanced egg-protein structure breakdown at 300 MPa for 30 min at ambient temperature with all egg samples exhibiting a time-dependent thixotropic behavior. Hayashi *et al.* (1989) demonstrated that pressurization of egg albumen and egg yolk at 400 MPa for 30 min at ambient temperature caused less severe gelation than conventional heat treatments.

Bonomi *et al.* (1998) evaluated the effects of HHP on the textural characteristics of egg white and egg yolks treated with solutes (sucrose and salt). They observed that in the presence of these protective agents, egg products did not form gels and were more likely to display beneficial properties from a nutritional and technological standpoint. Along the same line, Teramoto and Fuchigami (2002) studied the effect of high pressure and/or the addition of salts to improve the texture of frozen custard egg gel. The authors showed that pressure-shift freezing was effective in improving the quality of frozen egg custard gel. Pressure-induced egg gels present great academic and commercial interest as they have the ability to retain their original flavor and color and have better microbiological characteristics from a safety and quality standpoint (Hayashi *et al.*, 1989).

Ingestion of ovalbumin, one of the most abundant egg white proteins and one of the major allergens can result in severe food intolerance in humans (Tsukasa and Ryo, 1993). Since ovalbumin is normally resistant to proteolytic digestion by trypsin, Iametti *et al.* (1998) investigated the ability of high pressure to increase the sensitivity of ovalbumin to tryptic digestion. His findings revealed that minimal trypsin digestion occurred in pressure-insolubilized ovalbumin at pressures as high as 800 MPa. However, the addition of solutes such as sucrose and to a lesser extent sodium chloride enhanced pressure solubilization and subsequently increased the sensitivity of pressure-solubilized ovalbumin to trypsin digestion.

Hence, HHP offers many opportunities in liquid egg processing as well as in the manufacturing and optimization of novel egg-derived products because of its interesting influence on their structural, textural, and nutritional characteristics.

12.5.5 Effect of high hydrostatic pressure on fruit and vegetable products

Fruits and vegetable products have traditionally been processed using conventional heat-based processing methods known to reduce enzymatic activities and microbial populations. Thermal processing has the ability to enhance the microbiological safety and extend the shelf life of fruits and vegetable products albeit at the expense of their sensory quality. HHP on the other hand has the potential to achieve the same goals with respect to safety and shelf life while preserving the quality of fresh products to a greater extent. The application of high pressure in combination with mild heat, in the absence of additives and preservatives, also has great scope in produce processing and represents a vital objective for food researchers and industrial companies.

12.5.5.1 Effect of high hydrostatic pressure on microbial safety of plant-derived foods

The application of HHP to enhance the microbiological safety of fruit and vegetable products has been widely investigated by numerous researchers. Horie *et al.* (1991a, 1991b) reported that pressure treatment of jams at 294 MPa for 20 min was able to completely eliminate an initial burden of ≈5–6 log CFU/ml of yeasts and bacteria including *S. aureus*, *Salmonella* spp., and coliforms. Pilavtepe-Çelik *et al.* (2009) studied the survival of *E. coli* O157:H7 933 and *S. aureus* 485 in carrot juice treated at pressure levels ranging from 200 to 400 MPa at 40°C. They showed that carrot juice had a protective effect on *E. coli* O157:H7 933 whereas it had a sensitizing effect on *S. aureus*. Neetoo *et al.* (2008, 2009a, 2009b) and Neetoo and Chen (2010) evaluated the potential of using HHP technology for decontaminating alfalfa seeds inoculated with *E. coli* O157:H7 and *Salmonella* spp. The authors showed that high pressure at a level of 650 MPa for 15 min completely eliminated a ≈5 log burden of *E. coli* O157:H7. The use high pressure in conjunction with mild heat (Neetoo *et al.*, 2009a; Neetoo and Chen, 2010) or high pressure preceded by a soaking step (Neetoo *et al.*, 2009b; Neetoo and Chen, 2010) significantly enhanced the pressure inactivation of inoculated *E. coli* O157:H7 and *Salmonella* spp. with variable impact on the seeds' germinability.

High pressure has also been used in combination with antimicrobial compounds including lysozyme, chitosans, and nisin (Roberts and Hoover, 1996). Aleman *et al.* (1994, 1996) studied the effect of oscillatory pressurization on the inactivation of *S. cerevisiae* in pineapple juice. These authors found that cyclic pressurization was more effective than continuous pressurization for the same overall global time. Results garnered on the effect of HHP on high-acid foods as with most fruit juices suggest that shelf-stable (commercially sterile) products can be achieved by processing at 580 MPa with a holding time of 3 min. This treatment has been demonstrated to inactivate a ≈6 log CFU/g burden of *E. coli* O157:H7, *Listeria* spp., *Salmonella* spp., or *Staphylococcus* spp. in high-acid foods such as apple juice. Similarly, Iuchi *et al.* (1996) and Sato *et al.* (1996) demonstrated the efficacy of high pressure to sterilize Sudachi mandarin and tomato juice, respectively.

12.5.5.2 Effect of high hydrostatic pressure on the microbial quality of plant-derived foods

The inactivation of microorganisms by HHP is a multidimensional process consisting of a combination of pressure, treatment time, and temperature (Ting *et al.*, 2002). Kuribayashi *et al.* (1996) showed that high pressure at a level of 300 MPa for at least 10 min or 400 MPa was effective in inactivating the background microbiota in a traditional pickled vegetable. El Moueffak *et al.* (1996) studied the effects of combined HHP (550 MPa for 30 min) and low temperature (−18 and 4°C) treatments on the microbiological status and organoleptic characteristics (aroma, taste, exudation) of black truffle samples and demonstrated that HHP treatments brought about a significant reduction in the mesophilic counts with minimal adverse impact on the aroma. Krebbers *et al.* (2002) compared the effects of HHP on the microbial load, texture, color, and ascorbic acid content of green beans with conventional processing. The authors showed that pressure at a level of 500 MPa at ambient temperature effectively inactivated vegetative cells of background flora with retention of color, firmness, and ascorbic acid content. In addition, the authors demonstrated that a two-cycle HHP treatment reduced vegetative and sporal counts below detection limits but to the detriment of its natural color. Several authors have also shown that combination treatments involving a hurdle approach are more desirable for plant foods as sensory quality retention is better (Leistner and Gorris, 1995).

12.5.5.3 Effect of high hydrostatic pressure on enzyme inactivation in fruits and vegetables

Several researchers have studied the effect of HHP in inactivating enzymes implicated in deteriorative processes (Table 12.2). Hendrickx *et al.* (1998) described the effect of HHP at pressure levels ranging from 600 to 1000 MPa on the inactivation of browning enzyme polyphenol oxidase (PPO) in mushrooms as well as the flavor and color of the treated product. They reported that HHP treatment at 600 MPa resulted in the activation of the enzyme while higher pressure was required to completely inactivate PPO with concomitant textural and color changes. Cano *et al.* (1997) reported that high pressure at 230 MPa in combination with mild heat (43°C) resulted in an optimum inactivation of strawberry peroxidase. Anese *et al.* (1995) observed the inactivation of peroxidase in a carrot cell-free extract after a brief pressure treatment of 1 min at 900 MPa. The same author also studied the effect of HHP and mild heat on PPO activity in apple cell-free extracts and observed a concomitant reduction in enzymatic activity following pressure treatment at a level of 900 MPa for 1 min. Pectic enzymes also hold special interest in the processing of plant-derived products. Pectinesterase enzyme was inactivated in satsuma mandarin juices when pressure-treated between 300 and 400 MPa with very low residual enzymatic activity remaining post-treatment. On the other hand, Shook *et al.* (2001) observed that pectinesterase was highly resistant to pressure in diced tomatoes while lipoxygenase activity in diced tomatoes was completely inactivated when subjected to pressure of 800 MPa.

Although HHP treatments at high pressure levels or combined with mild heat have been shown to successfully inactivate several enzymes involved in food deterioration, the final storage conditions (temperature, packaging atmosphere, and relative humidity) of each product remain to be optimized.

12.5.5.4 Effect of high hydrostatic pressure on the structure and texture of plant-derived foods

A fundamental principle underlying HHP is that high pressure is applied in an isostatic manner such that all parts of the food experience a uniform pressure. As an isostatic process, pressure is transmitted rapidly and uniformly throughout the plant food (macroscopically) and throughout the individual food cells (microscopically). However, a major challenge when it comes to high-pressure treatment on certain plant tissues is the ubiquity of intercellular air spaces. Because air is highly compressible, during HHP the plant tissue is severely compressed, ultimately leading to cellular and organellar membrane damage and leakage of cellular materials. Several studies, on the other hand, have found that HHP of vegetable tissue can result in tissue firming (Fuchigami and Teramoto, 1996; Kasai *et al.*, 1995a, 1995b; Stute *et al.*, 1996) in certain plant foods or softening of texture in others (Basak and Ramaswamy, 1996).

Michel and Autio (2001) compared the textural changes in various fruits and vegetables subjected to different pressure levels and showed that major changes occurred with celery and apples that have been pressure-treated at 200 MPa or higher. At pressures of 100 and 200 MPa, selected fruits such as pears, oranges, and pineapples displayed a recovery in texture while apples showed signs of permanent damage. Selected vegetables such as carrots and green peppers were also adversely affected at pressure in excess of 200 MPa. Prestamo and Arroyo (1998) viewed the ultrastructure of pressure treated cauliflower and spinach leaves and reported that high pressure altered the plant cell permeability by promoting the efflux of water and cellular metabolites. Electron microscopy revealed the disappearance of parenchyma organization in spinach leaf and the presence of cavities after treatment. Cauliflower, on the

contrary, retained a firmer structure with a slight "soaked-like" appearance and thus appeared to be more amenable for HHP treatment than spinach.

12.5.5.5 Effect of high hydrostatic pressure on the sensorial quality of fruit and vegetable products

Butz *et al.* (2002) studied the influence of high-pressure treatment on the sensorial and nutritional value of various fruits and vegetable products. The authors reported that pressure-treated orange juice had a longer refrigerated storage life than their untreated counterparts. In addition, the authors could not detect any significant differences in the nutritional profiles or the antioxidant activity between treated and untreated juices. Ogawa *et al.* (1990) and Takahashi *et al.* (1993) reported that satsuma mandarin juice maintained its freshness and original flavor during high-pressure processing. Similarly, results of extensive sensory testing on orange and grapefruit juices allowed Mermelstein (1999) to infer that sensory panelists could not distinguish between the flavor of pressure-treated citrus fruit juices and their untreated counterparts derived from the same raw material. Donsì *et al.* (1996) studied the effect of high pressure on the individual aroma compounds by comparing the gas chromatography profile of pressure-treated and untreated orange juice. His findings showed that the level of the least-stable component – limonene – was comparable to untreated juice. Unlike citrus fruits, the flavor characteristics of tomato juice and onions were largely impacted by high pressure. Sensory panels found that the intensity of the aroma of pressure-treated onions was weaker than fresh ones with a characteristic "cooked or fried onions" smell. Pressure-processed tomato juice was judged to be unfit for consumption and described to have a "strong rancid taste" (Poretta *et al.*, 1995).

Fonberg-Broczek *et al.* (1999) studied the impact of high pressure on the sensorial aspects of various berry, pome, and stone fruits and did not observe any effects on their pH, soluble solids, and total acidity content and these quality parameters were almost unaffected over the subsequent 3 months' storage at refrigeration temperature. Pressure-treated strawberry desserts also did not display any significant changes in taste, aroma, color, and consistency during a sensorial evaluation.

As mentioned above, the fresh characteristics of produce are retained in many, albeit not all, produce commodities during HHP. Hence, the prospects for commercial implementation of HHP to process produce commodities is ever-expanding as more and more research findings confirm its suitability for certain niche plant-derived products.

12.5.5.6 Commercial relevance of high hydrostatic pressure for fruit and vegetable products

HHP has been demonstrated to be successful with various fruit and vegetable product categories. Pressure-treated strawberry, apple, and kiwi jams and fruit jellies have been the first HHP products to be marketed in Japan after their introduction by the Meidi-ya Food Co. in 1990 (Thakur and Nelson, 1998). Other fruit-based products that have also joined the same market include fruit purées and yogurt sauces. These products are typically pressure-treated at levels ranging from 400 to 600 MPa for 1–30 min to enhance permeation of sugars into fruits as well as to achieve sterilization. Citrus fruit juices such as grapefruit and mandarin juice treated by HHP were also introduced on the Japanese market in 1990 by Pokka Corp and Wakayama Food Industry, respectively (Henry, 1997). Pressure is applied at a level of 200 MPa for 10 min at 5°C in order to inactivate enzymes involved in the synthesis of limonene, the bitter constituent present in citrus juices, as well as to extend the shelf life to

≈3 months at room temperature. In addition, pressure-treated orange juice has also been commercialized in France by Ultifruit® (Yaldagard *et al*., 2008).

Ready-to-eat fruit products such as avocado purée and guacamole have a relatively short shelf life due to enzymatic browning caused by PPO as well as the growth of microorganisms. HHP applied at a level ≈600 MPa for a few minutes has the ability to kill spoilage organisms and inactivate the browning enzyme to generate a product with an extended refrigerated shelf life of ≈30 days. Fresherized Foods (previously known as Avomex) was the first company to begin the industrial production of pressure-processed guacamole in the USA in 1997. This product is widely distributed around the western USA and Mexico. Moreover, in the USA other commercially available pressure-treated fruit and vegetable products include salsa (Fresherized Foods), pressure-processed chopped Spanish onions (Winsoms of Walla Walla), green peppers, apple sauce (Leahy Orchards), fruit blends, and fruit smoothie products.

12.6 CONCLUSIONS

This review describes the scope of HHP as a food-processing and -preservation technology, its current applications in the USA and international markets, as well as potential extension for other commercial applications. Capitalizing on the unique abilities of HHP to destroy pathogens, spoilage microorganisms, and enzymes involved in food deterioration with minimal impact on the organoleptic properties may allow this processing technology to become the dominating intervention for certain niche commodities in the future. Moreover, the possible combination of high pressure with other intervention technologies such as heat and antimicrobials would allow the lowering of the pressure requirement and may thus be the goal of future process and product developments. As far as cost is concerned, it is likely that the capital cost may limit its large-scale use initially. However, in the long run it is hoped that the manufacturers of HHP systems will continue to improve their capabilities and optimize the design of industrial-scale units. Ultimately, it is anticipated that greater technological and instrumental advancement will bring down the cost of the equipment and increase efficiency, allowing high pressure-processed safe, nutritious, and high-quality products to be widely available at an affordable cost.

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13 Pulsed Electric Fields for Food Preservation: An Update on Technological Progress

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Abstract: Preservation of fresh produce with minimal loss of nutrients is of major concern. Most food-processing methods involve heat (thermal processing), which leads to deterioration in the overall quality (nutritional and functional properties) of foods. Recent studies have indicated pulsed electric fields to be a better alternative for thermal treatments, especially when liquid food preservation is concerned. In the present chapter, we discuss this novel technology and the recent developments involved.

Keywords: antioxidants; food preservation; pulsed electric fields; solid textures; starch modification

13.1 INTRODUCTION

Today, consumers' expectations of food suppliers and manufacturers have increased tremendously and they are constantly looking for quality food. With increased knowledge about health benefits, the consumption of natural and fresh food has increased rapidly. Hence, there is tremendous pressure on food technologists and food engineers to design appropriate processing methods which can preserve and retain the quality of fresh produce. Even though thermal processing of food has a wide range of applications as a vital preservation technique, it is known to reduce the quality and freshness of foods by degrading organoleptic and nutritional qualities (Jeyamkondan *et al.*, 1999). To achieve microbial decontamination, several novel preservation techniques (e.g. irradiation, sonication at low temperature) have been proposed which have been proved to be better than thermal processing. Novel processes based on applying heat in a very short period of time (high temperature short time (HTST), ohmic heating, microwaves) or applying low to moderate temperatures in combination with physical or chemical factors have also been proposed and been successful (Barsotti *et al.*, 1999). The general aim of all these methods is not only to extend shelf life, but also to maintain freshness and organoleptic quality.

Inactivation mechanisms of microorganisms and enzymes in the absence of heat are totally different to those that occur in thermal processing. Generally, physical factors in non-thermal processing are more efficient than chemicals such as hydrogen peroxide and organic acids.

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Some of the common non-thermal processing methods include high hydrostatic pressure, ultraviolet light, ultrasound, ionizing radiation, pulsed magnetic fields, and pulsed electric fields (PEFs) (Raso and Barbosa-Cánovas, 2003). These processing methods have their own historical background, mechanism of action, and safety challenges (Rahman, 2007).

In PEF processing, short bursts of electricity is used to inactivate microorganisms. Liquid, semi-liquid, and solid food products can undergo PEF processing. PEF processing provides high-quality fresh-like foods with good shelf life, nutritional value, and flavor (Sun, 2005). In this chapter we discuss and update various aspects relevant to PEF technology: its history, the technology, and processing with regard to future scope in the food industry.

13.2 HISTORICAL BACKGROUND OF PULSED ELECTRIC FIELDS

In 1967, Sale and Hamilton were the pioneers who proposed the concept of PEF to change the behavior of microorganisms (Sale and Hamilton, 1967). Further, during the 1980s the electric-field phenomenon was identified with the membrane rupture theory (Ravishankar *et al*., 2008; Jiahui *et al*., 2009; Yao *et al*., 2009). PEF has been reported to improve cell permeability and assist in cellular extraction and transfer of genetic material across the cell membrane (Li *et al*., 2009; Baldwin *et al*., 2010; Saulis, 2010). During the 1990s, studies on PEF in food processing were initiated by developing laboratory-scale equipments to evaluate the effect of PEF on microorganisms (Barbosa-Cánovas, 1999). Lethal effects of PEF on microorganisms without major effects on food quality parameters led to the rapid development of PEF technology. The first commercial applications of PEF were reported for processing apple cider products in the USA (in 2005) to extend storage life (Wan *et al*., 2009). Nowadays, apart from microbial decontamination and enzyme inactivation, PEF technology finds wide applications (Pizzichemi, 2009; Pataro *et al*., 2010; Saldaña *et al*., 2010; Zhao *et al*., 2010). It is also used in cell hybridization in biotechnology and genetic engineering (Jeyamkondan *et al*., 1999; Saulis, 2010), to improve of the extraction of cell contents (López *et al*., 2009a; Loginova *et al*., 2010; Puértolas *et al*., 2010b), and for wastewater treatments (Gusbeth *et al*., 2009a, 2009b; Su *et al*., 2009).

13.3 PULSED ELECTRIC FIELD PROCESSING

Pulsed power has a simple concept, wherein the electrical energy stored in a capacitor over an extended period of time at low power levels can be instantaneously discharged at a very high level of power (Barsotti *et al*., 1999). A typical PEF-generation system requires a pulse modulator and a treatment chamber which can convert pulsed voltage to a pulsed electric field. A processing chamber provides space for applying PEFs to foods between two electrodes. The chamber can either be static or continuous for laboratory or industrial scales, respectively, and the shape can vary in the form of parallel plates, coaxial cylinders, or co-field flow (Jeyamkondan *et al*., 1999). PEF equipment design has been comprehensively reviewed recently and there are guidelines for proper designing of PEFs for certain applications (Alkhafaji and Farid, 2009).

PEF processing utilizes intensive electrical pulses between electrodes to treat foods, which thereby inactivates microorganisms; it can be a continuous process. The field intensity is generally in the range of 15–50 kV whereas at high voltage the pulse range is between 20 and 80 kV (Yin and He, 2008). In majority of cases pulses are only applied for a couple of

microseconds. However, of late, nanosecond applications have also been reported (Eing *et al.*, 2009; Ito *et al.*, 2010; Nuccitelli *et al.*, 2010). Electric fields are applied in the form of square wave, bipolar, exponentially decaying, or oscillatory pulses. The energy required in PEF processing is less than for thermal processing and the rise in temperature is very minimal. Hence, this process is considered to be a non-thermal processing technology (Barsotti *et al.*, 1999; Raso and Barbosa-Cánovas, 2003).

Application of a series of electric fields breaks down the lipid membrane of cells. This is due to electroporation, involving the expansion of the existing pores or creation of new pores. Based on the intensity of the electric field, and duration and number of pulses, pore formation can be reversible or irreversible. When these factors go beyond critical range, a transmembrane potential difference is created with permanent membrane damage that leads to inactivation of microorganisms (Sun, 2005; Mosqueda-Melgar *et al.*, 2008; Ravishankar *et al.*, 2008).

PEF is mostly applied for liquid or semi-solid media. This is due to the fact that electric pulses should pass through the material, and liquid foods can pass electricity more easily than solid foods. However, PEFs have been envisaged to have applications in tumor therapy or may be applied on solid textures to improve extraction or drying processes (Esser *et al.*, 2009; López *et al.*, 2009b; Salerno *et al.*, 2009; Gachovska *et al.*, 2010).

13.4 MECHANISMS AND FACTORS AFFECTING PULSED ELECTRIC FIELDS

PEF treatments cause electroporation (generation of pores) in the cell membrane, consequently leading to microbial inactivation and destruction (Pliquett, 2009; Saulis, 2010). Even though formation of pores in the protein or lipid matrices is still unclear, it is believed that structural changes in microbial cells induced by electric fields are based on osmotic imbalance, transmembrane potential, and the electromechanical compression theories. (Barbosa-Cánovas *et al.*, 2001; Zhao *et al.*, 2008; Yu, 2009). Saulis (2010) has divided PEF actions on cells into four main stages: increase in transmembrane potential, pore initiation, evolution of pores, and cell death. These stages are discussed below:

13.4.1 Increase in transmembrane potential

In this stage, application of electric fields causes transmembrane potential to increase, leading to charging of the cell plasma membrane. A rapid membrane capacitor discharge is caused by increasing the membrane's electrical permeability. Therefore membrane breakdown at the cell's poles happens by increasing the intensity of the external field (Ho and Mittal, 1996; Pliquett *et al.*, 2007). The required field strength for breakdown of the membrane is in the range of 1 to 20 kV/cm, depending on cell radius. The voltage of breakdown is of the order of 1V itself, which in turn depends on field duration and temperature, among others. The breakdown voltage for other membrane sites is reached at higher field strength (Ohshima and Sato, 2004). The shape, duration and frequency of pulse, shape, size and orientation of cells, and conductivity of cell materials and medium are the most influencing factors in this stage (Vernhes *et al.*, 2002; Saulis, 2010).

13.4.2 Pore-initiation stage

This stage involves creation of metastable small hydrophilic pores. Two theories have been proposed for this stage: the electromechanical and the statistical model. The first theory states

that the application of an electric field to the membrane leads to compression and rupture when the electric field exceeds the critical potential. The second theory states that local electric fields and combined effects of thermal fluctuations create transient pores. Creation of hydrophilic pores strongly depends on the transmembrane potential in the first stage. Temperature, lipid composition, and oxidation of membrane components also affects the pore-formation stage (Levine and Vernier, 2010).

13.4.3 Evolution of the pore population

By application of an external electrical field and due to charging of the plasma membrane the transmembrane potential increases. This stage of the mechanism can range from nanoseconds to milliseconds (Schoenbach *et al.*, 2004; Saulis, 2010). In this stage, the size and number of pores tends to change. The membrane potential, temperature, and ionic strength of the medium are some of the affecting factors at this stage. Fluidity of the membrane can also affect the evolution of pore population (Thomas-Vernier *et al.*, 2004).

13.4.4 Pore resealing or cell death

This is the final step, wherein the treated cell could be "resealed and alive" or "ruptured and dead." If the pore population in the previous step is not sufficient to permeabilize the membrane to transfer molecules then the cell may have capability to reseal, grow, and multiply, wherein even some of the charged molecules and ions can pass through the membrane. If time and intensity of the field are adjusted properly, small molecules can be transferred through the membrane that can finally cause an imbalance of ions leading to rupture of the membrane. After PEF, substantial damage or increase in the cell membrane permeability can lead to the release of intracellular substances and consequently cell death (Gonzalez and Barrett, 2010). It is also believed that osmotic imbalance causes the cell membrane to rupture. This imbalance is generated by leakage of small molecules and ions induced by the PEF (Ho and Mittal, 1996). The swelling of the cell begins due to the osmotic pressure of the cytoplasm contents. Swelling of the cell causes an increase in cell volume. Rupture and lysis of the cell membrane happens when the cell volume reaches 155% of its original volume (Tsong, 1990). The most important factor at this stage is the medium composition because, after the previous stage, the cell membrane becomes permeable and various molecules and ions (small enough to transfer through the pores) tend to leak out of the cell. Other vital factors, such as temperature, pH, and osmotic pressure, can also play an important role in cell death or cell resealing (Ho and Mittal, 1996).

In the past few years, more than 300 research publications have appeared on PEF processing, which highlights the worldwide importance of this novel method. The principle of PEF has been described clearly in some of the previous publications (Barbosa-Cánovas, 1999; Barbosa-Cánovas *et al.*, 2001; Sun, 2005; Raso and Heinz, 2006; Lelieveld *et al.*, 2007). Here we have only dealt with PEF as relevant to food processing.

13.5 PULSED ELECTRIC FIELD APPLICATIONS IN FOOD PROCESSING

Of late, there is a growing interest in PEF applications for food preservation and processing (Barbosa-Cánovas *et al.*, 2001; Fleischman *et al.*, 2004; Lelieveld *et al.*, 2007; Muthukumaran *et al.*, 2009; Wan *et al.*, 2009; Puértolas *et al.*, 2010a). Generally, PEF

applications in food preservation and processing have fallen into two main categories: (i) liquid food preservation by inactivation of microorganisms; and (ii) the improvement of the texture of and mass-transfer coefficient of solids(/liquids). Most of the research work on PEF has been focused on extension of shelf life by reduction of microbial load and ensuring safety in liquid or semi-solid foods. The products that have been recently studied include milk (Jaeger *et al*., 2009; Noci *et al*., 2009; Riener *et al*., 2009; Sepulveda *et al*., 2009; Valizadeh *et al*., 2009; Walkling-Ribeiro *et al*., 2009a; Yu *et al*., 2009; Guerrero-Beltrán *et al*., 2010); apple juice (Noci *et al*., 2008; Azhu Valappil *et al*., 2009; Chen *et al*., 2009; García *et al*., 2009; El-Hag *et al*., 2010;); orange juice (Sampedro *et al*., 2009; Wang *et al*., 2009; Walkling-Ribeiro *et al*., 2009c; Gurtler *et al*., 2010; McNamee *et al*., 2010); other juices (tomato, strawberry, and watermelon) (Aguiló-Aguayo *et al*., 2009a, 2009b, 2009c, 2010; Odriozola-Serrano *et al*., 2009a), and liquid egg (Jin *et al*., 2009; Marco-Molés *et al*., 2009; Zhao *et al*., 2009a; Monfort *et al*., 2010a, 2010b). All of these researchers have reported PEF to successfully inactivate food spoilage and pathogenic microorganisms as well as some enzymes. Also, most of them have reported PEF treatment to result in better retention of nutrients and flavors, and to produce a fresher taste, compared to heat-processed products (Schilling *et al*., 2008a; Zhao and Yang, 2008; Sanchez-Vega *et al*., 2009; Aguiló-Aguayo *et al*., 2010). Recently, PEF has also been shown to exhibit great potential on pre-treatment of plant tissues to enhance the extraction of phenolic compounds (Odriozola-Serrano *et al*., 2009a; Puértolas *et al*., 2010b, 2010c), anthocyanins (Luo *et al*., 2009; Gachovska *et al*., 2010), and pectins (Yin *et al*., 2009; Yu and Yin, 2009), and for drying (Amami *et al*., 2008; Gachovska *et al*., 2008, 2009).

13.6 NANOSECOND PULSED ELECTRIC FIELDS

Generally, a PEF system includes four main parts: (i) a high-voltage generator; (ii) a liquid-handling system; (iii) a chamber; and (iv) monitoring and control devices. Key parameters of PEF used in treatment include field intensity of 15–50 kV/cm, pulse frequency 200–400 Hz, and pulse width of 1–5 μs (Wan *et al*., 2009).

New research has focused on decreasing the treatment time to nanosecond levels in order to decrease the side effects of PEF on a food's main structure. Nano-level application of PEF is being used for cancer and tumor therapy in human cells (Zygulska and Pawlega, 2008; Beebe *et al*., 2009; Donthula *et al*., 2009; Mi *et al*., 2009a, 2009b; Yang *et al*., 2009).

Katsuki and others (2002) applied PEF in ultra short pulses (nanosecond PEF) and set the electric field amplitude to 130 kV/s and pulse width to 45 ns. A pressurized field distortion spark gap switch allowed shortening of the voltage pulses to around 2 ns. The results showed that in killing of *Bacillus subtilis* ATCC 6051 short pulses was more efficient in phosphate-buffered saline (Katsuki *et al*., 2002).

Floury *et al*. (2006) applied PEF in a pilot plant to *Salmonella enteritidis* (10^7 cells/ml) in non-fat milk. They applied PEF with an electric field strength ranging between 45 and 55 kV/cm, a pulse width of 250–500 ns, and a pulse frequency of 40–120 Hz at low temperatures (<50 °C) to provide input energy equivalent to 0–100 kJ/kg. The authors confirmed that the effectiveness of the microbial inactivation by PEF processing was very limited, wherein a 1.4 log reduction of total *S. enteritidis* and microflora was the maximal inactivation.

In another report by Pleiquett (2009), the electroporation of cells at high field strength (>10 kV/cm) on nanosecond timescales, and at moderate strength (<2 kV/cm) for longer times were compared. In nanosecond PEF there was not enough time for pore creation to

occur, so the membrane charges rapidly as per the theory of electroporation and finally the membrane becomes overcharged. The high and fast decay in conductivity after applying a pulse are key features of nanosecond PEF. Pleiquett (2009) hypothesized the mechanism of action in nanosecond PEF on the bilayer membrane. Nanosecond PEF is in its infancy and requires more development for applications in food preservation.

13.7 IMPACTS OF PULSED ELECTRIC FIELDS ON ANTIOXIDANT FEATURES

Consumption of fruits and vegetables provides potential health benefits and can prevent the onset of cancer, cardiovascular illnesses, aging, and several other diseases (Block *et al.*, 2001; Sánchez-Moreno *et al.*, 2003). The health benefits are mainly attributed to the presence of bioactive compounds. Fresh fruit and vegetables cannot be stored for long periods and need to be processed immediately to extend their shelf life. The common methods for increasing shelf life include squeezing and extracting the juice followed by applying heat to deactivate the enzymes and microorganisms. Antioxidant components such as vitamin C, anthocyanins, lycopene, phenolic compounds, and carotenoids are very sensitive to heat. Hence, thermal processing might lead to the loss of bioactive compounds. Extension of shelf life of fresh produce by PEF has been reported (Sampedro *et al.*, 2009; Sepulveda *et al.*, 2009; Walkling-Ribeiro *et al.*, 2009a, 2009b, 2010; Zhao *et al.*, 2009b). However, there are still gaps wherein further studies need to be performed regarding the stability and the quality parameters of PEF-treated foods after long-term storage.

Reports on the effect of PEF on antioxidants or bioactive compounds are scarce. Soliva-Fortuny *et al.* (2009) reported that high-intensity PEF (HIPEF) treatment can be an effective alternative for traditional preservation by pasteurization, while low-to-moderate-intensity PEF can improve extractability of bioactive components. Research on HIPEF was accelerated as a proposed alternative to pasteurization and consequently the possible effects of HIPEF with respect to microorganism and enzyme deactivation to improve shelf life have been considered (Mosqueda-Melgar *et al.*, 2008; Huang and Wang, 2009).

Application of PEF as a proposed pasteurization method has still several uncertain aspects that should be resolved before this technology is industrialized. Table 13.1 summarizes some of the recent applications of PEF on health benefits of food with different PEF parameters. In all of of these studies, HIPEF effects are compared with the traditional methods (thermal processing).

13.7.1 Antioxidants and vitamin C

Fruit juices have a large quantity of bioactive compounds such as phenolic compounds and vitamins (Kitts, 1997; Prior, 2003). The important role of vitamin C as a water-soluble vitamin that can contributes to defense against oxidative stress is well known (Padayatty *et al.*, 2003). Likewise, there is a correlation between reduced risk of coronary heart diseases and a diet rich in phenolic compounds (Lam *et al.*, 2007).

Effects of PEF on vitamin C have been reported. Yeom and others (2000) applied PEF at 35 kV/cm for 59 μs on orange juice and compared this with pasteurization. They found PEF to prevent growth of microorganisms until 112 days (same as pasteurization), but retain a significantly greater amount of vitamin C than hot pasteurization (Yeom *et al.*, 2000). In 2003 commercial-scale PEF was used by Min *et al.* (2003) to compare the quality of tomato juice with thermal processing (2003). PEF was applied with a field intensity of 40 kV/cm for 57 μs.

Table 13.1 PEF applications and their effects on health-benefit components.

Sample	PEF parameters						Parameters improved compared with traditional methods	References
	Treatment temperature (°C)	Field intensity (kV/cm)	Total duration (μs)	Pulse frequency (Hz)	Pulse width (μs)	Pulse mode		
Orange juice	24	35	59	600	1.4	–	Vitamin C, flavor compounds	Yeom *et al.* (2000)
Tomato juice	45	40	57	–	2	–	Ascorbic acid, lycopene, color	Min *et al.* (2003)
Tomato juice	45	40	57	–	2	–	Ascorbic acid, flavor compounds, color	Min and Zhang (2003)
Orange juice	<50	35	750	800	4	Bipolar	Vitamin C, carotenoid, clavonoid, antioxidant capacity, viscosity, color	Sanchez-Moreno *et al.* (2005)
Tomato juice	<50	35	1000	50–250	1–7	Monopolar/ bipolar	Lycopene, vitamin C, antioxidant capacity	Odriozola-Serrano *et al.* (2007)
Apple juice	<50	40	–	15	1	–	Antioxidant capacity, total polyphenol	Noci *et al.* (2008)
Green tea drink	20	18.1–38.4	–	N/A	2	Bipolar	Free amino acids, green tea polyphenol	Zhao *et al.* (2008)
Tomato juice	<40	35	1500	100	4	Bipolar	Carotenoids, phenolic compounds,	Odriozola-Serrano *et al.* (2009a)
Strawberry juice	<40	35	1000	50–250	1–7	Monopolar/ bipolar	Vitamin C, anthocyanins, antioxidant capacity	Odriozola-Serrano *et al.* (2009b)
Watermelon juice	<40	30–35	50–2050	50–250	1–7	Monopolar/ bipolar	Vitamin C, lycopene, antioxidant capacity	Oms-Oliu *et al.* (2009)

(*Continued*)

Table 13.1 (*Continued*)

Sample	PEF parameters						Parameters improved compared with traditional methods	References
	Treatment temperature (°C)	Field intensity (kV/cm)	Total duration (μs)	Pulse frequency (Hz)	Pulse width (μs)	Pulse mode		
Carrot juice	<40	35	1500	200	6	Bipolar	Vitamin C, β-carotene, total phenolic content, antioxidant capacity	Quitão-Teixeira *et al.* (2009)
Bovine raw milk	30	15–35	12.5–75	15	1	N/A	Thiamin, riboflavin, retinol, α-tocopherol	Riener *et al.* (2009)
Apple juice	30–50	30.8, 38.5	200	150–300	1	N/A	Soluble solid	Sanchez-Vega *et al.* (2009)
Green tea infusions	<15	20–40	200	667	2	Bipolar	Total polyphenols, free amino acids, tea catechins, color	Zhao *et al.* (2009c)
Orange juice	<56	20–40	25–150	15	1	Monopolar	Ascorbic acid, color	Walkling-Ribeiro *et al.* (2009c)
Fruit juice/soymilk	<32	35	800–1400	200	4	Bipolar	Vitamin C, total phenolic compounds, antioxidant capacity	Morales-de la Peña *et al.* (2010)
Red wine	20 ± 2	2–7	410	300	3	–	Phenolic compounds, anthocyanin, color	Puértolas *et al.* (2010b)
Red wine	Ambient ± 2	30	410	300	3	–	Phenolic compounds, color	Puértolas *et al.* (2010d)
Orange juice/milk	N/A	15–40	40–700	–	–	–	Carotenoids, vitamin A	Zulueta *et al.* (2010a)
Orange juice/milk	N/A	15–40	40–700	–	–	–	Ascorbic acid	Zulueta *et al.* (2010b)
Peanut oil	<40	20–50	–	1008	40	–	Fatty acid composition	Zeng *et al.* (2010)
Sour cherry juice	Ambient	17–30	66–210	–	3	–	Ascorbic acid, anthocyanin, metal ion, color	Altuntas *et al.* (2010)

The results showed inactivation of microorganisms along with ascorbic acid retention in PEF-treated samples compared to thermal pasteurization (Min *et al.*, 2003).

Sanchez-Moreno *et al.* (2005) compared high pressure and PEF (as novel methods) with traditional thermal processing on antioxidant and bioactive components of orange juice. They applied PEF at 35 kV/cm for 750 μs and estimated the bioactive components and 2,2-diphenyl-1-picrylhydrazyl (DPPH)radical-scavenging capacity (RSC). Vitamin C in all methods showed loss at least by 9%. However, increasing the temperature of the processing method increased the total vitamin C. Application of PEF did not have any significant effect on carotenoids and flavanoids; whereas high pressure increased them. Also, PEF and the high-pressure method did not show any significant effects on DPPH (RSC). In another study on tomato juice processed with HIPEFs carried out by Odriozola-Serrano *et al.* (2007), increasing the pulse width and frequency lead to an increase in lycopene content, but with decreased vitamin C retention. HIPEF treatment of strawberry at a frequency of 232 Hz, bipolar mode, and with 1 μs pulse width has been reported to exhibit the best antioxidant profiles, and linearly this profile was dependent on the research variables (Odriozola-Serrano *et al.*, 2009b). Unfortunately, the critical parameters of PEF (field strength and treatment time) have not been investigated in these research reports and further research is required to optimize these parameters.

In a similar approach, surface-response methodology was carried out on the important parameters affecting HIPEF applied on watermelon juice (Oms-Oliu *et al.*, 2009). HIPEF was applied at an electric field strength of 30–35 kV/cm, treatment time 50–2050 μs, pulse width 1–7 μs, pulse frequency 50–250, and pulse polarity mono or bipolar on fresh watermelon juice to optimize parameters for lycopene retention, antioxidant capacity, and vitamin C. However, vitamin C in all the processing conditions showed a loss, while lycopene retention and antioxidant capacity increased with an increase in the field strength, frequency, and pulse width.

Recently Morales-de la Peña *et al.* (2010) applied HIPEF to a fruit (kiwi, orange, and pinapple) juice/soymilk beverage and evaluated for microbial load, antioxidant profiles, and quality parameters of the beverage compared to those gained from traditional thermal pasteurization. The treatment parameters applied in PEF were bipolar pulse at 200 Hz and width 4 μs with a field intensity of 35 (kV/cm) for 800–1400 μs. Increasing the time of PEF treatment from 800 to 1400 μs extended shelf life from 31 to 56 days, equivalent to the storage life resulting from thermal processing at 90°C for 60 s. As expected, vitamin C and antioxidant activity decreased during storage, but for PEF treatment the loss of vitamin C and antioxidant was lower than with thermal pasteurization. Quality parameters such as color, pH, and solids content were not changed during storage time.

13.7.2 Carotenoids and vitamin A

Carotenoids, which considered as health-promoting components, undergo rapid degradation in the traditional thermal-processing methods. Carrot, orange, and tomato are rich sources of carotenoids, and recently some reports have been published on the effects of PEF on carotenoids. Carrot juice exposed to HIPEF (35 kV/cm for 1500 μs, 200 Hz bipolar pulse frequency with 6 μs width) was evaluated for antioxidant components such as β-carotene and vitamin C and were compared with heat pasteurized (90 °C for 30 or 60 s) juice samples (Quitão-Teixeira *et al.*, 2009). The degradation rate of β-carotene after HIPEF treatment was around 1.8×10^{-2} (day^{-1}) and for vitamin C it was 1.7×10^{-2} (day^{-1}) throughout the storage period, whereas the degradation rates for hot pasteurized juice were around 3.5×10^{-2}

(day^{-1}) and 2.3×10^{-2} (day^{-1}) for vitamin C and β-carotene, respectively. These results indicated PEF treatment to substantially improve the stability of vitamin C and β-carotene.

Odriozola-Serrano *et al.* (2009a) compared tomato juice treated with HIPEF with pasteurized samples (thermal). The processing parameters for PEF of fixed field strength were 35 kV/cm at 1500 μs and 100 Hz bipolar pulse frequency with 4 μs width, while pasteurization was carried out at 90 °C. Results showed that after processing and during storage HIPEF tends to maintain better quality than pasteurization.

Recently, a combination of orange juice and milk was exposed to PEF treatment to evaluate the levels of carotenoids and vitamin A after processing and during storage at 4 and 10 °C (Zulueta *et al.*, 2010a). The PEF parameters applied included field strength ranging from 15 to 40 kV/cm with a treatment time of 40–700 μs. Increasing the electric field strength resulted in a corresponding decrease in the carotenoids, whereas applying a lower field strength led to an increase in carotenoids. Additionally, an increase in field strength from 15 to 40 kV/cm enhanced lutein and zeaxanthin. The results indicated that PEF treatments have no adverse effect on carotenoids and can significantly enhance the xanthophyll concentration in the produce.

13.8 EFFECTS OF PULSED ELECTRIC FIELDS ON SOLID TEXTURES

Research in the last decade has shown that moderate PEFs can improve some processing parameters, such as pressing, drying, extraction, and diffusion, of solid textures (Soliva-Fortuny *et al.*, 2009). The fundamental action mechanisms of PEF on plant tissues, effects of critical parameters of PEF, and synergistic effects of this method with traditional methods have been comprehensively reported (Vorobiev and Lebovka, 2006, 2009, 2010; Donsì *et al.*, 2010). Table 13.2 shows some applications of PEF on solid textures and results of some of the recent research reports. The extraction processes that have undergone pre-treatment are mostly carried out with low to medium field intensity (Soliva-Fortuny *et al.*, 2009). Application of high-intensity fields causes degradation of extracted material and consumes more energy (Yongguang *et al.*, 2006). However, to improve the extraction process, especially at low temperatures, and to avoid damaging the structure of the extracted material, PEF is recommended.

13.9 STARCH MODIFICATION BY PULSED ELECTRIC FIELDS

Starch, due to certain limitations such as low solubility and tendency to retrograde, needs to be suitably modified. This modification could be chemical, physical, enzymatic, or genetic (Huber and BeMiller, 2009). UV, gamma irradiation, electron beams, and hydrostatic high pressure are novel process that have been proposed to physically modify starch in the last decade (Bhat and Karim, 2009). Decomposition effects of HIPEF have been reported by Yongguang *et al.* (2006). Based on the proposed concept, modification methods in various biopolymers have been attempted. Han *et al.* (2009b) exposed corn starch suspension to HIPEF with an intensity of 30–50 kV/cm and adjusted suspension electric conductivity by potassium chloride at a temperature below gelatinization (<50°C) and were able to characterize HIPEF-modified corn starch. Intra-granular molecular rearrangement of corn starch observed by scanning electron microscopy revealed modifications in the corn starch's properties. Results of differential scanning calorimetry showed a decrease in gelatinization

Table 13.2 Effect of PEFs on solid textures.

Process	Applied PEF parameters				Effects of PEF	References
	Treatment temperature (°C)	Field intensity (kV/cm)	Total duration (μs)	Number of pulses		
Paprika juice extraction	Ambient	1.7	300	30	10% increase in juice yield, improvement in color, 60% more β-carotene compared to traditional method	Ade-Omowaye *et al.* (2001)
Soluble substance extracted from apple slices	20	0.5	100	1000	Diffusion coefficient shows increase of about 56% at 20°C and 31% at 75°C compared to control without PEF (calculated based on original data)	Jemai and Vorobiev (2002)
Sugar extracted from sugar beet slices	20–50	0.3–0.8	100	5–1000	Extraction process enhanced, field intensity 0.67 with 250 pulses are optimal parameter for extraction; increasing temperature from 20 to 50°C, duplicate diffusion coefficient	El-Belghiti *et al.* (2005)
Extraction of polysaccharide from *Rana temporaria chensinensis* David	Ambient	10–40	2–8	–	Increase intensity from 10 to 20 kV/cm, increase extraction ratio (ER) from ≈ 17 to ≈ 27% but intensity increase from 20 to 40 kV/cm decreases ER to ≈ 21% due to polysaccharide decomposition; pulse duration from 2 to 6 μs increases ER from ≈ 20 to ≈ 34%, from 6 to 8 μs decreases ER to 25%	Yongguang *et al.* (2006)
Extraction of components from carrot slices	Ambient	0.25–1	100	–	Juice yield and °Brix increase, especially for larger slices	Grimi *et al.* (2007)
Disintegration of apple tissue	20–50	0.1–0.4	10–1000	2–20	Textural relaxation data supported the higher damage efficiency of longer pulse duration	De Vito *et al.* (2008)
Extraction of solutes from fennel	Ambient	0–0.6	100	0–850	Low energy consumption, more vitamin C and E (approx. twice the extraction of that at 90°C), high yield of extraction but longer time	El-Belghiti *et al.* (2008)
Extraction of anthocyanin from red raspberry	<40	5–15	0.5–15	60–420	Extraction yield of anthocyanins from raspberry was linearly correlated to the treatment pulses of PEF; increasing pulse number from 0–420 increased extraction yield from ≈ 15 to 55%	Luo *et al.* (2008)

(*Continued*)

Table 13.2 (*Continued*)

Process	Applied PEF parameters				Effects of PEF	References
	Treatment temperature (°C)	Field intensity (kV/cm)	Total duration (μs)	Number of pulses		
Juice extracted from apple mash	Ambient	3	Input energy equal to 10kJ/kg		No difference in extraction yield with enzymatic method but delay observed; increase in antioxidant capacity	Schilling *et al.* (2008b)
Extraction of dissoluble calcium from bone	Ambient	0–70	–	0–12	Field intensity increase from 10 to 30 kV/cm, increased calcium extracted from ≈ 2500 to 4000 mg/L, and from 30 to 70 kV/cm no significant changes. Pulse number increase from 2 to 4 leads to a rapid increase in calcium extraction, and small changes shown by increasing up to 12.	Yin and He (2008)
Juice extracted from Chardonnay white grape	Ambient	0.4	1000	1–1000	Increasing juice yield by 67–75%, and 15% increase in polyphenol extraction	Grimi *et al.* (2009)
Extract of betanine from red beetroot	<30	0–9	2	5–100	PEF intensity 7 kV/cm yield maximum betanine extraction 4.2 times untreated food, and extraction time decreased by 16-fold. All betanine can be released by PEF.	López *et al.* (2009a)
Solid-liquid extraction of sucrose from sugar beet	<30	1–7	–	5–40	Increasing field strength increase sucrose extraction and decrease time of extraction in low temperature. At temperature 20°C, 20 pulse at 7 kV/cm increased yield of extraction by seven times, and reduced thermal energy by 50%	López *et al.* (2009b)
Anthocyanin extracted from red cabbage	<30	2.5	–	50	2.15 times increase in total anthocyanin extraction	Gachovska *et al.* (2010)
Soluble matter extracted from chicory	20–80	0.1–0.6	–	1–1000	PEF significantly accelerated diffusion, even at 20–40°C	Loginova *et al.* (2010)
Phenolic extraction during fermentation of red grapes	20	2–7	–	–	Increasing field strength 2–7 kV/cm increases extraction rate of anthocyanin and phenolic contents	Puértolas *et al.* (2010b)

temperature and enthalpy as a effect of PEF. Increasing the field intensity resulted in a decrease of thermal transition property of corn starch. By application of very high field intensity (50 kV/cm), starch granules tended to lose their original shape and degree of crystallinity, which was proved by X-ray diffractometry. The authors also showed that the pasting property of corn starch (by Brabender rheology method) increased with corresponding increases in field intensity. Previously, in the same approach, potato starch treated with HIPEF (field intensity 30 kV/cm and especially 40 kV/cm) showed the presence of some pits on the granules. However, the original oval shape of the granules did not exhibit any changes. Increasing the field intensity damaged granule shape, with some granules aggregating together and showing a gel-like structure (Han *et al*., 2009a). Luo *et al.* (2010) applied PEFs up to 25 kV/cm on chitosan and found that HIPEF can decrease the molecular weight of chitosan and can be used as a novel method to produce low-molecular-weight chitosan (Luo *et al*., 2010).

However, to date there have been no reports available on the application of PEF for biopolymer modification. Hence further studies are required to optimize the critical parameters for achieving expected target properties.

13.10 CONCLUSIONS

Much evidence is available on the application of PEF which indicates that this technology could be used as an alternative for thermal processing to produce excellent-quality food products. PEF has been shown to retain the color, nutrients, and bioactive compounds in fresh produce, better than with thermal treatments. PEF as a pre-treatment step in extraction and dehydration can successfully show positive effects that can be exploited on a commercial scale. The ability of PEF to destroy the basic structure of biopolymers is a new approach and can be considered as a new modification method for producing low-molecular-weight biopolymers for industrial applications. Additionally, the acceptability of PEF-treated food products among consumers needs to be pursued in detail.

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14 Salting Technology in Fish Processing

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Abstract: Salting is a fish-preservation method and it is used in many countries of the world. It is also used in the preprocessing of fish before processing technologies such as smoking, drying, and canning are employed. The main function of salting is the removal of some of the water from fish flesh and its partial replacement by salt. Salting reduces the water activity of fish; hence, microbial and enzymatic activities are also reduced. The ripening of salted fish is the biochemical process that causes the change in chemical and physicochemical characteristics of the fish tissues. The end of the salting process is the moment when all the fish has reached the required salinity, and acquired the appropriate taste, consistency, and odor.

Keywords: autolysis; freezing technology; reddening; ripening salted fish; salting

14.1 INTRODUCTION

It is possible to preserve fishery products in different ways, such as by salting, drying, smoking, marination, canning, chilling, and freezing. Among these, salting is a processing technology which requires neither high costs nor modern plants and equipment. Furthermore, salted fishery products can be consumed for a long time without any nutritional value loss. However, whether or not salted fishery products have the quality of being consumable for a long time is dependent upon the application of the key points in salting technology.

Salt is the most significant and effective chemical used in the preservation of fish. Salting technology is employed not only for obtaining durable products but also as a pretreatment in some other processing technologies.

14.1.1 Purpose and principles of salting

Basically, salting is a fish-preservation method; it is a process where fishery products come into contact with salt in appropriate containers or barrels and consequently the product attains a desired structure and salinity. Salted fish can be preserved for a long time because of the water discharged from the body of the fish by osmosis and because the fact that the water content in the fish is reduced. The water which is discharged as the salt penetrates the fish forms a concentrated salt solution together with the outer dry salt. At the beginning of salting, the outer salt concentration is higher whereas the salt concentration of the fish is lower. Due to the concentration difference between these two environments, the osmotic pressure of the outer solution is higher than that of the fish, and thus salt intake and water discharge take place

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by osmosis. Osmosis continues until the salt concentration in fish and the outer salt concentration equalize.

However, concentration equilibration does not practically occur. In both salting methods (dry salting, see section 14.2.3.1; and brine salting, see section 14.2.3.2), the water content rate of the fish decreases because the water in the fish is discharged. The decrease is higher in the dry salting method (Altınkurt and Gülyavuz, 1990). In codfish, for instance, 25% of the water in fish leaves the flesh within 4 or 5 days and following this water loss decelerates. After a long time it increases to 30%. On the contrary, salt absorption by the fish reaches approximately 18% within 6 or 8 days and gradually increases to a maximum of 20%. As soon as the salt in the fish reaches 9–10%, irreversible changes take place in the proteins, causing denaturation. In brine salting the weight of the fish fluctuates. For instance, herring and European pilchard loose 20% in weight within the first few days. The salt absorbed within this time reaches 18%. In the following days, the fish starts to gain weight and reaches to its original weight within approximately 10 days (Göğüş and Kolsarıcı, 1992; Horner, 1992).

The water content required for bacterial reproduction is 50–60%. Salted fish can be preserved for a long time since the decrease in water content of the salted fish reduces bacterial action. Furthermore, Cl^- ions present in the structure of salt hinder bacterial action due to a sterilizing effect. Solubility of oxygen in salt solution is lower than in distilled water. Therefore, the activity of aerobic bacteria in particular is decreased in salted products. Salt entering the fish penetrates any bacterial cells present and kills them. The amount of salt that destroys the activity of most bacteria is approximately 10% of fish weight. However, halophilic bacteria are not affected by salt and are active even in fish containing 15–20% salt. Salt reduces the effect of enzymes as well. Putrefaction is delayed due to the decrease in autolysis. As a result of salting and water loss, muscle shrinks and hardens (Altınkurt and Gülyavuz, 1990).

14.2 PROCESS STEPS IN SALTING TECHNOLOGY

14.2.1 Salt quality

Common salt originating from the land (rock salt) is used in salting. Sea or lake salts are not suitable since they carry the microorganisms, leading to pink spoilage and browning (mold) in fish. The salt to be used in salting should not contain $MgCl_2$, $CaCl_2$, $MgSO_4$, or $CaSO_4$. But tolerable levels are a maximum of 0.3% $MgCl_2$ and $CaCl_2$ and 0.6% sulfate compounds. Magnesium and calcium compounds result in moisture absorption in salt and consequently spoilage of the fish. Furthermore, the said compounds make the processed product decay and cause a bitter taste (Omurtag and Omurtag, 1968). If salt contains iron higher than 30 ppm and copper higher than 0.2–0.4 ppm, discoloration is observed in the fish. These chemical substances adulterating the salt's purity also delay salt intake of the fish. They also allow protein decomposition (Van Klaveren and Legendre, 1965). Fish salted with pure salt are brittle and amber-colored (straw yellow) whereas fish salted with a salt containing 0.5–1% Ca^{2+} or Mg^{2+} have a hard tissue and chalk white color. Since it is desired by consumers, the use of impure salt continues in the industry. Fish processed with Ca^{2+}- and Mg^{2+}-containing salts have an unpleasant sour and strong odor (Omurtag and Omurtag, 1968; Ismail and Wootton, 1992).

The dimensions of the salt crystals are important in salting. In general, fine salt is advantageous because it spreads over the whole fish more uniformly. Since, however, fine salt

rapidly absorbs water from surface tissue within the first days of salting, it coagulates protein and hardens the tissue. As a result, it may lead to spoilage because of slower salt intake. In the tissues which are closer to the surface, albumins precipitating due to dehydration within a short period of time prevent salt from penetrating deeper and consequently salt burn occurs (Tunalı, 1984). In addition, fine-grained salt causes fish to stick together and subsequently be damaged. Due to the fact that salt penetrates fish more slowly when salting is carried out with coarse-grained salt alone, inner spoilage may be observed. For the abovementioned reasons a mixture comprising equal volumes of fine- and coarse-grained crystals should be used in salting. The recommended diameter for course salt crystals is approximately 6 mm (Van Klaveren and Legendre, 1965)

14.2.2 Fish preparation

The foremost condition for success is very fresh fish. Otherwise, inner sections of the fish start to be spoiled before the salt penetrates them. In the event that fish is put in salt as long as 24 h after being caught, it is impossible to obtain a premium-quality product. On the other hand, the season during which salting occurs is highly significant as well. For instance, the quality of a European pilchard which is put into salt 20 h after being caught in a cold season is higher than the one salted 9 or 10 h after being caught in a hot season. Furthermore, post-salting quality of fish which are put in salt 36–40 h after being caught in cold weather is lower; for example, flesh around the bone goes dark (Tunalı, 1984).

Generally, freshwater fish that are salted include trout, lake herring, lake bluefish, European catfish, blue and yellow perch, pike, all types of carp, river herring and eels. Sea-fish types include anchovy, cod, berlam, bluefish, whiting, trout, shad, seabass, rock fish, mackerel, herring, and red mullet (Jarvis, 1953). Among shellfish and molluscs, mussels can be salted.

In order to obtain a high-quality product, the viscera are removed and, if necessary, the head is removed immediately after landing (Tunalı, 1984). Small fish are split ventrally to produce two adjoined flat pieces. Big fish can be filleted or sliced (Jarvis, 1953). Cleaning should be performed very quickly, otherwise bacteria which have been transmitted to the fish from sea water attack the tissues following the death of the fish and lead to spoilage. The second purpose of cleaning is the easier and quicker penetration of saline water into the ventral cavity and inner sections of the fish. Cleaned fish can be classified according to their length, if required. It is preferred that viscera are removed from the fish to be salted and preliminary salting is carried out on board the fishing vessel. When they are disembarked, they are either re-salted or put directly into boxes. Sometimes, preliminary salting on board is performed with coarse-grained salt and following debarkation they are re-salted and put into barrels (Tunalı, 1984).

14.2.3 Salting methods

14.2.3.1 Dry salting method

In general, white lean fish are salted by this method but ammonia or amines may pose a problem in cartilaginous fish (Connell, 1980). Fish salted with dry salt lose water due to the hygroscopic characteristics of salt and osmosis. Exported water forms a concentrated salt solution together with the outer dry salt. Since the outer salt concentration is higher than the salt concentration inside the fish, salt intake and water discharge continue by

decreasing gradually with osmosis. In dry salting, fish are split ventrally or sliced in circles, washed with very clean sea water or a salt solution of 2–5% and then soaked in this solution for 20–60 min. Thanks to this process, the blood and any dirt on the skin are removed. Afterwards, it is dewatered. In some applications, small fish are salted without removing the viscera (Gülyavuz and Ünlüsayın, 1999). In herrings, gills and a certain part of the viscera are removed outright by a process called gibbing; the pyloric ceaca and gonads are left. Enzymes in the pyloric ceaca are seen as necessary for good ripening (Connell, 1980).

To ensure a good distribution of salting, the fish should be about the same size. Plastic or wooden barrels or concrete tanks can be used in salting. It is recommended that tank bases are slightly inclined towards a certain direction and have a hole in the deepest part to enable water from the fish to flow out. Open salting is possible as well (Gülyavuz and Ünlüsayın, 1999). Generally, in strong salting, 1 unit of salt is used for 3 units of fish whereas, in light salting, 1 unit of salt is used for 8 units of fish (Regenstein and Regenstein, 1991). On average, 1 unit of salt is used for 5 units of fish, so the amount of salt should be 20–25% of the cleaned fish (Tunalı, 1984). During salting, the required amount of salt is weighed and then the bottom of a barrel or tank or, if it is an open salting, of the stowage is dusted with a certain amount of salt. The base fins and ventral cavity of big- and medium-size fish to be salted are rubbed well with salt. The thickest fish parts require more salt. Starting from the tank base, fish are laid together in such a way that skin comes into contact with the salt. Of the juxtaposed fish, one fish's head should be next to the tail of another, if possible. Furthermore, there should be no air between the fish. After a layer of fish is placed, a certain amount of salt is put in between. Then, another layer of fish is placed. This process is repeated until the barrel or tank is full. Again, it is recommended that the arrangement of each fish layer should be endways to that of the previous layer. While salting small-size fish such as anchovy, horse mackerel, and sardine, a certain amount of salt is put down, and then a fish layer of 4–5 cm thickness is formed and then again a certain amount of salt is put down. The recommended stowage thickness is a maximum of 1 m in order to prevent fish from being crushed and the ideal stowage height is approximately 30 cm. Additionally, allowing for settling, it better to stow a little more fish than the required amount. Skins of the fish placed on top should face upwards. Salt is added and salting process is completed. Afterwards, tanks are covered with a mat or wood and the fish are left to settle for 1 day. Later on, if necessary, pressure is applied by stone or other materials in such a way that the fish are not crushed. The salting period is 7–10 days. In some situations it is recommended that the fish are re-stowed whereby the fish on top are placed at the bottom and that they are re-salted (Altınkurt and Gülyavuz, 1990).

In dry salting, a brine, which absorbs the water in the fish, is formed in the area where salt comes into contact with the fish. Because of being in contact with dry salt as well, the brine preserves its concentration, although it continuously absorbs water from the fish. This exchange continues as long as salt crystals are present in the solution. (Tunalı, 1984) Yield is lower in dry salting since water loss from the fish is higher. However, higher water loss extends the product's period of preservation. Salt rate and dehydration are not equal in all areas because salt is not equally dispersed all around the fish body or the fish in the stowage. The fish surface comes into contact with air and, consequently, the fat is oxidized, the odor gets stronger, and the color gets darker. Lower layers of fish can be crushed by the weight of the upper layers. A certain amount of salt used may be lost with the running water. If a drying process is carried out after salting, it is easier with dry salting (Gülyavuz and Ünlüsayın, 1999).

Table 14.1 Brine strengths as measured on various hydrometer scales at 16°C (source: Clucas and Ward, 1996).

Salinometer degrees	Specific gravity	Baumé degrees	Twaddell degrees	Percentage of salt by weight	Salt (g)/liter of water
0	1.000	0.0	0.0	0.000	0.0
10	1.019	2.7	3.8	2.640	27.0
20	1.038	5.3	7.6	5.279	55.6
30	1.058	7.9	11.6	7.919	85.8
40	1.078	10.5	15.6	10.558	117.7
50	1.098	12.9	19.6	13.198	151.7
55	1.108	14.1	21.6	14.517	169.5
60	1.118	15.3	23.6	15.837	187.9
65	1.128	16.5	25.6	17.157	206.6
70	1.139	17.7	27.8	18.477	226.2
75	1.149	18.8	29.8	19.796	246.3
80	1.160	20.0	32.0	21.116	267.1
85	1.171	21.2	34.2	22.436	288.7
90	1.182	22.3	36.4	23.755	310.8
95	1.193	23.5	38.6	25.075	333.9
100	1.204	24.6	40.8	26.395	357.9

14.2.3.2 Brine salting method

Brines can be prepared with common salt in various concentrations (see Table 14.1). The solubility of salt in water is 35.8 g/100 g of water at 16°C. Accordingly, a saturated salt solution contains $(35.8) \times 100/135.8 = 26.4\%$ salt. Solubility of salt changes slightly depending upon temperature. The amount of salt in saturated salt solutions at different temperatures is as follows:

35.5% at 0°C
36% at 20°C
37% at 50°C
39.5% at 100°C (Tunalı, 1984)

Brines can be classified as light brines, medium brines, and heavy brines depending on concentration. One of the abovementioned classes can be used according to the quality of fish to be salted or the desired salting form. Brine contains 9–11% salt in light salting, 14–16% salt in medium salting, and 24% salt in heavy salting.

If brine is saturated to less than 12% then the fish absorbs the brine whereas if it is saturated to more than 12% then the fish loses water and soluble substances (Regenstein and Regenstein, 1991). Some processors add a coloring agent to the brine to give the fish a vivid color, especially for anchovies. The most commonly used color is red ruddle. Furthermore, in special salting, some aromatic or spicy substances can be added to give a characteristic aroma to the fish. These may include clove, pepper, and ginger (Tunalı, 1984).

The brining method of salting is mainly used in fatty fish (Connell, 1980; Regenstein and Regenstein, 1991). In this method, a salt solution with the desired concentration is prepared in concrete, plastic or water-tight tanks or barrels. Later on, the fish to be salted are put into the solution or the brine prepared is poured onto the stowed fish. Weight is applied to the salted fish by placing a mat or wood pieces on top. There is no need for any weight on the first day. However,

it is recommended that a weight equal to 20% of the fish weight is applied on the second day and a weight equal to 40% of the fish weight is applied after 4 or 5 days. Weights prevent fish from rising up and coming into contact with the air (Gülyavuz and Ünlüsayın, 1999). By the end of the first day precipitation should take place in the tank. The gap at the top is filled with a sufficient quantity of fish that were prepared on the same date and were placed in another tank. In brining, the distribution of salt on the fish is more uniform compared to dry salting. However, salt absorption by the fish is slower and lower than in dry salting because the salt in the brine is absorbed by the fish as the fish discharges its water to the brine (Tunalı, 1984). Dry salt should be added to the brine and the solution should be mixed well from time to time since the water discharged by the fish dilutes the solution. Addition of salt from one or two sections of the barrel does not meet this need because the diffusion of salt to equalize concentrations across the barrel takes place very slowly. Thus, a deceleration occurs in salt intake (Voskresensky, 1965). Due to the fact that fish salted by brining contain a higher amount of water and decompose more rapidly than those salted by dry salting, their storage life is shorter. However, the brining method is preferred for zestier salted fish (Tunalı, 1984). Since fish salted by the brining method do not come into contact with air, oxidation of fats is lower. The taste and appearance of the product is better (Gülyavuz and Ünlüsayın, 1999). In dry salting halophilic organisms can still survive because they are aerobic, but they cannot reproduce in brine and below 5°C (Horner, 1992).

During the storage of fish salted by brining, significant changes occur in the brine. Brine, which is transparent and odorless at the beginning, becomes opaque and dull. Brine that has completed its salting function includes 0.16–0.19% phosphoric acid, 0.4% potassium salts, and 1–2% nitrogen. Brine rich in nitrogenous substances and albumins as well as amino acids and fatty acids can allow microorganisms to reproduce, and the brine turns into a culture medium. Brine which is neutral or slightly acidic at the beginning of the process increases in acidity and decreases in pH (to pH 4.5) and fatty acids come into existence with micro-organismal activity. As a result of brine refreshment, pH increases and the bacterial flora changes. Upon the increase in pH, a malodor is produced due to production of hydrogen sulfide (H_2S) and mercaptans. Whether or not the brine remains suitable for salting should be determined with chemical and bacteriological analyses. Determination by conducting organoleptic tests results in mistakes and, consequently, products of poor quality. Therefore, as brine color changes (as soon as it becomes opaque) and as soon as the pH decreases with the effect of fatty acids, brine should be renewed. This should be performed before the brine turns reddish. Brine renewal is not preparing new brine but oxidizing the organic substances which increase in concentration in the brine. Sodium hypochlorite is used as the oxidizing agent. It covers the organic substances and increases oxygen in the atmosphere. Furthermore, it is transformed into NaCl and enriches the brine with salt. It is not possible to state the amount of sodium hypochlorite to be used. Practically, oxidizing is carried out by adding sodium hypochlorite until the brine stops emitting the chlorine odor. In the meantime, brine is continuously mixed (Tunalı, 1984).

14.2.3.3 Mixed salting method

It is possible to obtain a higher-quality product by employing the dry salting and brine salting methods. The salted product can be preserved for a longer time. In this method, first of all fish are dry salted. Water discharged by the fish forms a concentrated salt solution with the available salt. Water discharged by fish with osmosis is not discharged. In the event that the said water does not cover the fish surface, a certain amount of saturated salt solution is added 1 or 2 days later (Hansen, 1980; Regenstein and Regenstein, 1991; Horner, 1992). Here, dry salt

on the fish surface prevents the brine from gradually being diluted. As salt dissolves in the water discharged by the fish, extra salt water is formed and the brine remains saturated. In this way, the negative aspects of the previous two methods are eliminated. In general, the tank is filled with fish to such an amount that allows for settling or, after settling, fish are added from another tank prepared on the same day (Voskresensky, 1965).

In this method, it is possible to perform first brine salting and then dry salting or, in some situations, both salting method with brining. Generally, in salting processes carried out in summer, preliminary salting should be performed with salt solution. In the event that dry salt is used in the salting process, infestation may take place in fish which comes into contact with air and, consequently, product quality decreases. Due to high air temperature, penetration of salt by the product becomes difficult and the product may go bad. For the purpose of eliminating such disadvantages, both salting processes should be performed with brining in the two-stage salting process performed in the summer. In the two-stage salting process, the first salting stage takes a shorter time whereas the second salting stage takes a longer time (Gülyavuz and Ünlüsayın, 1999).

14.2.3.4 Salting and chilling

Fish salting and chilling can take place together. The process may be called warm salting, chilled salting, or cold salting depending on the amount of ice used. In *warm salting* there is no chilling; it is employed in cold seasons of the year. In *chilled salting* fish is salted after being chilled to 0–5°C. Thanks to chilling, autolytic and bacterial activities on fish tissue are decelerated; thus, fish quality is preserved during storage (Voskresensky, 1965). In this method, salt and ice are used together. Salting is performed in open containers. Fish are mixed with coarse-grained salt and fragmented ice. In general, 10–12 kg of salt is used for 100 kg of ice. Salted fish are kept in 0°C storages (Tunalı, 1984). In *cold salting* pre-freezing and salting are combined. It prevents bacterial activity and spoilage of the inner layers of fish. Frozen fish are put into barrels and salted by the dry or mixed salting methods. The brine is cold at the beginning. According to the degree of defrosting, the salt gradually penetrates into the fish. Salt penetration increases as the fish is defrosted. This method is applied mainly to large and fatty fish (approximately 1 kg of herrings with 20% fat) (Voskresensky, 1965).

14.2.3.5 Salting by pressing

Fish salted in accordance with the brine salting method are kept in a saturated salt solution for 5–20 days. After being taken out of the saturated salt solution, they are pressed in tanks with holes at the bottom and covered by a wooden clamp system. At the beginning, pressing should be performed very slowly. During pressing, the system should be clamped every 30 min for the first 10 h and every 1 h for the second 10 h. Thus, the water which has not been discharged in the course of salting leaves the fish and runs through the holes at the bottom of the tank together with the salt on the fish surface (Gülyavuz and Ünlüsayın, 1999).

14.2.3.6 Quick salting methods

Salting in a vacuum

Fish which are salted with fine salt are put in a boiler with a hermetic cover and double-wall structure. First pressure and then a vacuum are applied to the boiler. In the meantime, slight heat

may be employed as well. A vacuum is formed by de-aeration. Salt quickly penetrates into the fish since the water in fish is discharged faster under a vacuum. As a result, the fish are salted quickly, and then the salted fish are packaged. It is an expensive method due to the fact that a vacuuming plant is required and all compartments of the system should be made of stainless steel. Thus, it is not commonly employed (Tunalı, 1984; Gülyavuz and Ünlüsayın, 1999).

Salting by injector

Land animals and big fish are salted by this method. Salting time is one-third of the normal salting time. It is not employed in large-scale production since it is a labor-intensive method (Gülyavuz and Ünlüsayın, 1999).

14.2.4 Additives used in the salting process

During salting, different additives are used, such as potassium nitrate (KNO_3) for preserving fish color, various spices for making the taste and smell better, various antioxidants for preventing fat oxidation, and sugar for reducing the excessively salty taste of the salt (Espejo and Orejena, 1980; Gülyavuz and Ünlüsayın, 1999).

14.3 FACTORS AFFECTING THE PENETRATION OF SALT

14.3.1 Salting method

In dry salting, water discharged by fish forms a concentrated solution together with the salt present in the environment. The penetration rate of salt is high since the salt solution around the fish has a high concentration. In the brining method, however, water discharged by fish reduces the salt concentration of the surrounding environment. The rate of salt penetrating into fish from a weaker solution is lower. However, in dry salting, the distribution of salt around the fish is not homogenous (Gülyavuz and Ünlüsayın, 1999). Turan and Erkoyuncu (1997) revealed the salt content to be 15.4% in dry-salted trout and 8.5% in brined trout on the fifteenth day of salting and 21.3% in dry-salted trout and 15% in brined trout on the 165th day (Figure 14.1).

Turan *et al.* (2007) determined the amount of salt in the tissue to be 16.50% in dry-salted mussels and 11.24% in brine-salted mussels after 3 days. The value of 20.92% detected in dry-salted mussels on the twelfth day of salting was reached on approximately the 120th day in brine-salted mussels (Figure 14.2).

14.3.2 Salt concentration

The higher the salt concentration of a solution, the higher is the salt penetration rate. A salt level of 8–10% is the critical limit. When the salt in fish reaches this level, proteins are rapidly denatured and water loss and salt penetration accelerate. A temperature between 0 and 20°C has no effect, but the process accelerates at temperatures above this range. Within the first 4–5 days water loss is 25% of the weight, and then it rises to 30% (Horner, 1992).

14.3.3 Salt quality

Impurities in salt decelerate salt penetration. For instance, compounds such as $MgCl_2$, $CaCl_2$, $MgSO_4$, and $CaSO_4$ have this effect (Table 14.2). Salt containing 4.7% $MgCl_2$ penetrates fish

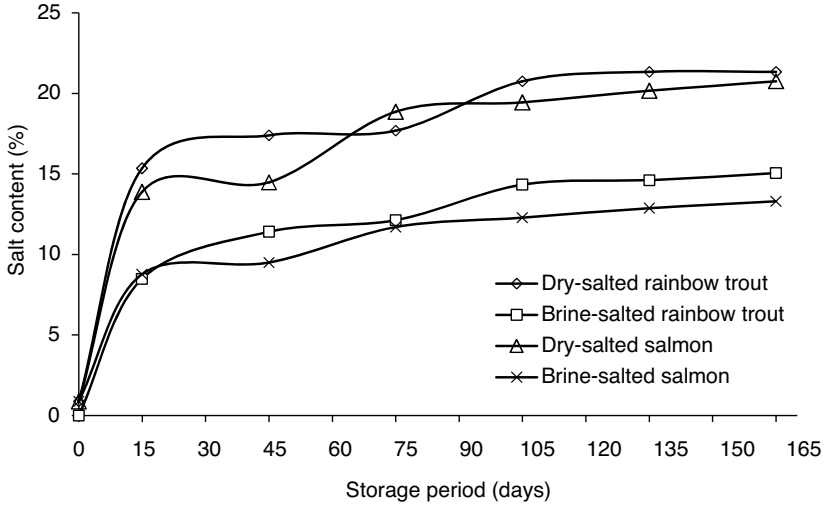

Figure 14.1 Changes in the salt amount of dry-salted and brine-salted rainbow trout and salmon during storage.

within 5 days whereas it takes 3 days for pure salt. Calcium salts bind to proteins and prevents salt from penetrating the fish. However, salt containing 0.5–1% calcium and magnesium (sulfate salts) brings a desired whiteness and hardness to the product. Higher concentrations are not desirable because they result in bitterness and brittleness (easy frangibility) (Omurtag and Omurtag, 1968; Espejo and Orejena, 1980; Horner, 1992; Ismail and Wootton, 1992; Gülyavuz and Ünlüsayın, 1999).

14.3.4 Fish freshness

Salt penetration of fresh fish is relatively slow. Salt absorption increases with stale fish. Fish in rigor takes a longer time to salt than fish in autolysis (Espejo and Orejena, 1980).

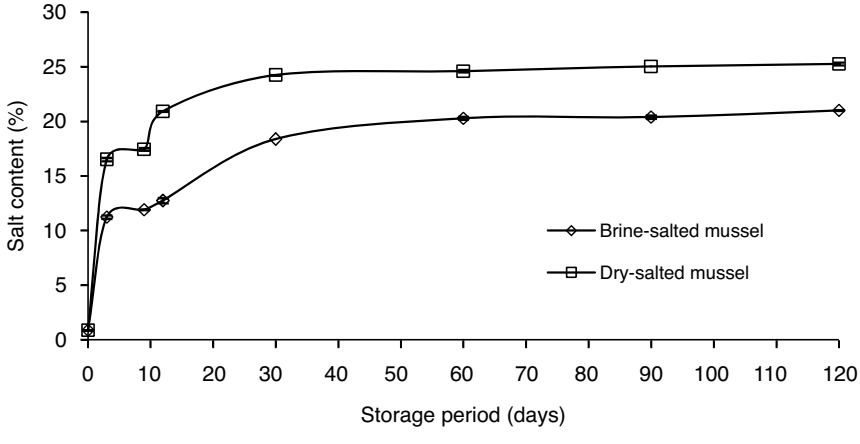

Figure 14.2 Changes in the salt amount of dry-salted and brine-salted mussels according to salting method.

Table 14.2 The amount of salt penetrating a fish depending upon the amount of impurities contained in the salt (source: Gülyavuz and Ünlüsayın, 1999).

Salt composition	Body part	Rate of salt penetration (%)			
		1 day	4 days	7 days	10 days
100% NaCl	Surface flesh	9.8	16.2	19.6	19.5
	Inner flesh	2.6	11.0	16.0	18.7
99% NaCl + 1% $CaCl_2$	Surface flesh	8.7	10.8	15.2	16.6
	Inner flesh	2.5	7.9	14.1	14.4
99% NaCl + 1% $MgCl_2$	Inner flesh	6.5	15.7	18.1	19.0
95.3% NaCl + 4.7% $MgCl_2$	Surface flesh	10.1	17.1	17.8	18.1
	Inner flesh	5.9	12.7	17.1	18.1
99% NaCl + 1% $MgSO_4$	Inner flesh	7.1	10.5	15.3	17.1

Surface flesh is from the surface to a depth of 0.5 cm and inner flesh is between 0.5 and 1 cm deep.

14.3.5 Amount of fat

The amount of fat in a fish is inversely proportional to the salt penetration rate. Fat reduces salt penetration and water loss (Espejo and Orejena, 1980). High protein content (18–19%) elongates the time necessary to reach osmotic balance (Horner, 1992). Although both trout containing 3.5% fat and salmon containing 6.6% fat contain 0.8% salt at the beginning, the level of salt was determined to be 15.4% in the trout and 13.9% in the salmon on the fifteenth day of salting and 21.3% in the trout and 20.8% in the salmon on the 165th day. The salt content of 20.8% detected in the salmon on day 165 was reached on approximately the 105th day in the trout (Figure 14.1) (Turan and Erkoyuncu, 1997).

14.3.6 Size of the fish

The amount of salt penetrating into the flesh of a big fish is relatively lower than salt entering a small fish. The middle part of a dry-salted fish of 2.5 cm thickness has 10% salt after 24 h. On the other hand, this rate is hardly reached in 3 days in flesh of 5 cm thickness. In thick-skinned fish, the skin obstructs salt penetration. Thus, in big fish, fish scales and skin are removed and the fish are cut into fillets. The visceral cavity is well rubbed with salt. Sometimes, the penetration rate of salt is accelerated by splitting the back of the fish. The ventral region is where salt penetrates most quickly because ventral flesh is soft (Horner, 1992; Gülyavuz and Ünlüsayın, 1999).

14.3.7 Temperature

Penetration of salt into fish gets easier as the ambient temperature increases. However, since a rise in temperature increases the rate of autolysis and the level of bacterial activity, putrefaction quickens (Espejo and Orejena, 1980). Fish heavier than 35 g cannot be salted at room temperature. For instance, at room temperature in tropical climates the fish decays before salt penetrataion can occur. Thus, temperatures below 10°C are appropriate in salting (Regenstein and Regenstein, 1991). The best salting is performed between 3 and 4°C and should be completed within 2–4 h (Omurtag and Omurtag, 1968).

14.4 RIPENING OF SALTED FISH

Basically, ripening takes place as a result of a substance exchange between brine and salt fish. The system reaches equilibrium after a certain period as a consequence of the diffusion of substances in brine (NaCl, water) and substances in fish (water, nitrogenous substances, fat). The process is completed when salt concentrations in the fish tissue and the ambient solution equalize. Upon the movement of salt molecules from brine to fish tissue, a layer of salt solution which covers the fish and has a concentration lower than that of brine is formed because water is discharged from the fish faster than salt molecules enter the fish. In other words, there is a kind of traffic where substances move from brine to the fish and another substance is discharged by the fish. When the salt concentration in cells located close to the surface of the fish muscular tissue hits a peak, the salt front slowly moves to inner parts. In flesh where a sufficient amount of salt has accumulated the tissue darkens, and can be distinguished by the naked eye from the unsalted, light-colored parts of the fish. As salting proceeds, this light-colored layer increases in salt content and decreases in thickness. The outward diffusion rate of water decreases as salt penetrates into deeper parts (Voskresensky, 1965). After water is transmitted to the surrounding brine with exosmosis, a weight loss of up to 20% takes place. However, the fish reaches its original weight up to 10 days from the start of salting (Horner, 1992). When the salt concentration in a layer of fish reaches the salt concentration in the brine, water discharge from the fish ceases. The size of this layer considerably affects the rate of salting and generally activates the movement of salt molecules into the fish. Therefore, the salting rate is significantly lower in unmixed brine than in circulated brine or the dry-salting process. Water discharge stops prior to the end of salt penetration. This is why there is a slight increase occurs in fish weight at the end of the salting period (Voskresensky, 1965).

Fish muscular tissue contains 30–35% bound water. Bound water becomes free after the salt concentration in the cells in various parts of the body reaches 15–20%. As bound water in myosin and actomyosin attains a free form, salt concentration in the cells decreases. It leads to an additional movement of salt from brine to fish and, as a result, fish weight increases. Water loss causes a certain constriction and is not always uniform. The loss of water molecules between coagulated protein molecules increases the electrostatic effect. Molecules get closer and fish muscular tissue contracts, constricts, and hardens. In the brining method, less water comes out of the muscle yet, if other conditions are equal, salt penetration is higher than in dry salting (Voskresensky, 1965).

The salting process can be divided into three periods, as follows:

- First period: the fish is exposed to high osmotic pressure. The movement of salt to fish takes place along with the movement of water from fish to brine. The amount of water decreases while the amount of salt increases in the tissue. No chemical change occurs in this period. The fish has the smell and taste of a raw fish. Salt has not penetrated into the inner layers and the ventral cavity.
- Second period: osmotic pressure is effective although it has decreased. There is no significant difference between the salt absorbed and the water discharged. Towards the end of this period, water discharge and weight loss stop. the level of salt in the surface layer of the muscular tissue rises to the concentration in the surrounding brine. A barrier comes into existence and prevents water from moving further into or out of the flesh. Any decrease in the amount of salt present in the outer part of the flesh is immediately compensated from the brine.

- Third period: although water discharge comes to an end, the fish absorbs a little salt. As a result, the weight of the increases slightly. Salt concentration in the cells of all body parts is same as the salt concentration in the outer part of the fish. Flesh becomes dense and contracts. The fish has a sharp, salty taste, and the smell and taste of raw fish disappear.

The stages described up to here apply to all fish salted by the dry or mixed method (Voskresensky, 1965).

Salted fish attain their unique color, smell, and taste during ripening. Ripening of salted fish is dependent upon the original chemical structure of the raw fish, salt composition used, temperature, brine composition, and amount of salt in the fish tissue. Ripening of salted fish is a biochemical phenomenon and changes the chemical and physicochemical characteristics of fish tissues. These changes are carried out in particular by enzymes causing decomposition of proteins and fats. Tissue structure of muscles and body organs are affected as well (Voskresensky, 1965). Enzymes responsible for ripening may stem from the fish digestive system (sometimes from pyloric caeca left without being cleaned), fish muscles, and bacteria developing in the brine. Proteolysis and lipolysis products are the most significant processes in ripening (Horner, 1992). During ripening, small-molecule nitrogenous compounds and fatty acids (triglycerides) are transmitted to brine from the fish. The amounts of protein and fat in the fish decrease. The most apparent changes take place in proteins. The increase in the amount of free amino acids and other non-protein nitrogen compounds in fish tissue can be measured by the increase in the amount of non-protein nitrogen in brine. Over time, the said substances lose their colloidal characteristics and are transmitted to the brine. Here they undergo further changes. In fatty fish, most of the fat is transmitted to brine. These transmissions reach a peak within 30 days. The best measure of ripening is the organoleptic test (Voskresensky, 1965).

Optimum temperature selection for ripening of salted fish depends on various factors, such as fish type, cleaning method, chemical structure of raw and salted fish, and environment (brine or air). Highly fatty fish which are slightly salted (8–10%) and not separated from their viscera should be ripened under cold conditions (-2 to -4°C). Under these conditions, ripening takes place slowly and the product's taste and smell are better. The sour taste of salted fish kept at or above 10°C are not observed in ones ripened at cold temperatures. Additionally, sometimes higher temperatures are applied to accelerate ripening. On the other hand, highly salted herring ripens slowly and the typical quality of taste particular to the slightly salted herring cannot be acquired. Following ripening, high-fat herrings are considerably tastier than low-fat herrings. Ripening in airtight packages or in barrels which are filled with brine and tightly sealed provides better-quality product than does ripening in open containers, because no active fat oxidation occurs (Voskresensky, 1965). Ripening starts from the twentieth day and takes 4–8 weeks (Tunalı, 1984).

14.4.1 Storage of salted fish

Storage life of salted products depends on salt concentration, moisture content, and temperature. Storage life may be up to 1–2 years under certain conditions. Low salt concentration, high humidity, and high temperature shorten the shelf life of salted fish (Connell, 1980). In general, strongly salted fish in saturated brine can endure 1 month at normal ambient temperature. Slightly salted fish are stored at 2–8°C according to the amount of salt used (Tunalı, 1984). Storage life is a few days with light salting at room temperature. If storage life is desired to be longer than 1 week, the temperature should be around 0°C

(Connell, 1980). Fish salted with brine containing less than 24% NaCl should be stored at low temperatures (Tunalı, 1984). In the event that salted products are not stored under suitable conditions, changes such as oxidation, autolysis, fermentation, putrefaction, and molding occur. No putrefaction takes place in products which are salted with 30% dry salt and stored at 7–8°C, because the amount of salt in the fish reaches 15% after 30–40 days. However, the same product undergoes significant putrefaction if kept at or above 20°C (Altınkurt and Gülyavuz, 1990). Brined fish such as herring the viscera of which have been partially removed may be preserved for 6–9 months at room temperature but then rancidity starts. On the other hand, storage life of fish which have not had the viscera removed is shorter because enzymes and probable bacteria create softening and a rotten smell. Regardless of the method and rate of salting, fish should be definitely kept in cold storage. Good packaging may considerably decrease spoilage since it prevents moisture being taken in from the environment. Tight packaging of fish creates anaerobic conditions. Storage life of products with a high level of fat and which have been salted without the removal of viscera and gills is shorter than the times listed above since the enzymes and probable bacteria in the said parts create softening and a rotten smell (Connell, 1980). It is necessary to increase the salting time and the amount of salt used to increase the storage life of big and fatty fish. Even when fatty fish such as shad are salted with salt above 20%, the amount of salt in the fish does not reach 15% before 20–30 days. If non-fat fish such as whiting are salted with 20% salt, the salt in the flesh reaches 15% after 5–6 days. In order to ensure a long storage life in salted fish, salt in the flesh should reach 15% and storage should be at low temperatures (Altınkurt and Gülyavuz, 1990).

14.4.2 Undesirable changes in salted products

14.4.2.1 Fat change

If fish fats contain a considerable amount of unsaturated, especially polyunsaturated, fatty acids, the fish becomes very sensitive to auto-oxidation (Huss, 1995). Auto-oxidation is the chemical decomposition of fat in the presence of oxygen and is hard to control. It can continue by inactivating antioxidants, specifically in very fatty fish (Pigott and Tucker, 1990). Pro-oxidants which are naturally present in fish are affected by factors such as temperature, oxygen, pH, sodium chlorite addition, metal contamination, and light during processing and storage. Additionally, low-molecular-weight nucleotides, amino acids, and organic acids are significant factors in the initiation of fat oxidation (Hultin, 1992). The said changes are observed even though salted products are stored in cold storages. Besides, salt accelerates oxidation. Therefore, fat rancidity is more common in salted products. Oxidation speeds up further if salt containing a high amount of copper and iron is used (Gülyavuz and Ünlüsayın, 1999). Trace amounts of hemoglobin and iron and copper ions increase preliminary hydroperoxide formation. Metallic pro-oxidants act as hydroperoxide-decomposing agents. If hydroperoxides decompose, by-products such as hydroxyl, keto acids, and aldehydes occur. Moreover, it leads to taste and smell loss and to yellowish colors. Fat oxidation products react with carotenoids, tocoferoles, ascorbate, thiamine, pyridoxine, and pentatonic acid and decrease in nutritional value. Free radicals and peroxides damage vitamins A, C, and E. As a result, they gradually lose their antioxidant characteristics. Due to the reactions of peroxides, pigments are damaged as well and toxins and carcinogens are formed. Excessive peroxide formation in fat oxidation results in the catabolism of essential fatty acids. Oxidized unsaturated fats bind proteins and form insoluble protein-fat complex. Together with nutrient catabolism, organoleptic characteristics deteriorate and product quality decreases. Existing

sugars and aldehydes, ketones, and carbonyls resulting from fat oxidation react with the amines, amino acids, peptides, and proteins present in the environment and create a brown color. This reaction is called non-enzymatic browning (Maillard reaction) or the "carbonyl amine" reaction. In the Maillard reaction, there is serious loss of amino acids containing sulfur, and of lysine. Maillard reaction affects fish color, smell, taste, appearance, and texture (Pigott and Tucker, 1990; Huss, 1995).

The concentrations in fish muscular tissue of pro-oxidants and antioxidants which are generally present in all biological tissues are especially high in skin, the hypodermic fat layer, and red muscular tissues. Fat oxidation is faster in skin compared to white or red muscles since skin contains more fat-soluble pro-oxidants. Fish containing more fat are more inclined to fat oxidation. It is estimated that the higher fat oxidation in red muscular tissues stems from not only high fat content but also higher heme and non-heme iron content. Iron ions which may reach to 1 ppm in white fish and 60 ppm in red fish are extremely significant catalysts in the formation of free oxygen radicals (Hultin, 1992). Various protective methods are recommended for preventing fat oxidation. Methods used in the prevention of fat oxidation include packaging with light- and gas-tight packaging materials, keeping the storage temperature low and regular, storing in the dark, and the addition of various antioxidants (such as vitamin E, vitamin C, ethylenediaminetetra-acetic acid (EDTA), tert-butylhydroquinone (TBHQ)) (Licciardello, 1990; Pigott and Tucker, 1990; Hultin, 1992; Porter *et al.*, 1992). Furthermore, in order to reduce oxidation, fatty fish should be brine salted.

14.4.2.2 Autolysis

Autolysis does not stop but decelerates in salted products. The amount of water is higher in fish at the beginning of salting and consequently autolysis is faster. Fish should be kept under cold conditions at least until sufficient salt intake is ensured because temperature increases autolysis rate. In the course of time, the amount of salt in the product increases as the amount of water decreases, and autolysis proceeds more slowly. To reduce autolysis, the ventral cavity, where enzymes causing autolysis most commonly exist, should be rubbed with salt (Gülyavuz and Ünlüsayın, 1999).

14.4.2.3 Changes caused by microorganisms

Most of the microorganisms normally associated with fish spoilage – for example, *Pseudomonas* spp. – are halophobic and will not grow in salt concentrations exceeding 5%. There are, however, certain organisms that may be both common and pathogenic, and which are halotolerant, growing in a 10%, or even 20% salt environment. *Staphylococcus aureus* is a highly significant example. The most important spoilage microorganisms are the halophiles which actually require salt for growth and will not grow unless 10% salt is present. These bacteria are responsible for pink spoilage, so-called because of the color of their colonies and consequent appearance of the cured fish (Horner, 1992).

Texture deformation

Under conditions of high temperature, high humidity, and insufficient salt intake, some microorganisms can reproduce in 6–12% salt concentrations and cause fish to be covered with a slippery and sticky layer and to produce a noxious smell. If this state of stickiness has not considerably advanced, a solution may be provided by decreasing the temperature and humidity and drying the outer layers by means of ventilation. Bacteria can reproduce in thick

parts of the fish where salt intake is slow. If the temperature is very high, it can make fish become pasty (Voskresensky, 1965). Mesophyllic bacteria can reproduce in thick parts of a fish which are kept above 5°C, giving rise to a sour taste. Insufficient salting, extension of soaking time, use of stale fish, high humidity, and high temperature are the reasons for the observed pastiness. Suitable brining conditions can create an anaerobic environment and prevent the development of microorganisms. Use of sugar in herrings may create sticky conditions due to the formation of a polysaccharide (levan) by bacterial action (Connell, 1980).

Molding

Molding means the development of brown or light brown spots which are observed especially in fleshy parts of the fish and which originate from *Sporendonema epizoum* (*Wallemia sebi*, syn. *S. epizoum*) (brown mold) that is capable of growing in salt concentrations up to 10–15%. Molding can be observed even if the amount of water is low in fish. It is not capable of developing at high temperatures. Molding is observed mostly in brines with lower salt concentrations. Products which have the lowest extent of molding are those containing 15–20% salt. In moldy products, product quality and value decrease. Mold spots can be removed by careful brushing and product made marketable. It is possible to prevent this situation by preparing solutions such as acetic acid, benzoic acid, propionic acid, potassium sorbate, sodium propionate (3%), sorbic acid (0.3%), and sodium metabisulfate and spraying onto the product or immersing the product in the solutions or adding the solutions to the salt solution. Dry conditions, good ventilation, and painted buildings should be used (Connell, 1980; Regenstein and Regenstein, 1991). Some molds are capable of producing mycotoxin, the most significant of which is aflatoxin (*Aspergillus flavus*, *Aspergillus parasiticus*). However, to date, no intoxication has occurred due to the presence of mycotoxin in salted fish.

Reddening

The most significant microorganisms which give rise to spoilage in salted products are halophiles and they need salt to develop. If salted products are stored in hot and humid environments, especially in the summer, they first turn pink and then red (Connell, 1980; Hansen, 1980). Aerobic and red-pigment-producing thermophilic and halophilic microorganisms such as *Halobacterium salinaria*, *Halobacterium cutirubum*, *Sarcina morrhuae*, and *Sarcina litoralis* cause this situation. They do not reproduce in brine since they are aerobic. They are thermophilic and their development temperature is a minimum of 5°C and an optimum of 10°C. They are not capable of developing if at least 10% salt is not present since they are halophilic (Horner, 1992). In general, the bacteria involved are bacilli and cocci. Spoilage is observed at the edges of the salted fish which are salted and stored at 15–20°C. These bacteria attack specifically fish which are dry salted or not sufficiently covered with brine.

The most important symptom observed in such fish is pink spots and pink shadows on the surface or a pink gloss in the form of reddening of the outer parts. It is possible to remove the redness by rubbing or washing. However, if they spread to the inner parts, bacteria affect proteins and soften the muscular tissue and an astringent smell may arise. Such a product does not have any commercial value. In this spoilage, the efficiency of enzymatic activity varies depending on the microorganism type and salt concentration. It is possible to prevent recurrence by preserving under cold conditions (below 10°C), exposing to formaldehyde or sulfur dioxide vapor or by submerging in sodium metabisulfite (Horner, 1992). Furthermore, halophilic microorganisms may be killed by adding 3% sodium acid phosphate and 0.25%

sodium benzoate to the salt to be used in salting. Their reproduction is prevented by reducing brine pH value to 5.1–5.5 or by adding 0.01% citric or tartaric acid or 0.3% sorbic acid. Bacteria giving rise to the said spoilage exist mainly in some types of salt, especially rock salt.

It is difficult to kill halophiles in salt. High temperatures such as at 160–180°C may be necessary to kill them. It is effective to expose the salt to microwaves for 10–15 min or to cook the salt in an oil bath. Salts should be sterile (Regenstein and Regenstein, 1991). It has been detected that the bacteria in question disappear in salts stored for 1 year or more since contamination tends to reduce over the course of time. It is possible to take precautions such as storing in a cool place or disinfecting tools with disinfectants that do not harm human health. Moreover, fumigation with sulfur dioxide is useful (Connell, 1980). Pink bacteria are neither toxic nor pathogenic; they do not cause intoxication. Intoxications which are stated to arise from eating fish with pink spoilage are probably caused by exotoxins produced by *Staph. aureus* (Horner, 1992). They are human-related and stop reproducing below 5°C and pH 4.5. Again, they are said to not produce toxins in salt concentrations higher than 12%. For a long storage life, the amount of salt in the fish should reach 15%.

14.5 CONCLUSION

Salting is a traditional method of processing fish in many countries of the world. Besides, salt is used in almost all processes involved in fish preparation, either as a condiment or as an accessory preservative. It is also used in preprocessing before fish-processing technologies such as smoking, drying, and canning. Salt and salting methods will always be an important instrument in fish-processing technology in the future.

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15 Hypoxanthine Levels, Chemical Studies and Bacterial Flora of Alternate Frozen/Thawed Market-Simulated Marine Fish Species

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Abstract: In this chapter the results obtained in one of our interesting studies on alternate frozen/thawed market-simulated marine fish species is discussed. Hypoxanthine levels were standardized with chemical indices and bacterial flora/count of three marine fish species: *Pseudotolithus senegalensis* (croaker), *Scomber japonicus* (chub mackerel) and *Sardinella eba* (sardine). Fish were exposed to 12 h of thawing (weekly) to simulate market conditions before refreezing and analysis for a 12-week cold storage period at −4°C. Organoleptic assessment of the cooked fish was best for the fresh basal fish sample, while chemical studies and bacteria flora revealed that *S. eba* had the highest spoilage rate with 12 bacteria species isolated followed by *Sc. japonicus* with 10 bacteria species and *P. senegalensis* with seven bacterial species. A total viable count range of $(1.60–5.58) \times 10^5$ colony-forming units/g, trimethylamine of 24.12–31.20 mg/100 g fish, peroxide value of 22.50–29.40 mEq/kg fish, free fatty acid of 1.53–2.16% and hypoxanthine levels of 25.32–33.84 mg/100 mg fish were recorded for *P. senegalensis*, which were much lower than in the other two species studied. Incidence of *Lactococcus acidophilus* was observed in all three fish species stored from week 0 (basal sample), possibly due to contamination during handling. The bacteria of highest prevalence in the study were *Bacillus cereus*, *L. acidophilus* and *Pseudomonas aeruginosa*.

Keywords: chemical studies; contamination; handling; hypoxanthine; marine fish species; preservation; shelf life; spoilage bacteria

15.1 INTRODUCTION

Frozen fish constitutes a major commodity in international trade and despite the introduction of quality assurance schemes in the sector, various sampling methods and controlled analyses of end products are carried out, particularly of imported fish at the port of entry (Food and Drug Administration (FDA), 2002). According to Connell (1995), fish qualities are those attributes which consciously or unconsciously the consumers or buyers of fish consider should be present in a fish. Quality embraces intrinsic composition, degree of contamination with undesirable materials, nutritive value, degree of spoilage, damage, deterioration during processing, storage, distribution, sale and presentation to the consumer, hazard to health,

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satisfaction on buying and eating, aesthetic considerations, yield and profitability to the producer and middlemen (Connell, 1995).

The freezing process cannot improve the quality of fish; therefore, the best frozen fish products are those made from first-class raw materials. Frozen fish belongs to the class of foods in which respiration processes are suspended, but in which biochemical, microbial and other decomposition processes which must be taken into account still proceed (Huss *et al.*, 1992).

Fish is considered properly frozen if after storage in a cold store it cannot be differentiated from fresh fish on thawing (Eyo, 2001). Also, freezing cannot reverse the deterioration that has already occurred (Connell, 1995). The main factors affecting the quality of frozen fish are the freshness of the fish at the time of freezing, the rate of freezing and the storage temperature, the amount by which the storage temperature is allowed to fluctuate and desiccation during cold storage. Even a temporary rise in temperature of a few degrees can have a marked effect on quality or storage life (Food and Agricultural Organization, 1976).

15.2 SOURCES OF CONTAMINATION OF FISH

The maximum contamination of freshly caught fish that could reduce its quality may originate from the following:

(i) contamination of the raw material at the fishing ground;
(ii) use of polluted water in washing the fish;
(iii) use of unclean equipment, including fish boxes during handling;
(iv) high level of unhygienic conditions in the processing factory;
(v) lack of personal hygiene among fish handlers;
(vi) contamination in markets or during the hawking process by buyers pricing and selecting the fish; there is no limit to what fish is touched.

15.3 FISH AS A PERISHABLE FOOD

Fish is one of the most perishable foods of all the good-quality protein sources. This perishability is due to the production of high content of low-molecular-weight metabolites useful for microbial nutrition. Also, the total body weight and low amount of connective tissue makes it highly susceptible to bacterial attack. The high temperature of the tropics makes fish spoil within 12 h of death (Frazier and Westhoff, 1988).

Just after the death of a fish, the protein components are initially decomposed by protease enzymes. This increases the content of the non-protein nitrogenous compounds, such as indole, skatole, trimethylamine *N*-oxide (TMAO) and dimethylamine *N*-oxide (DMAO); all these make the fish flesh more alkaline and therefore deterioration sets in quickly. These compounds are responsible for the offensive smell, rancid odours and off-flavours in a spoiling fish.

Fish lipid comprises fatty acid groups (triglycerides) with much unsaturation, which are more susceptible to oxidation due to their unsaturated state, thus further contributing to the rancid odour and off-flavours. Stansby (1976) observed that there is a universal relationship between lipid and water content of fish muscle such that the sum of the two is close or equal to 80%. When water content is high in fish it makes the fish liable to microbial attack

immediately after death, because bacteria grow rapidly in an environment with high levels of water or moisture.

Clucas (1990) stated that carbohydrates are minor components of fish flesh. The carbohydrate content of fish is 1%, which can vary seasonally. In any case it declines rapidly during the stress and struggle associated with capture. After fish death, glycogen is hydrolysed to lactic acid during the first few hours, resulting in fall in pH from 6.8–6.0 to 3.0 depending on the species and the condition of the fish when the process is completed.

The prime causes of spoilage in fish according to Eyo (2001) are bacteria, enzymatic action which results in the production of various volatile compounds and chemical action involving oxygen in the air and fat in the fish flesh. Obvious signs of spoilage according to Huss (1994) are slime formation, detection of off-flavour, discoloration and changes in texture, and these developments are due to a combination of microbiological, chemical and autolytic activities.

15.3.1 Autolytic spoilage

In frozen fish, autolytic enzymes break down TMAO to dimethylamine and formaldehyde. The effect of formaldehyde in frozen fish is to increase the denaturation of fish tissue, and to induce changes in texture and loss of water-binding capacity (Huss, 1994). The enzymatic spoilage also known as autolysis (self digestion) is a process whereby enzymes against which the fish are normally protected when alive, under optimal conditions for enzymatic activity, digest the fish tissue post-mortem (Eyo, 2001). Enzymes act as catalysts to chemical reactions both in the gut and in the flesh and they remain active after death, resulting in self digestion, affecting the flavour, texture and appearance of the fish (Food and Agricultural Organization, 1995). The visceral organs (guts) of fish contain enzymes, which during life are responsible for digesting food. On death, these powerful digestive proteolyic enzymes attack the organs themselves and their surrounding tissues (Cornell, 1995). The rate of attack is particularly great in heavily feeding fish and the organs quite quickly become degraded to a structureless mass and the belly walls are either digested away or weakened such that the slightest abrasion or pressure causes them to tear. The well-known phenomenon 'belly burst', which can occur only a few hours after catch in sardines, herring and some other fish, is caused simply by weakening of the belly wall due to self digestion (Food and Agricultural Organization, 1995).

During autolysis, the non-protein nitrogenous compounds are also broken down into compounds such as trimethylamine (TMA), urea, butane and sulphur compounds with their characteristic obnoxious pungent smell of 'rotten eggs'. However, TMAO, which is reduced to TMA, is only present in marine fish species and not in freshwater fish species.

15.3.2 Microbiological spoilage

Although the flesh of a newly caught fish is sterile, the skin, gills and intestines can carry a considerable load of bacteria (Zhang *et al.*, 2010; Iregui *et al.*, 2011). These bacteria exist in a state of equilibrium when the fish is alive. After death the bacteria can intrude into the tissue and spoil the fish (Clucas, 1990).

When the fish dies, the defence against bacteria attack breaks down and the mechanical barriers such as the skin and membranes lose their impermeability. The bacteria then multiply rapidly and invade the fish flesh, entering it through the gills, kidney, along the veins and arteries, and directly through the skin (Borgstrom, 1965). They consequently break down the flesh and feed from the smaller compounds produced by autolytic action.

The specific spoilage organisms are producers of metabolites responsible for the off-flavour associated with spoilage (Huss, 1994). Bacteria also reduce TMAO to give TMA, which imparts an off-odour to the fish. Other bacterial by-products are ammonia and hydrogen sulphide, both of which have objectionable smells (Clucas, 1990). Although individual bacteria are microscopically small, they are present in such large numbers on the skin at the time of spoilage that they can be seen in aggregate as a yellow slime (flora) (Aitken *et al.*, 1982). However, only a part of this yellow flora contributes to spoilage (Eyo, 2001).

Spoilage bacteria also cause changes in the palatability of the fish. Significantly, they operate positively in most cases by the production of bad-smelling or -tasting substances, mainly ammonia and amines, and also by the production of various keto acids and carboxyl compounds (Liston, 1980). Similarly, bacterial breakdown of TMAO to TMA and oxygen leads to deteriorative changes in spoiling fish.

15.4 INDICATORS OF DETERIORATION IN FROZEN FISH

Frozen fish may dry slowly in cold storage; fish get badly dehydrated and their surface becomes dry, opaque and spongy, and they lose weight. This is known as 'freezer burn'. Drying also accelerates denaturation of proteins and the oxidation of fats (Food and Drug Administration, 1986). Some of the reactions that occur in unfrozen fish continue in the frozen state, albeit more slowly; in general, however, the deteriorative changes in frozen fish are quite different.

A badly stored fillet or a fillet from a badly stored whole fish will feel hard and stiff and will copiously drip fluids. There is a loss of the sweet meaty flavour of fish that is variously described as musty, turnipy, leathery or singed (Aitken *et al.*, 1982).

In frozen fish stored at a low temperature, the bacteria are kept in a state of suspended animation. Immediately that the fish begins to thaw out, however, the bacteria become active again and continue the process of spoilage (Burgress *et al.*, 1967). During freezing, when the temperature falls below the freezing point of water (0°C), microbial activity is slowed down; as the temperature approaches −30°C most bacteria die. Freezing therefore enhances the microbial stability of fish and thereby extends the shelf life (Eyo, 2001). According to Twiddy and Reilly (1994) the predominant kinds of bacteria causing spoilage vary with the temperature at which the fish are held. At freezing temperatures, species of *Pseudomonas* are most likely to predominate with *Acinetobacter*, *Moraxella* and *Flavobacterium* species next in order of importance. *Micrococcus*, Enterobacteriaceae and *Bacillus* largely replace the dominant microflora of *Pseudomonas* as temperature increases (Huss *et al.*, 1992). There are marked changes in texture and flavour on thawing when fish has been frozen very slowly at temperatures only a little below 0°C (Horde, 1973).

15.5 BACTERIAL FOOD POISONING IN SEAFOOD

The presence of harmful microorganisms in fish may cause illness through infection or poisoning. The presence of large numbers of organisms indicates gross contamination or spoilage (Connell, 1995). The term bacterial food poisoning is usually applied to gastro-intestinal disturbance caused by bacteria that have multiplied in a food during storage at too low a temperature. The common symptoms are acute abdominal pain and diarrhoea, often accompanied by vomiting and headache. The organisms may cause their effect either by

direct infection of the alimentary tract or by a toxin, produced in the food during storage (Aitken *et al.*, 1982). Causative organisms of food poisoning include *Aeromonas hydrophila*, *Acinetobacter baumanii*, *Bacillus cereus*, *Clostridium perfringens*, *Clostridium botulinum*, *Enterobacter* spp., *Staphylococcus aureus*, *Salmonella* spp., *Vibrio parahaemolyticus* and *Vibrio cholerae* (Jones and Disney, 1977; Shewan and Murray, 1979; Aitken *et al.*, 1982; Clucas, 1990; Böhme *et al.*, 2010).

Scromboid food poisoning is usually associated with mackerel in the UK (Aitken *et al.*, 1982). *V. parahaemolyticus* occurs naturally in fish. *C. botulinum* has been isolated repeatedly from waters, sediments and fish in various parts of the world. It has been reported to show seasonal occurrence with counts highest in summer when temperatures are high. Botulism is a neuroparalytic disease resulting from the ingestion of toxins produced by *C. botulinum.* There are seven types of *C. botulinum*, referred to as types A–G (Clucas, 1990; Li *et al.*, 2010; Zhang *et al.*, 2010). For the disease to occur, the organism must be present in the fish and must have an opportunity to grow and produce toxin. Even then the fish must be consumed without or with only inadequate cooking since the toxin is susceptible to heat (Hobbs, 1987).

Bacteria in the genus *Pseudomonas*, which are Gram-negative motile rods, cause urinary infections, melioidosis (pneumoenteritis, pseudocholera, Stanton disease, Whitmore disease), respiratory infections and septicaemia (Cheesebrough, 1984) is naturally present in fish. According to Huss (1994) some freshwater fish and many fish from tropical waters during iced/aerobic storage are characterized by a *Pseudomonas* type of spoilage, which is described as having a strong fruity or sickening aroma.

Eschericha coli, according to Hobbs (1987), has been used as an index of faecal contamination, *E. coli* can occur as motile or non-motile rods, and can cause gastroenteritis, bacteraemia, wound infection and urinary infection in humans (Cheesebrough, 1984).

The spores formed by spore-forming species like *B. cereus* and *Bacillus licheniformis* enable them to survive when conditions for vegetative growth are not favourable, especially when carbon and nitrogen become unavailable (Cheesebrough, 1984). *Bacillus* also causes anthrax in humans.

Micrococcus acidophilus replaces *Pseudomonas* when there is an increase in the temperature of stored fish. *Lactococcus acidophilus* serves a preservative function. They have the ability to reduce the pH to a level where the normal spoilage flora is inhibited, the usual mechanism being the production of lactic acid from the carbohydrate in the food source; this thereby elongates the product shelf life (Clucas, 1990). *Staph. aureus* produces a poisonous toxin which differs from most other microbial toxins. The toxins can withstand boiling for up to 30 min before being destroyed. The number of *Staph. aureus* in fish is also currently used in commercial specifications and standards (Aitken *et al.*, 1982). *Streptococcus* bacteria produce extracellular enzymes called streptokinases, which assist the organism in spreading around the human body by breaking down fibrin, which is formed by the host as a protective barrier. *Streptococcus faecium* are cocci in chains and they cause wound and ulcer infection, septicaemia and urinary tract infections (Cheesebrough, 1984).

15.6 METHODS USED FOR ASSESSING DETERIORATIVE CHANGES IN FISH

The primary aim of fish quality control, according to Borgstrom (1965), is to find some methods by which deteriorative changes can be accurately and quantitatively measured.

Quality assessment describes the suitability or excellence of fish for processing or eating and it embraces intrinsic composition, degree of contamination with undesirable materials, nutritive value, degree of spoilage, storage, sales and presentation to consumers.

The purpose and applications of the methods used for fish quality assessment vary. As a result, the application of the methods used and the assessment of fish quality or rate of spoilage can be classified under three headings, namely:

(i) organoleptic or sensory assessment;
(ii) chemical assessment;
(iii) bacteriological assessment (microbiological analysis).

15.6.1 Organoleptic or sensory assessment

The oldest and still most widespread means of evaluating the acceptability and edibility of fish are the senses: smell and sight supplemented by taste and touch (Borgstrom, 1965). No special laboratory equipment is needed, the fish can be examined wherever they happen to be, the tests can be carried out quickly and many samples can be evaluated in a relatively short time using a linear scale (1–8), with 1 meaning excellent condition and 8 extremely unacceptable.

This method depends solely on human senses of sight, touch, smell and taste, and may occasionally be aided with simple devices such as a ruler or weighing scale.

15.6.2 Chemical assessment

15.6.2.1 Use of hypoxanthine

Hypoxanthine is the end product of a series of enzymatic reactions occurring in the flesh of a fish. At death of a fish, the balance of the enzymatic reactions is disturbed and hypoxanthine levels increase (Aitken *et al.*, 1982). Hypoxanthine is a degradation product of the nucleotide adenosine triphosphate (ATP) and the levels present reflects evidence of autolytic deterioration (Kirk and Sawyer, 1999). Hypoxanthine gives the characteristic bitter after taste to cooked 'stale' spoiling fish when eaten.

The degradative changes in fish tissues associated with the hydrolysis of adenosine monophosphate (AMP) have shown that the hypoxanthine content rises throughout the commercially important period of storage (Burt, 1977). ATP has a widespread and a near-uniform degradative pathway in most tissues and hypoxanthine is a result of this ATP degradation (Eyo, 2001). The way in which hypoxanthine concentration increases with storage time is more variable and is a better predictor of spoilage over a wide range of quantities and it is applicable to a wider range of species products than both TMA and total volatile bases (TVB) (Howgate, 1982). Shortly after the death of the fish, hypoxanthine begins to accumulate. However, the total volatile amine and TMA tests measure the various stages of spoilage caused by bacteria, and they do not increase at the beginning of storage, but nucleotides (ATP, ADP, AMP) and hypoxanthine levels reflect enzymatic spoilage (Spinelli, 1967). Thus nucleotide and hypoxanthine measurement have some advantages over TMA and TVB analysis (Metin *et al.*, 2001).

ATP is degraded via inosine to hypoxanthine mainly due to autolytic processes, but in the latter phases bacterial action may also be involved. Measurement of hypoxanthine (shown as HX in the scheme below) should therefore give a good indication of early post-mortem

changes in fish. Hypoxanthine is a normal constituent of fish flesh, though present in very low concentrations in live fish. According to Kassemsam *et al.* (1963) ATP is degraded at the post-mortem stage by endogenous enzymes in the flesh.

$$\text{ATP} \rightarrow \text{ADP} \rightarrow \text{AMP} \rightarrow \text{IMP} \rightarrow \text{HXR} \rightarrow \text{HX}$$

Here, IMP means inosine monophosphate, HXR means inosine and HX means hypoxanthine. Hypoxanthine is regarded as the major catabolite of ATP and it is a useful indicator of freshness because of its gradual accumulation in flat fish (Greene *et al.*, 1990). The usual trend according to Eyo (2001) is that the formation of hypoxanthine begins slowly at first following the activities of autolytic enymes and more rapidly as spoilage progresses through the activities of bacterial enzymes.

These autolytic degradative products have been found to possess different organoleptic properties. Inosine monophosphate produces a sweet flavour at very low concentrations and it is regarded as a strong flavour enhancer. Methods for the analysis of hypoxanthine according to Eyo (2001) include colorimetric, gravimetric, K-value and test paper methods (Burt, 1977), Colorimetry involves the use of the enzyme xanthine oxidase and also 2,6-dichlorophenol-indophenol (DCPIP). Gibb's reagents are the colour indicators used. The colour change is measured at a wavelength of 618 nm using a spectrometer. The K-value is the ratio (expressed as a percentage) of inosine plus hypoxanthine to the total amount of ATP-related compounds.

15.6.2.2 Determination of hypoxanthine

Two grams of crushed fish sample are weighed into a 250 ml beaker; 1 g of active carbon, 100 ml of distilled water and 5 ml of Carrez solutions I and II are added and mixed for 30 min. The mixture is filtered through a Whatman no. 2 filter paper. Five millilitres of clear colourless filtrate is pipetted into a 15 ml test tube, 5 ml of 4-dimethylaminoborane solution is added, and the solution is mixed and placed in a water bath at 20°C. The absorbance of the mixture is taken after colour development on a spectronic 21D spectrophotometer at a wavelength of 460 nm. Standard hypoxanthine of range 2–10 ppm is also treated in the same manner as the sample and absorbance taken at the same wavelength.

$$\text{Concentration of hypoxanthine} = \frac{\text{Absorbance of sample} \times \text{gradient factor of standard} \times \text{dilution factor}}{\text{Weight of sample}}$$

15.6.2.3 Determination of peroxide value

Two grams of crushed fish sample are weighed into a 25 ml beaker. Then 20 ml of chloroform and 10 ml of glacial acetic acid are added to the fish sample in the beaker and mixed. The mixture is filtered into a 250 ml conical flask; 1 ml of 5% (aq) saturated potassium iodide solution is added and the mixture shaken thoroughly. The homogenous mixture is placed on a hot plate to boil for 30 s. Then 25 ml of distilled water are added and the vessel is shaken; 1 ml of 1% starch is added and the hot mixture titrated against 0.002 M $Na_2S_2O_3$. A blank

determination is also carried out at the same time. Peroxide value is the number of millilitres of $Na_2S_2O_3$ (0.002 M) used for the titration in mEq/kg:

$$\text{Peroxide value (PV) (mEq/kg)}$$

$$= \frac{\text{Titre value of sample} - \text{titre value of blank} \times \text{molarity of } Na_2S_2O_3 \times 10^3}{\text{Weight of sample}}$$

15.6.2.4 Determinations of free fatty acid

One gram of well-macerated flesh sample is weighed into a 100 ml beaker. Fifty milllitres of of chloroform are added and stirred for 5 min to ensure complete extraction of fat from the flesh sample. The mixture is filtered through Whatman no. 1 filter paper into a 250 ml conical flask. Then 25 ml of the filtrate is dissolved in 250 ml of mixed neutral solvent (mixture of diethyl ether and alcohol neutralized with 0.1 M NaOH). Then 1 ml of 1% phenolphthalein solution is added and the mixture is titrated against 0.1 M NaOH until a pink colour, which persists for 15 s, is obtained.

$$\text{Free fatty acid (FFA)} = {}^1/_2 \times \frac{\text{Titre value} \times 5.61}{\text{Weight of sample used}}$$

15.6.2.5 Determination of TMA

Two grams of well-ground fish sample is homogenized with 6 ml of 5% trichloroacetic acid); it must be properly homogenized to obtain a uniform slurry. The slurry is filtered into a 50 ml volumetric flask to obtain a clear filtrate. Then 5 ml of the clear filtrate is pipetted into a semi-distillation apparatus to which 5 ml of 2 M NaOH IS added. The moisture is steam distilled in the distillation apparatus into 15 ml of 0.01 M HCl solution in a 50 ml conical flask. One millilitre of 1% resolic acid indicator solution is added to give a bluish colour. This is titrated to give a pale pink endpoint with 0.01 M NaOH to obtain V_2 (the volume standard acid released in the second titration). Note that to every 10 ml of liquid in the titration flask, 1 ml of 16% neuturalized formaldehyde solution should be added.

$$\text{TMA} = \frac{14(300 \times 10) \times V_2 \text{ml}/100\text{ g}}{500}$$

where V_2 ml is the volume standard acid released in the second titration and W is the weight of sample taken.

15.6.3 Bacteriological assessment (microbiological analysis)

15.6.3.1 Preparation of media, serial dilution, pouring of plates and identification

Twenty eight grams of nutrient agar is weighed with an analytical balance into a clean 1 litre beaker, boiled in water to dissolve and distributed into McCartney bottles, which are placed inside an autoclave and sterilized at 121°C for 15 min at a pressure of 1.03 bar. Further, 9 ml of dissolved water is pipetted into a clean test tube, which is plugged with cotton wool and

wrapped with aluminum foil. The tube is placed inside an autoclave and sterilized at 121°C for 15 min at a pressure of 1.03 bar.

Ten grams of sample are weighed into the cooled sterile water in the test tube and allowed to stand for some time to enable the sample to dissolve, with occasional shaking, before serial dilution is carried out. To do this, 1 ml is pipetted from the test tube with a sterile pipette and transferred into a second tube; this is repeated using the second tube until the desired tube dilution factor is reached. One millilitre is then pipetted onto a sterile agar plate and appropriate medium is added, mixed very well and then incubated for 24–48 h at 37°C.

Bacteria are then identified based on shape and colour of the isolated colonies. Further biochemical tests, which include oxidase, indole, urease, mobility, catalase, casein hydrolysis, Voges-Proskauer and methyl red tests, are carried out.

15.7 STUDY OF THREE MARINE FISH SPECIES

Three economically important marine fish species commonly encountered in African regions were studied as a model system for their chemical and microbial qualities. The species were *Pseudotolithus senegalensis*, *Scomber japonicus* and *Sardinella eba*.

P. senegalensis (croaker, ladyfish) is an economically important edible fish that is commonly encountered in coastal waters of West African countries. It feeds on small fishes or shrimps. *S. japonicus* (club mackerel, Pacific mackerel, blue mackerel) is a coastal pelagic species that lives between the surface and about 250 or 300 m. It is a species of high economic importance and has good market value. It is assumed to be an opportunistic feeder and is non-selective in its food habitat. It can feed on small crustaceans, copepods and other fish. *S. eba* (sardine, flat sardine, herring) is commonly found in regions of low salinity and is routinely encountered near river estuaries. This fish does not migrate and is rarely found below 40 m. Sardines possess high commercial value in West African countries.

A study was undertaken to determine the chemical and microbial qualities of these species, and their tendency for spoilage. The study used the methods described above.

15.7.1 Proximate composition of marine fish samples

The initial and final proximate composition of three marine fish species studied under cold storage (−40°C) conditions for 12 weeks is presented in Table 15.1. These species were *P. senegalensis* (croaker), *S. japonicus* (chub mackerel) and *S. eba* (sardine). Croaker had the highest mean moisture content, of 79.60%, while the lowest was recorded in chub mackerel, with 71.81%. Chub mackerel had the highest mean crude protein of 20.50% while sardine had the lowest, with 16.59%. Crude fat was highest in chub mackerel (17.14%), which far exceeded values recorded in croaker (4.35%) and sardine (4.00%). Croaker had the highest crude fibre (4.92%) while the lowest value of 2.60% was recorded in chub mackerel. However, chub mackerel had the highest ash content (3.02%) while the lowest value of 2.50% was recorded in croaker.

The best quality was recorded from organoleptic assessment (chemical analysis is more accurate) in sardines throughout the 12-week storage period with an average score of 1.0–4.0; second was croaker (1.2–4.0) while the poorest storage quality was recorded in chub mackerel with the widest range of 1.8–5.6 (Table 15.2). All the measured parameters increased steadily at least from 0 to 12 weeks; however, some fluctuations were observed in some cases between the eighth and twelfth weeks. The highest hypoxanthine range of

Table 15.1 Initial (week 0) and final (week 12) proximate composition of the three marine fish species studied.

Parameter	*Scomber japonicus* (chub mackerel)		*Pseudotolithus senagalensis* (croaker)		*Sardinella eba* (sardine)	
	Initial	**Final**	**Initial**	**Final**	**Initial**	**Final**
Moisture	69.25	74.36	77.64	81.56	71.11	74.96
Mean	(71.81)		(79.60)		(73.04)	
Crude protein	19.65	21.34	16.32	17.64	15.59	17.59
Mean	(20.50)		(16.98)		(16.59)	
Crude fat	17.38	16.89	4.28	4.41	4.71	3.29
Mean	(17.14)		(4.35)		(4.00)	
Crude fibre	2.78	2.42	5.43	4.41	5.70	2.17
Mean	(2.60)		(4.92)		(3.94)	
Ash	3.18	2.86	2.82	2.18	2.99	1.99
Mean	(3.02)		(2.50)		(2.49)	

25.30–47.94 mg/100 g fish, peroxide value 31.40–44.37 mEq/kg, TMA 22.40–48.14 mg/100 g fish and free fatty acid 1.19–3.75% was recorded for sardines with the lowest ranges for all parameters recorded in croaker, whereas chub mackerel was second to sardine (Table 15.3). This implies that sardine has the greatest tendency for a higher rate of spoilage. These findings are further confirmed by the overall grand total of all indices (parameters measured; see Table 15.3) with sardine having the highest range of 80.29–144.20; second was chub mackerel with 86.00–113.66 and last was croaker with 73.47–96.60.

15.7.2 Results of bacteriological assessment

Sardine had the highest tendency for spoilage with the highest total viable count (TVC) of 98.68×10^5 colony-forming units (CFU)/g and also with the highest number of bacterial species (12) being present (see Table 15.4). Second was chub mackerel with a TVC of 29.36×10^5 CFU/g and 10 bacterial species prevalent. Croaker (which is also a common marine fish species sold in the open market) has the least tendency for spoilage with the lowest TVC of 23.11×10^5 CFU/g and only seven bacterial species prevalent.

However, among the total 12 bacteria species isolated in this study, *L. acidophilus* showed its earliest presence from the basal sample of the freshly killed fish in all three species, and also prevalence was recorded throughout the storage period of 12 weeks in all cases. The presence of *L. acidophilus* from the onset in the freshly killed fish could be attributed to contamination in the course of fish handling (washing, transportation, packaging, etc., and any activity that involves contact with personnel working on board or dirty rough working surfaces).

The most prevalent bacterial species, *Bacillus cereus*, was recorded in sardine (16.30×10^5 CFU/g) in the study throughout the 12-week storage period. However, it was never present in croaker, which further confirms the hardiness of this fish to cold storage and confirms consumer acceptance of croaker. *B. cereus* was only present in week 12 in chub mackerel. Also the second most prevalent bacterium, *L. acidophilus*, with a TVC of 13.38×10^5 CFU/g, and the third most prevalent bacterium, *P. aeruginosa*, with a TVC of 10.29×10^5 CFU/g, were most abundant in sardine, and they were also present in chub mackerel and croaker.

Table 15.2 Score sheet for organoleptic assessment of the fish samples under cold storage (−4°C).

	Mackerel	Croaker	Sardine	Mackerel	Croaker	Sardine	Mackerel	Croaker	Sardine	Mackerel	Croaker	Sardine	Mackerel	Croaker	Sardine	Mackerel	Croaker	Sardine	Mackerel	Croaker	Sardine
Storage period (weeks)	**0**			**2**			**4**			**6**			**8**			**10**			**12**		
Sample	**AB**			**A_1**			**A_2**			**A_3**			**A_4**			**A_5**			**A_6**		
Taste (cooked)	2	1	1	4	2	2	5	2	2	5	2	3	4	3	3	5	4	4	7	5	5
Odour	2	1	1	3	2	2	4	2	2	3	2	2	4	3	3	5	4	4	6	4	4
Texture	2	1	1	2	1	1	3	2	2	3	3	2	3	3	3	4	3	3	4	4	4
Appearance	1	2	1	5	1	1	4	2	2	5	2	2	4	2	2	3	3	2	6	3	3
Colour	2	1	1	3	1	1	4	2	2	4	2	2	4	2	2	4	3	3	5	4	4
Total score	9	6	5	17	7	7	20	10	10	20	11	11	19	13	13	21	17	16	28	20	20
Average score	1.8	1.2	1.0	3.4	1.4	1.4	4.0	2.0	2.0	4.0	2.2	2.2	3.8	2.6	2.6	4.2	3.4	3.2	5.6	4.0	4.0

1, Excellent; 2, very good; 3, good; 4, satisfactory; 5, fairly satisfactory; 6, fair; 7, poor; AB, baseline sample; A_1–A_6, stored samples.

Table 15.3 Biochemical assessment of samples under cold storage (−4°C).

	Storage period (weeks)	Peroxide value (mEq/kg)	TMA (mg/100 g)	Free fatty acid (%)	Hypoxanthine (mg/100 g)	Grand total (all parameters)
Mackerel	**0**	26.40	29.62	1.74	28.24	86.00
Croaker		22.50	24.12	1.53	25.32	73.47
Sardine		31.40	22.40	1.19	25.30	80.29
Mackerel	**2**	27.80	30.10	1.82	29.46	89.18
Croaker		24.20	25.60	1.58	26.42	77.80
Sardine		33.25	23.44	1.32	26.97	84.98
Mackerel	**4**	28.90	31.60	1.88	30.72	93.10
Croaker		25.40	26.50	1.64	27.54	81.08
Sardine		34.95	25.34	1.45	28.89	90.63
Mackerel	**6**	30.10	33.20	1.93	31.86	97.09
Croaker		26.20	27.60	1.72	28.34	83.86
Sardine		38.50	29.14	1.96	33.25	102.85
Mackerel	**8**	30.30	34.60	1.98	32.65	99.53
Croaker		26.40	28.60	1.84	29.26	86.10
Sardine		35.47	37.17	2.70	36.76	112.10
Mackerel	**10**	31.20	36.70	2.14	34.20	104.30
Croaker		28.20	29.90	1.92	30.42	90.44
Sardine		39.49	42.27	2.98	41.17	125.91
Mackerel	**12**	34.60	39.20	2.32	37.54	113.66
Croaker		29.40	31.20	2.16	33.84	96.60
Sardine		44.37	48.14	3.75	47.94	144.20

Table 15.4 Microbiological assessment of fish samples: isolated organisms and their counts under cold storage conditions of −4°C.

	Chub mackerel (5×10^5 CFU/g)					**Croaker ($\times 10^5$ CFU/g)**					**Sardine ($\times 10^5$ CFU/g)**				
Length of storage (4 weeks intervals)	**0**	**4**	**8**	**12**	**Prevalence**	**0**	**4**	**8**	**12**	**Prevalence**	**0**	**4**	**8**	**12**	**Prevalence**
Sample	**AB**	**A_1**	**A_2**	**A_3**		**AB**	**A_1**	**A_2**	**A_3**		**AB**	**A_1**	**A_2**	**A_3**	
Bacillus cereus	–	–	–	2.10	**2.10**	–	–	–	–	–	–	4.84	5.20	6.25	**16.30**
Bacillus licheniformis	–	–	–	–	–	–	–	–	–	–	–	–	–	2.56	**2.56**
Bacillus subtilis	–	–	1.50	0.90	**2.40**	–	–	1.27	2.10	**3.37**	–	–	1.82	3.85	**5.67**
Clostridium welchii	–	1.52	–	1.20	**2.72**	–	1.57	1.42	1.30	**4.29**	–	1.65	2.83	4.22	**8.70**
Eschericha coli	–	0.96	1.00	1.40	**2.36**	–	0.56	0.82	0.80	**2.18**	–	1.86	1.45	4.14	**7.45**
Lactococcus acidophilus	**1.82**	1.62	1.10	1.60	**6.14**	1.64	1.31	1.43	1.20	**5.58**	1.00	3.82	2.96	6.50	**13.38**
Proteus morganii	–	1.24	2.10	1.95	**2.40**	–	–	1.38	1.80	**3.18**	–	1.12	2.72	3.35	**7.19**
Pseudomonas aeruginosa	–	–	1.80	1.70	**3.50**	–	–	1.21	1.70	**2.91**	–	1.95	2.00	6.34	**10.29**
Staphylococcus aureus	–	–	–	1.30	**1.30**	–	–	–	–	–	–	1.00	1.75	4.66	**7.41**
Streptococcus faecium	–	–	–	1.10	**1.10**	–	–	–	–	–	–	–	3.22	5.25	**8.47**
Staphyloccus	–	–	–	–	–	–	–	–	–	–	–	–	2.40	2.50	**2.45**
Micrococcus acidiophilus	–	–	–	1.50	**1.50**	–	–	–	1.60	**1.60**	–	–	1.60	3.86	**2.73**
Total viable count	1.82	5.34	7.50	14.70	**29.36**	1.64	3.44	7.53	10.50	**23.11**	1.00	16.25	27.95	53.48	**98.68**

AB, baseline sample; A_1–A_3, stored samples.

15.8 CONCLUSIONS

Organoleptic assessment of stored fish products is deceptive and does not reflect the state of deterioration of the stored fish post-mortem under cold storage. In this study organoleptic assessment was only suitable for basal samples (AB) of freshly killed fish before cold storage. Chemical assessment (peroxide value, TMA, free fatty acid, hypoxanthine) and bacteriological assessment (TVC and bacterial identification) reflected the true deteriorative state (spoilage) of cold-stored fish products, as shown in this study.

Croaker had the best cold-storage keeping quality with the lowest TVC of 23.11×105 CFU/g, lowest hypoxanthine range of 25.32–33.84 mg/100 g fish (0–12 weeks), free fatty acid of 1.53–2.16% (0–12 weeks), TMA of 24.12–31.20 mg/100 g fish (0–12 weeks) and peroxide value of 22.50–29.40 mEq/kg (Tables 15.3 and 15.4).

From the study, the above ranges can be taken as the minimum and maximum tolerable values for cold-stored marine fish samples in detecting early spoilage in fish post-mortem. The incidence of the bacterium *L. acidophilus* is a result of contamination during handling and the maximum tolerable TVC value should be 5.58×10^5 CFU/g (the total prevalence recorded for croaker).

DEDICATION

This chapter is dedicated to the promotion of healthy growth and well-being of mankind through the consumption of quality fish food products.

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16 Preservation of Cassava (*Manihot esculenta* Crantz): A Major Crop to Nourish People Worldwide

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Abstract: This chapter considers the increase in cassava production and processing into a wide range of traditional and improved cassava-based products worldwide. Toxic cyanogenic glycosides are removed from high cyanogenic cassava cultivars by known techniques prior to further processing. Therefore we also discuss the overall detoxification processes and steps as well as the shelf life and end-use of different cassava-based products.

Keywords: cassava; cassava derivatives; cyanogenic glycosides; processing technologies; storage systems

16.1 INTRODUCTION: CASSAVA PRODUCTION AND IMPORTANCE

Cassava or manioc (*Manihot esculenta* Crantz, Euphorbiaceae) was originally a perennial shrub of the New World. It is an out-breeding species possessing $2n = 36$ chromosomes, and is considered to be amphidiploid or a sequential allopolyploid (El-Sharkawy, 2003). The crop is widely grown as a staple food and animal feed in countries of tropical and sub-tropical Africa, Asia and Latin America between 30°N and 30°S with a total cultivated area of over 13 million ha, more than 70% of it being in Africa and Asia (El-Sharkawy, 1993). Cassava ranks second place followed by sweet potato in the group of roots and tubers, with a world production of 224 131 501 tonnes in 2007 after Irish potato (323 543 199 tonnes) during the same year (Food and Agriculture Organization, FAO, 2008).

The main countries producing cassava are given in Table 16.1. Thus cassava is a very important staple food, nourishing millions of people in many parts of the world. More than half of the cassava produced is used for human consumption.

16.2 NUTRITIONAL VALUE

Even in comparison to food other than root crops cassava counts as one of the most important sources of food. Hence, after rice, sugarcane and maize, is of major importance as a source of carbohydrate for over 500 million people in developing countries of the tropics and

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Table 16.1 Cassava production in Africa, Latin America and Asia in 2007 (FAO, 2008).

Country	Production (Mt)
Angola	8 840 000
Benin	2 662 272
Brazil	26 541 200
Cambodia	6 727 000
Cameroon	2 100 000
Congo-Brazzaville	1 000 000
Congo (Democratic Republic of the Congo)	15 004 430
Cote d'Ivoire	2 342 158
Ghana	9 650 000
Malawi	3 238 943
Mozambique	5 038 623
Nigeria	43 410 000
Peru	1 158 042
Phillipines	1 871 138
Tanzania	6 600 000
Uganda	4 456 000
Vietnam	8 192 800
Zambia	1 100 000
Africa	114 021 873
Latin America	35 354 330
World	224 131 501

sub-tropics. Its main value is in its storage roots, which, when dried, contain more than 80% starch (El-Sarkawy, 2003). Due to a very low protein content in the storage roots (values range among cultivars from 5 to 19 g/kg dry matter, based on an average conservative Kjeldahl nitrogen-to-protein conversion factor of 2.49–3.67; Yeoh and Truong, 1996), human requirements for protein and other essential nutrients are commonly fulfilled by other food sources. In some areas where cassava is grown, young leaves are also harvested and processed for human consumption as a vegetable or as a constituent in a form of sauce eaten along with main staple meals (Lancaster and Brooks, 1983). Cassava leaves have value as a protein supplement (leaf crude protein content on a dry basis ranges among cultivars from 21 to 39%; Ravindra, 1993) for humans (Ndugi *et al.*, 2003) and in animal nutrition, either in feed formulations for monogastric animals or as a fresh forage to supplement low-quality roughage in ruminant feeds (Ravindra, 1993).

Worldwide, cassava occupies sixth place as a source of energy. Cassava produced in Africa is not generally consumed outside the sub-Saharan region where it is produced. About 70% of world cassava root production is used for human consumption either directly after cooking or in processed forms; the remaining 30% is used for animal feed (El-Sarkawy, 2003; FAO, 2008) and to make other industrial products such as starch, glucose and alcohol.

16.3 CASSAVA UTILIZATION

Cassava utilization differs from one region to another; however, the most important is its use in human consumption. In areas where cassava is used directly for human consumption (snacking, simple cooking), particularly in Africa and Latin America, cultivars low in cyanogens (sweet/cool varieties) are used in preference to avoid health hazards. In bitter

cultivars (high cyanogen content), much of the cyanogenic glycosides and their toxic products of degradation are removed from the roots and leaves by using a mix of complex traditional methods and modern technologies during food processing and preparation (Essers, 1995).

As already stated approximately 70% of world cassava root production is used for human consumption (FAO, 2008). In Africa, an average of about 65% of the cassava produced is used for human consumption with approximately 50% used in some form of processed food. In most regions the roots are the most important harvest as compared to the leaves; however, the opposite situation has also been reported (Knoth, 1993). Apart from the use of cassava for human consumption, it is also used for animal feed, representing about 20% of the African production as compared to 30% of the world production (FAO, 2008). Cassava is also used to produce starch for the agro-industry, and for textiles, papers and chemicals. In 2000 the International Food Policy Research Institute (IFPRI) predicted that world cassava utilization will increase from 172.7 million tonnes in 1993 to 275 million tonnes in 2020 (Scott *et al.*, 2000). Overall, cassava utilization in Africa represents about 62% of world production (Westby, 2002).

16.4 FACTORS THAT LIMIT CASSAVA UTILIZATION, AND ITS TOXICITY

There are two main factors that are responsible for limiting cassava utilization. These factors are the high perishability and the toxicity of the product.

Because of its high water content (around 60%), the root is bulky and highly perishable. The root thus undergoes rapid physiological deterioration that starts within 24–48 h of harvest (Beeching *et al.*, 1998), followed by a second stage (after 5–7 days) involving microbial decay. The biochemical processes and histological changes of the first stage are to be classified as post-harvest physiological deterioration or vascular streaking (Buschmann *et al.*, 2000). A discoloration of the vascular tissue to blue-black is the first visible sign of post-harvest physiological deterioration, followed by a browning of the parenchymic tissues. Microscopically, observations of the early processes reveal occlusions in the xylem vessels, as well as the occurrence of tyloses, a thickening of the horny layer of the skin (Rickard and Gahan, 1983). This is followed by a rapid accumulation of fluorescent compounds in the parenchyma; these have been described by different authors and identified as the hydro-coumarins scopolin, esculin and scopoletin (Tanaka *et al*., 1983; Uritani, 1999; Buschmann *et al.*, 2000). The microbial decay is characterized by disintegration and rot of the tissues as a result of fungal (Ikotun, 1989; Gnonlonfin *et al*., 2008a) and bacterial (Ikotun, 1983; Eggleston *et al*., 1992) attack. These deteriorations affect the quality of the roots and consequently that of the processed cassava-based food.

The other principal problem of cassava as a food crop is its toxicity. All varieties of cassava are poisonous because they contain cyanogenic glycosides (Jørgensen *et al*., 2005) such as D-glucose joined by a β-linkage to acetone cyanohydrin (linamarin), which constitutes the major part of the toxins present, and lotaustralin, both found in cassava roots as well as leaves (Obidoa and Obasi, 1991; Brimer, 2000), and 2-((6-*o*-(β-D-apiofuranosyl)-β-D-glucopyranoxyl)oxy)-2-methylbutanenitrile, found only in the cassava root cortex (Prawat *et al*., 1995).

The biosynthesis of the cyanogenic glucosides is shown in Figure 16.1. In general, the cyanogenic glycosides are typically derived from one of the hydrophobic amino acids such as

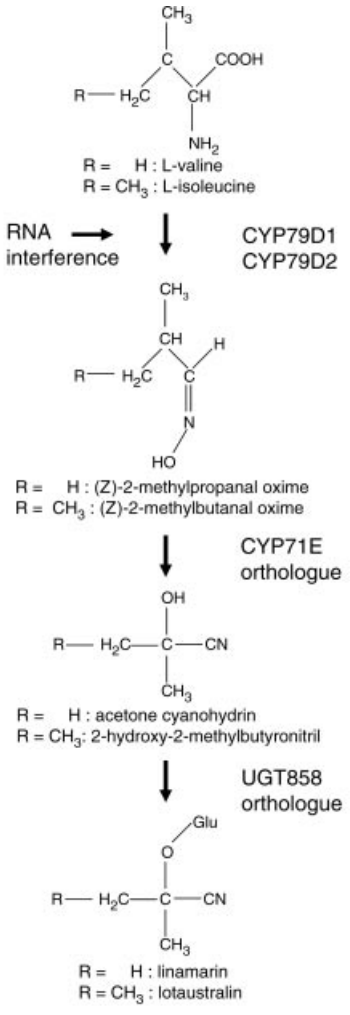

Figure 16.1 Biosynthesis pathway for the cyanogenic glucosides linamarin and lotaustralin in cassava. The enzyme step which has been experimentally blocked by RNA interference technology is indicated (source: Jørgensen *et al.*, 2005).

tyrosine, phenylalanine, valine, leucine or isoleucine. The glycoside is usually a monoglucoside, although there are a number of diglycosides and one triglycoside (Lechtenberg and Nahrstedt, 1999; Jørgensen *et al.*, 2005). The degradation of the cyanogenic glycosides into hydrocyanic acid (HCN; Figure 16.2) is a two-step process. First, the cyanogenic glycoside is cleaved, resulting in the production of a sugar and a cyanohydrin. The cyanohydrin is unstable and is further degraded, either spontaneously or in the presence of an α-hydroxynitrile lyase, to produce hydrogen cyanide and a carbonyl group (Poulton, 1988).

The amounts of these glycosides vary from harmless to lethal depending on the cassava variety, soil fertility status and soil moisture conditions (Obidoa and Obasi, 1991; Brimer, 2000). Depending on variety the concentration of cyanogenic glucosides in the root parenchyma may vary from about 1 ppm to approximately 6–700 ppm (fresh weight basis) when calculated as the total cyanogenic potential; i.e. as mg HCN eq kg^{-1} (El-Tinay *et al.*,

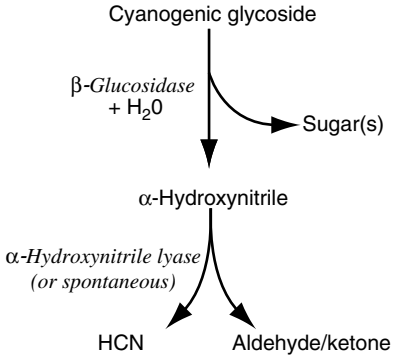

Figure 16.2 Degradation of cyanogenic glycosides into hydrocyanic acid (source: Gleadow and Woodrow, 2002).

1984; Bokanga, 1994a; Chiwona-Karltun *et al*., 2004). Based on the cyanogenic glycoside concentration, cassava plants can be categorized as a bitter variety (high cyanogenic glycoside concentration) or a sweet (cool) variety (low in cyanogens) (Essers, 1995; El-Sharkawy, 2003; Mkumbira *et al*., 2003).

The actual lethal toxic agent is the hydrocyanic acid readily released by β-glycosidase-catalysed enzymatic hydrolysis of the cyanogenic glycoside during processing and digestion (Herbert, 1988; Brimer, 2000) (Figure 16.2). Hydrocyanic acid can also be released by heat during cooking, by the action of mineral acids such as gastric hydrochloric acid and by megadoses of ascorbic acid, especially in the presence of blood (Herbert, 1988). The toxicity may in fact not be entirely due to the cyanogens. This view has been expressed by workers who based their arguments on the fact that a greater proportion of the HCN content in cassava is lost during processing, which is not the case with the coumarins such as scopoletin, the levels of which are not significantly altered during processing (Obidoa and Obasi, 1991).

As already mentioned, not only the roots but also the leaves are used as food and feed. The cyanogenic potential of leaves of the manihot plant is much higher (5–20 times) than that of the roots, but is in general less variable than that of the roots (Bokanga, 1994a; Kolind-Hansen and Brimer, 2010). The high concentrations of cyanogenic glycosides in the leaves do not normally present a problem for their use in food, taking the general methods of preparation in consideration (Bokanga, 1994b).

On several occasions it has been reported that a steady diet of cassava, in addition to causing acute poisoning with vomiting, dizziness and even death (Westby, 2002), also produces various human chronic toxicities. The supposed consequences are, among others, endemic goiter, cretinism and mental retardation (Dorozynski, 1978). Furthermore, chronic intoxication syndromes including, among others, visual loss, tropical ataxic neuropathy and konzo (a central nervous syndrome) have been related to the intake of cyanogens through a cassava-based diet (Howlett, 1994; Osuntokun, 1994; Tylleskar, 1994; Cliff *et al*., 2009).

However, cassava roots, even highly cyanogenic types, are a very valuable and irreplaceable crop. Small farmers have shown a preference for, and in more recent decades even increased use of, more highly cyanogenic cultivars (Brimer, 2000). A clear preference for *Kii* (a toxic variety) for most purposes has been reported in India (Dufour, 1993), while planting of some bitter cultivars, such as *New Guinea* and *Beqa*, was reported to be increasing by Aalbersberg and Limalevu (1991) in Fiji and Tonga (see also New Zealand Institute for Crop and Food Research, 2006).

Based on the data available for the toxicity of cyanide and cyanogenic glycosides, the Joint FAO/WHO Expert Committee on Food Additives (1993) tried to propose regulations concerning the safe level for the intake of cyanogenic glycosides for humans. However, the committee could only conclude that because of a lack of quantitative toxicological and epidemiological information, a safe level of intake for cyanogenic glycosides could not be estimated. However, the committee concluded that a level of up to 10 mg of hydrogen cyanide/kg of product in the Codex Standard for Cassava Flour (approved by the Codex Alimentarius Commission in 1991) is not associated with acute toxicity (Siegers *et al*., 1993). The European Union regulation of HCN in manioc by-products intended for animal feed is 100 mg/kg relative to foodstuff with a moisture content of 12% (European Food Safety Authority, 2007).

Referring to the above constraints, processing is required to ensure stability and preservation. This permits enhancement of product value by removing the naturally occurring toxins found in the root, and at the same time improves the sensory and organoleptic properties; reduces product weight, thereby facilitating its transportation to markets; lessens post-harvest losses arising from breakage of the roots; and extends the product's shelf life.

16.5 CASSAVA PROCESSING

The processing of cassava into more storable forms offers an opportunity to overcome the perishability of the fresh produce. The processing also helps to improve the sanitary and organoleptic properties of the derived products. A range of traditional processing techniques has been developed in cassava-producing countries around the world. Overall, these processing methods integrate the unit operations such as peeling, washing, slicing, grating, fermentation and drying. A number of processed cassava-based foods exist and these serve as a staple food, especially for rural populations.

16.5.1 Description of some cassava-based products

A number of cassava-based products, such as fresh cassava, cassava flour, sour starch, cassava starch, tapioca (*sago*), *gari*, *fufu*, *lafun* and chips, are the main foods processed in different regions in Africa and in different locations of the individual countries according to the food preference of the ethnic group.

16.5.1.1 Fresh cassava

Fresh cassava is common product encountered in cassava-producing countries. In Brazil, fresh cassava is available as frozen cassava chips and ready-to-use doughs. It is sold on the local markets for direct consumption (Chuzel, 2001). In Benin, in rainy season, fresh cassava are peeled and boiled before consumption (Padonou, 2006).

16.5.1.2 Cassava flour

Cassava flour is the main cassava-based product in Brazil. Its local name is *farinha*, which varies widely in appearance (particle size, colour) and flavour (based on crunchiness, whether or not it has been roasted, amount of fermentation, cooking level) (Chuzel, 2001). It is also available in Benin, Cameroon, Nigeria and Togo, among other places. However, the

procedure does involve the same stages: washing, stripping, peeling (in the most rustic production units), grating, pressing and sieving, cooking or roasting and possibly grinding and sieving. It is mostly used in baking for bread making and for making other types of snack (Hahn, 1988).

16.5.1.3 Sour starch

Sour starch is a typically Latin American product, found in Colombia, Ecuador, Bolivia, Paraguay, northern Argentina (*almidon agrio*) and Brazil (*polvilho azedo*). It is cassava starch extracted by the wet method, naturally fermented in tanks for 3–6 weeks and dried in the sun. Apart from being sold for direct household consumption, sour starch is a commodity used in secondary processing industries and bakeries, cake shops and cafes. In large urban centres, cheese breads (in Brazil: *pao de queijo*, *biscoito*) are popularly eaten as an accompaniment to coffee, as a snack food or as treats during the day. Numerous fast-food outlets have sprung up alongside traditional shops, specializing in cheese breads, often in the form of franchise networks targeting the middle and upper classes. In addition, new products based on sour starch (frozen dough, instant mixes, etc.), are marketed to counter the problems of availability facing urban consumers (Chuzel, 2001).

16.5.1.4 Cassava starch

Cassava starch is mostly found in the Latin American region. It is used in the food, meat, paper and textile industries.

16.5.1.5 Tapioca

Tapioca, called *sago* in Brazil and often called *tapioca* in many African countries, is a rolled, pre-cooked cassava starch. It has a 'pearl-like' appearance and is used for desserts and baby foods.

16.5.1.6 Gari

Fresh roots are peeled and grated. The grated pulp is put in sacks (jute or polypropylene) and the sacks are placed under heavy stones or pressed with a hydraulic jack between wooden platforms for 3–4 days to express the excess liquid from the pulp while it is fermenting. The lactic acid bacteria *Corynebacterium manihot* and *Goetrichum candidum* are the two microorganisms which are most involved in the fermentation process (Balagopalan and Padmaja, 1988; Moorthy and Mathew, 1998). Fermentation imparts an acidic taste to the final product. The dewatered and fermented lumps of pulp are crumbled by hand and most of the fibrous matter is removed. The remaining mass is sieved with traditional sieves (made of woven splinters of cane) or iron or polyethylene mesh. After being sieved, the fine pulp is then roasted in an iron pan or earthen pot over a fire. If the sieved pulp is too wet, it takes longer to roast, resulting in a finished lumpy product with dull colour. Palm oil may be added to prevent the pulp from burning during roasting and to give a light yellow colour to the *gari*. When palm oil is not added, a white *gari* is produced. Palm oil contains substantial quantities of vitamin A; therefore, yellow *gari* is 10–30% more nutritious and expensive than white *gari*. The garification or conversion rate of fresh roots into *gari* is 15–20% (Balagopalan and Padmaja, 1988); that is, 100 g of fresh root makes 15–20 g of *gari*. This value varies with cassava

varieties, time of harvesting, age of plant and other environmental factors. *Gari* is very popular in Nigeria and less so in Cameroon, Benin, Togo, Ghana, Liberia and Sierra Leone. In Brazil, this method is used for the production of *farinha de madioca*.

16.5.1.7 Fermented and dried cassava pulp

Lafun in Nigeria and Benin, *cossettes* in the Democratic Republic of the Congo and Rwanda, *kanyanga* and *mapanga* in Malawi and *makopa* in Tanzania are various names for fermented and dried cassava products. The processing method to ferment and dry cassava pulp is very simple and does not require much labor. It is thus widely used for processing high-cyanide cassava varieties in many parts of Africa where water for soaking is available. Whole or peeled roots are immersed in water for 3–4 days for fermentation and to soften the tissues. The fermenting roots are then removed and broken into small crumbs, sun-dried on mats, racks, flat rocks, cement floors or roofs of houses. Drying the fermented roots takes 1–3 days, depending on the prevailing weather. The dried crumbs are then milled into flour (Balagopalan and Padmaja, 1988; Padonou, 2006).

16.5.1.8 Wet pulp

The processing procedures for 'wet pulp' and of fermented and dried pulp production are similar except for the drying. The wet pulp may be molded into balls, 3–5 cm in diameter, put into boiling water and stirred thoroughly to obtain a stiff wet pulp. This pulp is packed in quantities of about 0.5–1 kg in plastic or polypropylene bags and marketed in cities in Nigeria, Ghana and Cameroon (Hahn, 1988).

16.5.1.9 Smoked cassava balls (kumkum)

Cassava is processed into smoked cassava balls in the same way as fermented and dried pulp is produced except that the fermented wet pulp is pounded and molded into round balls of about 4–7 cm in diameter. These balls are then smoked and dried on a platform above a fireplace in a special structure hung above the hearth. The dark coating caused by the smoke is cleaned off and the cleaned balls are milled into flour before reconstitution into *fufu* (Numfor and Ay, 1987).

16.5.1.10 Fufu

Peeled roots after cooking by steaming or boiling are pounded and the sticky dough eaten with soups made out of fish or vegetables. This is common in West Africa, particularly in Ghana (Balagopalan and Padmaja, 1988) and Nigeria (Oyewole and Odunfa, 1990).

16.5.1.11 Chickwangue

Chickwangue is the most popular cassava-based processed food in Democratic Republic of the Congo. *Myondo* and *bobolo* in Cameroon belong to this *chickwangue* group of foods. Similar products are produced in Congo, Central African Republic, Sudan, Gabon and Angola (Numfor and Ay, 1987).

Cassava roots are peeled, and steeped in water for 3–5 days to ferment and become soft. The fermented pulp is taken out and the flakes are removed from it. The pulp is then heaped

onto racks for further fermentation or the heap is covered with leaves and pressed with heavy objects to drain off the excess liquid. The pulp is then ground on a stone or pounded in a mortar to obtain a finer pulp. The fine pulp is wrapped in leaves of plantain or any plant of the Zingiberaceae family and tied firmly with fibres from banana. These are steamed in pots. *Chickwangue* is about 10 cm wide and 20 cm long. *Myondo* has a diameter of 1.5–2.0 cm and a length of 15–20 cm. *Bobolo* has a diameter of 2–4 cm and a length of 30–40 cm. The Gabon *chickwangue* is smaller in size than that from Democratic Republic of the Congo (Hahn, 1988).

16.5.1.12 Dried cassava

The roots are peeled, sliced into small pieces and sun-dried on racks or roofs for 4–5 days or sometimes up to 3 weeks, depending on the weather and the size of pieces (Hodges *et al*., 1985; Oyewole, 1992; Stumpf, 1998; Gnonlonfin *et al*., 2008b). Later, sun-dried pieces are milled into flour. This processing system is very simple. The method is widely used in many areas of Africa, particularly where the supply of water for fermentation is seriously limited. In parts of India, the chipped cassava is parboiled to gelatinize the texture before drying (Rajamma *et al*., 1994).

16.6 STORAGE OF PROCESSED CASSAVA PRODUCTS

Processing, particularly drying and roasting, increases the shelf life of cassava products. Good storage depends on the moisture content of the products and temperature and relative humidity of the storage environment (Magan and Aldred, 2007). The moisture content of *gari* for safe storage is below 12.7%. When temperature and relative humidity are above 27°C and 70% respectively, the *gari* goes bad (Igbeka, 1987). The type of bag used for packing also affects shelf life, depending on the ability of the material to maintain safe product moisture levels.

Jute and hessian bags are recommended in dry cool environments because they allow good ventilation (Igbeka, 1987). When *gari*, dried pulp and flour are well dried and properly packed, they can be stored without loss of quality for over 1 year. Dried cassava balls (*kumkum*) can be stored for up to 2 years (Numfor and Ay, 1987). *Chickwangue*, *myondo* and *bobolo* can be preserved for up to 1 week but they can be kept for several more days when recooked. Dried cassava can be stored for up to 3 or even 6 months if well packed (Gnonlonfin *et al*., 2008b).

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17 Use of Electron Beams in Food Preservation

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Abstract: Electron-beam irradiation (EBI) is a novel, non-thermal, physical method of food preservation (processing) technology which is effective in achieving microbial decontamination, insect disinfestation and shelf-life improvement of various food- and agriculture-based commodities. This technology is economical and environmental friendly and holds several advantages over other sources of food irradiation and conventional preservation techniques. Based on the available scientific reports, EBI could prove to be a potential alternative to the current chemical fumigants used for preservation purposes. Reports available have clearly indicated the effectiveness of employing electron beams in preserving the overall qualities and extending the shelf life of various fruits, vegetables, cereals, legumes, poultry, meat and seafoods. EBI can be highly effective when combined with other conventional and non-conventional food-processing technologies. Being a recently explored technology, there are still wide gaps prevailing in the body of research that need to be filled to provide appropriate scientific evidence. In the present chapter we aim to highlight a few vital reports on the use of EBI in various food commodities and emphasize certain vital areas which need to be explored in the near future for commercialization purposes and to overcome quarantine barriers of international trade. It is expected that this novel technology will benefit both processors and consumers in the near future.

Keywords: consumers; decontamination; dose; electron beams; food safety; ionizing radiation; pathogens; radiation processing

17.1 INTRODUCTION

Food contamination is a recurring problem all over the world and causes heavy economic losses. According to the Food and Agriculture Organization (FAO), an annual loss of nearly 25% of the world's food supply has been estimated to occur by food contamination (Sánchez-Bel *et al.*, 2008; Bhat *et al.*, 2010a), primarily attributed to microbial contamination, improper handling and the storage conditions employed. Additionally, insect infestation of agricultural produce during storage has become a major problem despite quarantine rules and strict regulations in the world market. Today, with increased knowledge and available databases on food safety, consumers are increasingly becoming aware of high-quality foods that are safe for consumption and free of any types of contaminants.

The expected ban on use of chemical fumigants (such as methyl bromide, ethylene dioxide) by the year 2015 for food preservation in developing countries, and the imposition of

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stringent laws by the world health governing bodies, have led researchers as well as governments and non-governmental agencies to explore the possibilities of applying physical, non-thermal and non-conventional alternative methods of food preservation (Anonymous, 1995; Wilkinson and Gould, 1998; Bhat, 2007; Bhat and Sridhar, 2008). There are several chemical-based food preservatives such as benzoic acid, butylated hydroxyanisole, potassium nitrate, sulphur dioxide, propyl *p*-hydroxybenozoate, propylparaben and paraben, which need to be avoided (see http://altmedangel.com/additive.htm). In this regard, the application of 'food irradiation' technology was recommended when the Joint Expert Committee on Irradiation (the FAO/World Health Organization (WHO)/International Atomic Energy Agency (IAEA)) declared that 'irradiation of food up to an overall dose of 10 kGy is safe and [such food] possess[es] no toxicological hazards with minimal compromise on the nutritional quality' (World Health Organization, 1981; Codex Alimentarius Commission, 2003). A series of conventions undertaken by the FAO/IAEA/WHO Study Group on the applications of high-dose irradiation concluded that food irradiation at dose levels ranging from 25 to 60 kGy employed to achieve the desired safety criteria does not pose any potential health risks, and that the irradiated foods are wholesome and safe for consumption, and are nutritionally and organoleptically acceptable (World Health Organization, 1994, 1999). Radiation processing has contributed substantially to enhancing food safety in developing countries by reduction of economic losses (Loaharanu, 1994). Bruhn (1998) has suggested that, given the opportunity, consumers will surely buy high-quality, safety-enhanced irradiated foods.

Although radiation processing of food is considered to be a new technology, historical evidence indicates research being effectively conducted during the early twentieth century, wherein the first US and British patents were issued (in 1905) for decontamination purposes. In the present chapter, we have discussed various aspects relevant to electron-beam (e-beam) radiation technology and certain important breakthroughs relevant to the effects of e-beam irradiation (EBI) on various food products. Some of the existing gaps in the research that need to be filled in the near future for commercial applications and for the benefit of consumers have also been discussed.

17.2 FOOD IRRADIATION, SOURCE AND TECHNOLOGY

Food irradiation involves exposure of food or agricultural commodities to ionizing energy in the form of gamma (γ) rays (cobalt-60 and caesium-137) or machine-generated X-rays (maximum 5 mega-electron volts, MeV) and high-energy accelerated electrons (8–10 MeV). Generally, the radiation exposure is measured as dose and expressed either as Grays or in kiloGrays (kGy) (1 kGy = 1000 kJ or megarads (MR); 1 MR = 1 000 000 ergs/g). The delivered dose of radiation varies depending on the food product or the raw material or purpose.

Food-irradiation technology (radiation processing) has been successfully employed across the world for commercial purposes (for at least one food product) in nearly 40 countries (IAEA, 1995; Loaharanu, 1996; Bhat, 2007). Food irradiation has been approved by the health and safety authorities for over 60 different types of foods, including spices, grains, meat, fruits and vegetables. The dose of radiation falls into three categories: low-dose treatments (<1 kGy) for disinfestation purposes (e.g. spices, grains, dry fruits), delay in ripening of fruits (e.g. banana) and for sprout inhibition (e.g. potatoes, ginger, garlic, onions); medium-dose treatments (1–10 kGy) for microbial decontamination and shelf-life extension (e.g. spices, spice powder, coffee beans, fruits and vegetables and their products, seafood,

poultry); and high-dose treatments (10–60 kGy), used to irradiate foods for immunocompromised/immunosuppressed patients and astronauts.

Even though the application of γ rays has been a well-accepted and established technology worldwide, some skepticism still exists among consumers on the use γ-irradiated foods. However, development of an electron accelerator during 1930s, which later led to the breakthrough research of Cleland during the late 1950s, resulted in more sophisticated and economical accelerators that could be used for food irradiation purposes. In 1957 in Germany there was the first commercial application of EBI of spices, which was a stepping stone for this novel technology. In an electron accelerator, a stream of electrons from a heated cathode is accelerated through an evacuated tube between cathode and anode with an energy input varying between 4 and 12 MeV. The number of mega-electron volts might vary depending on the accelerator used. To date, various e-beam accelerators have been developed and designed with varying beam power (10–200 kW), which includes cyclotrons, the Dynamitron, microtrons, linear particle accelerators (or linacs) and the Rhodotron. The exposure to radiation can be direct, wherein samples are placed in front of e-beams (like in a microtron) or the samples are placed on conveyor belts and irradiation is carried out just as with γ irradiation. However, unlike in γ irradiation, the speed of the conveyor belt can be automatically altered corresponding to the changes occurring in the beam current during processing.

The penetration of electrons in irradiated materials is directly dependent on the kinetic energy, while the processing rate increases with beam power. The electron accelerators used in industrial applications are classified based on their energy ranges, which includes: low-energy range (80–300 keV), medium-energy range (300 keV to 5 MeV), and high-energy range (>5 MeV) (Cleland and Parks, 2003). Electron accelerators found instant success compared to γ rays owing to many advantages, such as: the source (machine), whereby irradiation can be stopped at any moment; being non-nuclear, whereby accelerated generation of radiation is available as and when required; the absence of any radioactive materials to be used or transported; no risks of occupational hazards; manipulation of beam energy can be done to suit the size of the irradiation package; and being applicable for high-throughput and high-dose irradiation applications. Above all, electron accelerators are more economical than previous technologies, simple to operate and faster, and they are an effective method of cold pasteurization. Additionally, they are environmentally friendly and require shielding only during the irradiation process.

Controlling the absorbed dose as well as delivering a uniform radiation treatment in a food product is important, especially for fruits and vegetables, where it is very important to attain effective microbial decontamination, disinfestation and shelf-life extension (Bresica *et al.*, 2003). In a classical report by Bresica *et al.* (2003), Monte Carlo simulation was used to determine the dose distribution at the surface of an apple irradiated with e-beams generated by a Van de Graaf accelerator (1–2 MeV). The dose distribution obtained was employed to develop the best suitable irradiation angle while rotating an irregularly shaped food material (such as an apple) for uniform surface irradiation. Recently, Rivadeneira *et al.* (2007) reported on the dose mapping of complex-shaped foods using e-beam accelerators, the results of which supported the validity of using chemical surrogate techniques (to represent a complex-shaped food) for accurate planning of food irradiation treatments, especially concerning uniformity for e-beam treatments.

Recently, electron accelerators have found applicability in food industries (mainly for decontamination and disinfection purposes), in pharmaceutical industries (for sterilization of disposable medical instruments), in hospitals and medical applications (for radiotherapy,

brachytherapy), in chemical industries (for cross-linking, and polymerization of polyethylene and polypropylene) and environmental applications in various industries (disinfection of sewage sludge, monitoring and control of pollution). However, the major disadvantage of using electron accelerators is the requirement of a continuous power supply during the controlled radiation processing, which might pose a problem in developing countries where a continuous power supply might not be available. Low penetrability of e-beams also poses a problem. Also, for EBI the size, thickness, direction (single-sided or double-sided exposure) and packaging assumes importance: EBI is more effective in low-density and uniformly packed food products. As with any other preservation technology, radiation treatments are not capable of reversing the physiological and chemical processes involved in decay (Henson, 1995). Negative perceptions by consumers on the use of the terms 'radiation', 'radioactivity' and 'safety' (Osterholm and Norgan, 2004) have been a matter of concern. However, with the available means of knowledge dissemination such as databases and reports, this misconception has to some extent been cleared.

The present chapter is an attempt to highlight the importance of this novel technology (using e-beam accelerators) in food industry applications and the future scope for the technology as an alternative for conventional food disinfection, including fumigation. Even though several book chapters and excellent reviews have appeared in the field of food irradiation, only a few comprehensive research papers and reviews are available on the application of electron accelerators in the food industry. We have attempted to highlight some of the important research work employing electron accelerators in the food and agro-based sectors.

17.3 THE FOOD INDUSTRY AND ELECTRON-BEAM IRRADIATION

Even though the main purpose of food irradiation is to achieve microbial safety, reports available have shown its effectiveness in improving some quality attributes, such as nutrition, and in enhancing bioactive compounds. In the food industry, ionizing radiation has been successfully employed to attain microbial decontamination and insect disinfestation, for sterilization and hygienic purposes, and for improving shelf life of perishable products. Application of radiation treatments for food and agricultural commodities is mainly focused towards overcoming barriers in international trade as well as for consumer safety. Although several reports are available on the successful utilization of γ rays, scientific reports on the potential applications of e-beams still remain in the infant stage. In Table 17.1 and Table 17.2 we have summarized some of the important results reported in the past few years on the application of EBI in food products.

17.3.1 Fruits and vegetables

There is increasing consumer awareness, supported by the widely available supporting epidemiological data, of the rich health benefits associated with the consumption of fresh fruits, vegetables or their products (Bernal *et al.*, 2011; Key, 2011). This has led consumers to prefer a more 'natural' fruit- and vegetable-based diet along with ready-to-eat food products, which are considered to be safe and of better quality. Even though plant produce might seem to be fresh, it might be contaminated by various spoilage and pathogenic microorganisms during the pre- or post-harvest stages. Some recent cases from all over the world on the outbreaks of foodborne illnesses are related to fruits, vegetables or their products (Beuchat,

Table 17.1 Some selected reports on the application of e-beams in fruits, vegetables and other plant products.

Product	Purpose	Dose	Observation	Reference
Almond (*Prunus amygdalus*)	To evaluate the oil and sensory qualities during storage (up to 5 months)	Delivered dose of EBI: 0, 3, 7 and 10 kGy EBI was carried out using a Rhodotron circular electron accelerator at an energy level of 10 MeV	The fatty acid linolenic acid (18:3) showed (at 3 kGy) retention of the initial content during the whole storage period. However, at 7 and 10 kGy the content disappeared on irradiation. The quality indices of the oils (K232, K270) decreased at all the delivered dose and were stable during storage. The peroxide value significantly increased at 10 kGy. No differences in the sensory quality were recorded between the control and irradiated samples (at 3 or 7 kGy). However, at 10 kGy a rancid flavour with a significant decrease in the overall quality was observed.	Sánchez-Bel *et al.* (2005)
Almond (shelled almond variety Guara)	To study the effects of EBI on chemical composition (water content, proteins, neutral detergent fibre, sugars, lipid content, organic acids, colour) and sensorial properties (rancidity, sweetness, off-flavours and odours, texture, whiteness) of packed almonds in air atmosphere and stored for 5 months	Irradiation with accelerated electrons (dose delivered: 0, 3, 7 and 10 kGy) Rhodotron circular electron accelerator at an energy level of 10 MeV	Glucose content decreased in all irradiated samples, while citric acid content increased at doses >3 kGy. No effect was observed for sensorial qualities (sweetness, texture or colour). Rancidity occurred in samples treated with 10 kGy. Overall, irradiation up to 7 kGy was suitable for post-harvest sanitation treatment as no significant changes occurred in the sensorial quality or in the contents of protein, fibre, water or lipid compared to control samples (up to 5 months of storage).	Sánchez-Bel *et al.* (2008)
Almond skin	To study the effect of EBI on almond skin phenolic extracts	Dose delivered: range between 0 to 30 kGy	EBI increased the extraction yield by 23%. Irradiated samples extracted with acidified methanol showed enhancement in the extractable phenols at all the doses. Samples extracted with 52% methanol showed increase at 10 and 20 kGy. However,	Teets *et al.* (2009)

(*Continued*)

Table 17.1 (*Continued*)

Product	Purpose	Dose	Observation	Reference
			30 kGy revealed a decrease by 31%. Any enhancement in aglycones respective to their glycosides was not recorded after irradiation.	
Apricots (*Prunus armeniaca*, cv Búlida)	To extend shelf life after harvest	Dose delivered: 0.5 and 1.0 kGy Circular electron accelerator, energy level 10 MeV	Irradiation resulted in decrease in the ethylene concentration in the climacteric peak. Irradiation at 0.5 or 1.0 kGy was not effective in extending the shelf life as no significant differences between control and treated apricots were recorded during storage. Overall, EBI treatment was not effective for extending the shelf life.	Egea *et al.* (2007)
Barley (infected with *Fusarium* fungi)	To prevent *Fusarium* growth and mycotoxin production along with maintaining characteristic malt quality	Delivered dose: 0, 2, 4, 6, 8 and 10 kGy	The aerobic plate counts, mould and yeast counts for malts obtained from barley exposed to 8–10 kGy were slightly higher than other malted samples. The germinative energy of barley decreased significantly (3–15%) at 8–10 kGy. EBI did not have any effect on the mycotoxin deoxynivalenol (DON) in raw barley. However, DON decreased significantly (60–100%) in finished malts prepared from treated barley (6–10 kGy). From the results, it was concluded that 6–8 kGy is effective for reducing *Fusarium* infection in barley, and DON in malt with minimal effects on malt quality.	Kottapalli *et al.* (2006)
Blueberries (*Vaccinium corymbosum* L.)	To study the effects of EBI on packaged fresh blueberries at doses of >1.0 kGy Also, fruits were stored at 5°C at 70.4% relative humidity for 14 days and tested after 0, 3, 7 and 14 days for physicochemical,	Dose delivered: 1.0–3.2 kGy using a 10 MeV (18 kW) linear accelerator with single-beam fixture	Fruits exposed to 3.2 kGy were found unacceptable by the sensory panelists (for texture, colour and aroma). However, irradiation dose levels 1.0–3.2 kGy did not affect the density, pH, water activity, moisture content, acidity and juiciness of blueberries. EBI of blueberries up to 1.6 kGy was found to be	Moreno *et al.* (2007)

	textural, microstructural and sensory characteristics		optimal for decontamination along with maintaining overall fruit qualities.	
Broccoli seeds and broccoli sprouts	Comparison between e-beam and γ rays at doses up to 8 kGy	Varying doses (1–8 kGy)	E-beams and γ radiation had similar effects on the viability and functional properties of sprouts.	Waje *et al.* (2009)
Broccoli heads (*Brassica oleracea* L. *italica*)	Irradiation for improving shelf life, physicochemical properties and consumer acceptability	Delivered dose: 1, 2 and 3 kGy using a 10 MeV linear accelerator (at 22°C)	EBI affected the respiration rates on the first 5 days of storage. Vitamin C, chlorophyll and total carotenoids content decreased with storage time. In terms of overall acceptability: the colour, odour and texture of the irradiated samples were highly acceptable by the panelists with scores >5. EBI treatments up to 3 kGy maintained the overall quality of fresh broccoli.	Gomes *et al.* (2008a)
Cabbage (fresh-cut cabbage pre-inoculated with *Escherichia coli* K-12)	For microbial decontamination	Dose delivered: 0, 1.0, 2.3 or 4.0 kGy	At 2.3 kGy, <1.0 log microflora were detected, which was a >4.0 log reduction. At 4.0 kGy, >7 log reduction of *E. coli* K-12 occurred with the 90% reduction value (D_{10} value) for *E. coli* K-12 in fresh-cut cabbage being 0.564 kGy.	Grasso *et al.* (2011)
Cantaloupes (fresh cut)	To evaluate the effects of EBI on respiration, microbial quality and on colour and texture	Dose delivered: 0, 0.25, 0.5, 0.75, 1.0, 1.25 or 1.5 kGy/ 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 or 0.7 kGy and 0, 0.3, 0.6 or 0.9 kGy, respectively from e-beam accelerator with 90 mega-amps and 95% scan capacity	Increase in respiration was observed in irradiated samples with reduction in total plate counts occurring during storage in irradiated samples. EBI maintained firmness even after 7 days, while colour of non-irradiated and irradiated samples were retained.	Boyton *et al.* (2005)
Cantaloupe (*Cucumis melo*)	For microbial decontamination and for optimization of EBI treatment using dose distributions from Monte Carlo simulation	A 10 MeV e-beam linear accelerator (linac) and a lower energy source (Van der Graaf, 1.35 MeV) was used at a dose level of 1 kGy).	EBI of fresh-cut cantaloupe showed that the maximum dose of 1.1 kGy occurred at the 3.5 cm depth and the minimum dose of 0.81 kGy took place at 5.5 cm depth, with an average of 2.99 log reduction around the whole package.	Kim *et al.* (2010a)

(*Continued*)

Table 17.1 (*Continued*)

Product	Purpose	Dose	Observation	Reference
	and computed tomography (CT) scan data			
Grapefruits (*Citrus paradisi* Macf.), Rio Red and Marsh White varieties	To study the effect of EBI on bioactive compounds	Dose delivered: 0, 1.0, 2.5, 5.0 and 10.0 kGy using two separate 10 MeV linac accelerators	Low-dose irradiation had insignificant effects on bioactive compounds. Irradiation at >1 kGy significantly reduced the vitamin C content. Lycopene and β-carotene were not affected significantly on irradiation. Lycopene levels decreased with increase in dose, while naringin, a major flavonoid of grapefruit, significantly increased at 10 kGy (increase by 18.90 and 15.37% in Rio Red and Marsh White, respectively). At 1 kGy, nomilin decreased by 7.19 and 4.27%, whereas at 10 kGy it was significantly reduced by 19.02 and 21.56% in Rio Red and Marsh White, respectively. Limonin levels remained the same at all of the doses.	Girennavar *et al.* (2008)
Korean ginseng and red ginseng (*Panax ginseng* C.A. Meyer)	To study the effect of EBI on microbial growth and qualities of vacuum-packaged during storage	Dose delivered: 0, 2, 8 and 16 kGy	Populations of total bacteria, yeasts, moulds and total coliforms were decreased by 2–3 log CFU/g on irradiation. Thiobarbituric acid reactive substance values of the samples were enhanced during storage. No significant changes were observed in colour and odour and in Hunter's colour L, a and b values in the irradiated samples. Saponin, a leading compound in ginseng, was not affected by irradiation, even during storage.	Jin *et al.* (2007)
Lotus seed flour (*Nelumbo nucifera*)	To evaluate the effects of EBI on the nutritional and antinutritional qualities,	Dose delivered: 0, 2.5, 5, 7.5, 10, 15 and 30 kGy from a microtron source	A significant dose-dependent decrease in water absorption capacity with increase in oil-absorption capacity >10 kGy was observed. Irradiation resulted in significant increase in protein	Bhat and Sridhar (2008)

	and on functional properties		solubility (>5 kGy) and foaming capacity (>7.5 kGy). Least gelation capacity was improved at doses of 5 kGy and above. EBI showed significant enhancement in total phenolics and tannins, while phytic acid was eliminated at 5 kGy.	
Lotus seed	Comparison between γ irradiation and EBI for microbial decontamination	Dose delivered: 0, 2.5, 5, 7.5, 10, 15 and 30 kGy from a ^{60}Co source and from a microtron source (for e-beams)	Most of the bacteria and fungi could be reduced/eliminated at doses of 7.5 kGy and above. The contaminant yeasts could survive up to 10 kGy and could be completely eliminated at 15 kGy.	Bhat *et al.* (2010b)
Mango fruit (*Mangifera indica* L.)	To study irradiation effects on antioxidant constituents before and during post-harvest storage	A dose range of 1–3.1 kGy was delivered from a 10 MeV e-beam linear accelerator	Increase in flavonols occurred after 18 days of storage (3.1 kGy). Total phenolics and antioxidant capacity (ORAC) were not affected, while vitamin C decreased by 50–54% during storage in doses of >1.5 kGy. A delay in ripening occurred in irradiated mangoes at all the dose compared to control.	Reyes and Cisneros-Zevallos (2007)
Mucuna pruriens seeds (an underutilized, nutraceutically valued seed)	To study the effects of EBI on composition and functional properties	Doses delivered: 2.5, 5, 7.5, 10, 15 and 30 kGy from a microtron source	EBI significantly increased crude protein, *in vitro* protein digestibility and carbohydrates, while no significant changes occurred in amino acids even after 30 kGy of irradiation. Linoleic acid showed their presence increase after 10 kGy treatments (0 kGy 14.35 mg/g lipid). Water, oil and foaming capacities significantly increased on irradiation, while protein solubility decreased at 15 and 30 kGy. Gelation property increased while cooking time of seeds significantly reduced from 22 min up to 14 min at 30 kGy).	Bhat *et al.* (2008)
Peanut butter	To evaluate the effectiveness of EBI for reduction of *Salmonella* serovars Tennessee (ATCC 10722)	Dose delivered: ranged from 0 to 3.1 kGy	*Salmonella tennessee* was highly susceptible to EBI, with 5.00 and 6.75 log reduction of cells on TSA and XLD agars, respectively, at an approximate beam dose of 3.0 kGy.	Hvizdzak *et al.* (2010)

(*Continued*)

Table 17.1 (*Continued*)

Product	Purpose	Dose	Observation	Reference
	and Typhimurium (ATCC 14028)		*Salmonella Typhimurium* was reduced by 4.19 and 4.85 log on TSA and XLD agars, respectively, at the approximate beam dose of 3.0 kGy. The D_{10} values revealed *Salmonella Typhimurium* to be more resistant (0.82 and 0.73 kGy on TSA and XLD agars, respectively) compared to *Salmonella tennessee* (0.72 and 0.60 kGy on TSA and XLD agars, respectively).	
Pecan kernels (*Carya illinoinensis*)	To evaluate the effects of EBI on phytochemical constituents and antioxidant capacity during storage at 40°C, relative humidity 55–60% for 134 days	Dose delivered: 0, 1.5 and 3.0 kGy using a single e-beam linear accelerator	EBI did not have any major effects on antioxidants and colour. However, reduction in total phenolics and condensed tannins occurred. A certain level of lipid peroxidation was induced by EBI.	Villarreal-Lozoya *et al.* (2009)
Plant essential oils (*Thymus vulgaris thymoliferum*, *Eucalyptus radiata* and *Lavandula angustifolia*)	Comparing the effects of γ and e-beam radiations	25 kGy γ source: ^{60}Co chamber The e-beam facility used was a double-beam linear electron accelerator (linac)	Neither γ nor EBI induced any significant changes in the contents and yields of essential oils (qualitative or quantitative).	Haddad *et al.* (2007)
Raw rice (unhusked and husked)	To evaluate the efficacy of decontaminating rice with EBI	Dose delivered: 0, 1, 3 or 7 and 3 or 7 kGy, respectively Irradiation was carried out using a 10 MeV circular electron accelerator (Rhodotron)	The dose of 7.5 and 1.1 kGy of e-beams were required to destroy coliforms, *E. coli*, *Bacillus cereus*, sulphite-reducing clostridia and fungi in unhusked and husked rice. Dose of 7.5 kGy dose was sufficient for decontamination of husked rice and to reduce aerobic plate counts to an average level of 2.66 log CFU/g in unhusked rice. Irradiation at 1.1 kGy reduced aerobic plate counts to 4.03 log CFU/g in husked rice.	Sarrías *et al.* (2003)

Rice (brown)	For disinfestation against maize weevil, *Sitophilus zeamais* Motschulsky	Exposure to soft electrons voltage of 170 kV (beam current 4 mA) produced by a Van de Graaff electron accelerator	Soft-electron treatment effectively killed eggs and pupae of *S. zeamais*.	Imamura *et al.* (2009)
Soybeans	For decontamination and sterilization purposes	Treating with 'soft electrons' (electron energies of 300 kV) using a Van de Graaff electron accelerator	Voltages at 170 kV reduced the microbial count to an undetectable level. Pre-treatment of soybeans with soft electrons extended the shelf life of soymilk without sterilization. Gelling property of soymilk from soft-electron-treated beans was better than that of high-temperature sterilized soymilks.	Todoriki *et al.* (2002)
Spinach (baby spinach; *Spinacia oleracea*)	For reducing *Escherichia coli* O157:H7 and *Salmonella* (microbial decontamination)	Various low doses of EBI	Treatment by EBI at 0.40 kGy resulted in the reduction of *E. coli* O157:H7 and *Salmonella* by 3.7 and 3.4 log cycles, respectively. At 0.70 kGy, both of these pathogens were reduced by 4 log cycle. Doses >1.07 kGy showed reductions greater than 6 log and were decreased to undetectable levels on storing for 8 days.	Neal *et al.* (2008)
Spinach (baby spinach)	To study the efficacy of EBI on the microbiological and sensory characteristics	Various low doses of EBI	At 0.7 and 1.4 kGy, total aerobic plate counts were reduced by 2.6 and 3.2 log CFU/g, respectively. Lactic acid bacteria were reduced at both doses of e-beams, but grew steadily after 35 days. Yeasts and moulds were not reduced at 0.7 kGy; whereas 1.4 kGy significantly reduced microbial counts. With regard to sensory qualities, EBI did not affect the taste, aroma or mouth feel of fresh spinach. However, hardness decreased on increasing the irradiation dose. Also, the slimy attributes of fresh spinach were low in irradiated samples compared to controls.	Neal *et al.* (2010)
Spinach leaves (ready-to-eat)	To evaluate the safety and quality aspects and to study the simulated dose distribution	EBI up to 1 kGy using a 2 MeV Van de Graff accelerator and stored at 4°C for 15 days	Irradiation did not affect the chlorophyll and total carotenoids content. However, vitamin C content was significantly lowered on irradiation. After 15 days of storage, chlorophyll content was	Gomes *et al.* (2008b)

(*Continued*)

Table 17.1 (*Continued*)

Product	Purpose	Dose	Observation	Reference
	Simulation studies carried out using 10 MeV linear accelerator (0.3–1 kGy)		decreased, while the total carotenoids levels remained constant. Results on simulation studies confirmed that baby spinach leaves could be irradiated up to 1 kGy to eliminate *E. coli* O157:H7, along with maintenance of the overall quality.	
Sorghum grain	To study EBI effects on chemical composition, antinutritional contents and digestibility	Dose delivered: 0, 10, 15, 20, 25 and 30 kGy	Tannin content and phytate contents were reduced by 28, 30, 42, 83, 32 and 86% and by 39, 49, 66, 79 and 90%, respectively on irradiation. *In vivo* digestibility of dry matter, crude protein, true protein and gross energy were improved on irradiation.	Shawrang *et al.* (2011a)
Summer truffles (*Tuber aestivum*)	To study the effects of EBI on shelf life, microbial populations and sensory characteristics	Dose delivered: 1.5 and 2.5 kGy E-beam irradiator (Rhodotron) with a 10 MeV energy level, a 10 kW power level, and an 127 average 98 kGy/min dose rate	EBI reduced postharvest sensory losses and the presence of dominant microflora (*Pseudomonads* and *Enterobacteriaceae*). Combination of modified atmosphere packaging using P-Plus micro-perforated plastic together with irradiation at 2.5 kGy and storage at 4°C was successful in enhancing the shelf life of summer truffles up to 42 days without development of mycelium and yeast growth.	Rivera *et al.* (2011)
Spices (black pepper, turmeric, rosemary and coriander)	Microbial decontamination	Varying doses	A dose of 4 kGy was found to be successful in reducing the microbial load (coliforms, osmophilic moulds and fungi).	Hitoshi and Islam (1994)
White button mushroom (*Agaricus bisporus*)	To determine the postharvest quality and selected enzyme activities	Dose delivered: 0, 1, 2, 3 and 4 kGy Irradiation was performed by using a electron linear accelerator	EBI retarded post-harvest softening of mushroom and increased malondialdehyde levels. After 10 days of storage, polyphenoloxidase activity in irradiated samples (1–4 kGy) was found to be significantly low compared to controls. Superoxide dismutase and catalase activities were decreased in irradiated samples.	Duan *et al.* (2010)

CFU, colony-forming units.

Table 17.2 Some selected reports on the use of e-beams in poultry, meat and seafood.

Product	Purpose	Dose	Observation	Reference
Beef jerky	To achieve microbial safety	EBI dose delivered: 0, 1, 3, 5 and 10 kGy and stored at 20°C for 60 days	Populations of total aerobic bacteria significantly decreased with increasing irradiation dosage. Sensory evaluation results showed EBI not to affect overall sensory scores during storage.	Kim *et al.* (2010b)
Beef patties	To study the effects of fat content and post-slaughter ascorbic acid infusion on microbial and physicochemical qualities of beef patties processed by EBI	Dose delivered: 5 and 10 kGy using a linear e-beam accelerator	The addition of fat significantly increased aerobic, total coliform, *E. coli* and psychrotrophic bacteria counts in beef patties during storage. EBI at both doses exerted a pasteurization effect on psychrotrophic bacteria for up to 7 days of storage. No viable aerobic, total coliform or *E. coli* bacteria were detected in any irradiated beef patties during storage. Physicochemical changes caused by lipid oxidation and surface discoloration of beef patties were significantly increased by both addition of fat and irradiation.	Wong *et al.* (2005)
Beef sausage patties	Comparison on the effects of γ and EBI on TBARS value, hardness, colour, sensory characteristics, and total bacterial populations in vacuum-packaged samples	Dose delivered: 0, 5, 10, 15 and 20 kGy	Results of γ rays were similar to EBI with regard to lipid oxidation, hardness, colour and sensory scores. γ-Irradiated samples showed significantly lower total bacterial counts than e-beam-irradiated samples even during storage, irrespective of irradiation dose.	Park *et al.* (2010)
Beef (ground)	To improve safety and stabilize colour during irradiation using antioxidants	EBI at 0 and 2.0 kGy with storage time of 0, 3, 6, 9 days	Control samples showed highest TBARS value and low redness (a^*), proportion of oxymyoglobin and vividness. Irradiated samples were just as red and vivid on SRD day 9 as the non-irradiated, control at day 0. Treatments stabilized the colour and lipids of ground beef after irradiation and during SRD.	Duong *et al.* (2008)

(*Continued*)

Table 17.2 (*Continued*)

Product	Purpose	Dose	Observation	Reference
Broiler breasts and thighs	To determine the effects of high-energy irradiation on the number of aerobic microorganisms and *Salmonella*	Dose delivered: 100–700 kilorads (krads) delivered using a commercial-scale e-beam accelerator	E-beam dose levels as low as 100 krads eliminated *Salmonella*. The total number of aerobic organisms was reduced by 2–3 $\log_{10}$ cycles at irradiation levels of 100–700 krads. Increasing the dose above 100 krads did not provide any additional benefits.	Heath *et al.* (1990)
Chicken (boneless, skinless chicken breasts)	To determine the effectiveness of EBI on elimination of bacteria	Dose delivered: 0, 1.0 and 1.8 kGy	In control samples, mean counts for coliforms, *E. coli* and psychrotrophs were 3.13, 3.26 and 1.92 $\log_{10}$ CFU/200 mL rinsate, respectively. These pathogens were undetected after irradiation. Mean count for aerobic bacteria in the control samples were 4.60 $\log_{10}$ CFU/200 mL rinsate. Irradiation doses of 1.0 and 1.8 kGy reduced these levels to 2.23 and 1.62 $\log_{10}$ CFU/200 mL rinsate, respectively. No differences were observed between control and treatment groups for any of the quality attributes tested (product stored for 0, 14 and 28 days at 0°C). At 14 days, texture and flavour attributes were lower for the irradiated samples. At day 28, irradiated samples were less desirable with decreased texture, flavour and overall acceptability. Lipid oxidation also increased along with increase in storage time and level of irradiation.	Lewis *et al.* (2002)
Chicken meat (ready-to-eat poultry frankfurters, deboned)	To evaluate the sensory profile attributes over a 32 day refrigerated storage period	Dose delivered: 0, 1, 2 and 3 kGy	EBI affected: aroma attributes classified as being like that of chicken, cured meat, spice blend and "wet dog" (an aroma like that associated with wet dog hair); meaty and chickeny flavour attributes; off-flavours; and sour and sweet taste. The texture of the poultry frankfurters was not significantly affected by irradiation.	Johnson and Resurreccion (2009)

			Storage time had a more significant effect on the frankfurters than irradiation dose. EBI had significant effect on some aroma and flavour attributes, however, it did not have a significant effect on texture attributes.	
Commercially available egg white and ground turkey meat samples	Study of the effects of EBI in samples spiked with the low-pathogenic A/chicken/TX/2002 H5N3 avian influenza virus (AIV) and exposed to varying doses of high-energy e-beam irradiation	Exposure to high-energy (10 MeV) EBI at dose ranging between 0 and 8 kGy	The viral titres in irradiated samples showed a linear dose-dependent reduction. The dose required to achieve 90% reduction (D_{10} value) of viable AIV loads was 2.3 kGy in phosphate buffer, 1.6 kGy in egg white and 2.6 kGy in ground turkey meat samples.	Brahmakshatriya *et al.* (2009)
Fermented sausage	To study the survival of lactic acid bacteria during fermentation and aging (0, 7, 14, 21 days), and control the production of off-flavour and development of lipid oxidation	EBI at 0, 2, 4 and 6 kGy	A dose of 2 kGy of irradiation was highly effective in manufacturing a fermented sausage, and the addition of rosemary extracts was effective in controlling the production of off-flavour and development of lipid oxidation during cold storage.	Lim *et al.* (2008)
Cooked ham (ready-to-eat)	Controlling *Listeria monocytogenes* and evaluating the sensory properties (appearance, odour and flavour)	EBI of 1.0 and 2.5 kGy followed by storage time of 0, 8, 12, 15, 18, 20, 22, 30 and 41 days	Vacuum-packed ready-to-eat cooked ham subjected to EBI resulted in a microbiologically safe product. Additionally, the shelf life was doubled (from 20 to 40 days). By using a dose of 1 kGy the safety levels required by the European Union (FSO=10^2 CFU for *L. monocytogenes*) was achieved without noticeable changes in the sensory quality. However, high dose resulted in off-odours that may be detected by consumers but there is no reason to reject the product.	Cabeza *et al.* (2007)
Cold-smoked salmon	For reducing *L. monocytogenes* contamination	Varying dose of EBI	Low doses of 1 kGy reduced *L. monocytogenes* counts and 2.0 kGy eliminated the pathogen.	Su *et al.* (2004)

(*Continued*)

Table 17.2 (*Continued*)

Product	Purpose	Dose	Observation	Reference
Cold-smoked salmon	To evaluate the effectiveness of EBI and high-pressure treatments	EBI at 1–4 kGy at 10 MeV and pressure treatments at 450 MPa and 12°C at 5, 10, 15, 20 and 25 min in a model ACIP 6000 apparatus	EBI at 2 kGy showed microbial population of smoked salmon to be $<6 \log_{10}$ CFU/g after 35 days of storage at 5°C, with negligible or very light changes in odour. Pressurization at 450 MPa for 5 min also showed microbial population $<6 \log_{10}$ CFU/g after 35 days of storage at 5°C and did not alter odour, but affected negatively the visual aspects of smoked salmon.	Medina *et al.* (2009)
Hamburgers	To evaluate the changes in colour, texture and sensory quality and stability in presence of folic acid	EBI at dose of 0, 2, 3 and 4 kGy and folic acid at 0.6, 1.2 and 2.4 mg/100 g	The presence of folic acid negligibly influenced the quality of the meat products. Irradiation treatments caused loss of sensory quality and treatment with 4 kGy was not adequate. Folic acid levels decreased by 20–30% following irradiation with 2 kGy, and no additional decrease was observed at higher doses of radiation.	Galán *et al.* (2010)
Oyster (Oyster *Jeotkal*) salted, seasoned and fermented oyster	To study and compare the effects of γ and EBI on *L. monocytogenes*, *Staphylococcus aureus* and *Vibrio parahaemolyticus*	Dose delivered: 0, 0.5, 1, 2 and 5 kGy	γ Irradiation was more effective than e-beams. D_{10} values of EBI were 0.69, 0.94 and 0.29 kGy, respectively, for the three pathogens. Sensory quality was not affected by irradiation treatment.	Song *et al.* (2009)
Vacuum-packed slices of Iberian dry-cured loin	Evaluation of EBI on colour changes and lipid oxidation at two levels	EBI doses: 0, 5 and 10 kGy. E-beam irradiator used was a Rhodotron with a 10 MeV energy level, 10 kW power level, and an average 98 kGy/min dose rate	EBI induced changes in colour and lipid oxidation in sliced Iberian dry-cured loin immediately after treatment and subsequent refrigerated storage.	Cava *et al.* (2009)
Turkey breast rolls and turkey hams (oven roasted)	The study the effect of EBI on the survival and growth of *L. monocytogenes* and natural microflora	Samples were vacuum packed and irradiated at 0 (control), 1.0, 1.5, 2.0 or 2.5 kGy; and	Reduction in *L. monocytogenes* and natural flora was observed. But, *L. monocytogenes* and natural flora that survived	Zhu *et al.* (2008)

		stored at 4°C for up to 28 days	irradiation could multiply during the 28 days of storage at 4°C.	
Turkey breast rolls	To determine the effects of antimicrobial treatments on the survival and proliferation of *L. monocytogenes* following EBI	Vacuum-packaged samples were e-beam-irradiated at doses of: 0, 1.0, 1.5, 2.0 or 2.5 kGy	For microbial safety, 0.1% potassium benzoate + 2% sodium lactate and 2% sodium lactate + 0.1% sodium diacetate antimicrobial treatments combined with 1.0 or 2.0 kGy irradiation showed promising results.	Zhu *et al.* (2009)

FSO, Food Safety Objective; TBARS, 2-thiobarbituric acid reactive substances.

2002; Sivapalasingam *et al.*, 2004; FDA News, 2007). Doyle and Erickson (2008) have suggested that implementation of strict food safety measures are necessary throughout the production, processing and distribution chain for achieving and ensuring 'pathogen-free' fresh and fresh-cut produce.

Foodborne pathogens such as norovirus, *Escherichia coli* O157:H7, *Salmonella*, *Listeria*, *Aspergillus* and *Fusarium* species are of serious public health concern as they are the most common causal agents of foodborne illness from fresh produce (Sivapalasingam *et al.*, 2004; Bhat *et al.*, 2010a; Grasso *et al.*, 2011; Strawn *et al.*, 2011). Several novel processing techniques such as modified-atmosphere packaging, ozone treatment, ultrasound, UV treatment and others have been employed for achieving the microbial safety of fresh plant produce. However, most of these technologies have their limitations and still a paucity of evidence exists when it comes to evaluating their commercial importance and benefits. Previously, the benefits of employing γ radiation as an alternative for chemical preservatives in fruits and vegetables have been well documented in several research papers and reviews (Thomas, 1986; Thomas *et al.*, 1995; Thayer and Rajkowski, 1999; Arvanitoyannis *et al.*, 2009). Different doses of irradiation have been fixed, standardized and recommended for industrial applications. Irradiation at lower dose levels (<1.0 kGy) is recommended for disinfestation purposes and sprout inhibition; medium-dose levels (1.0–3.0 kGy) for delay in ripening or senescence of raw fruits and vegetables and microbial decontamination; and higher doses for extracting bioactive compounds. However, the main parameter that gets affected by e-beam radiation is texture, with such treatments causing undesirable changes (Egea *et al.*, 2007). Radiolysis of water due to the formation of free radicals during irradiation acts directly on the material, leading to the breakdown of nucleic acid molecules (DNA) and other constituents, which in certain cases can induce significant changes in the product, such as formation of hydroperoxides (Bhat, 2007).

However, compared to γ irradiation, only few studies have been conducted or reported on the effects of EBI on the quality of fresh produce. Some selected reports on the application of e-beams in fruits and vegetables are given in Table 17.1. As observed, most of the studies are focused towards improving shelf life, decontamination of the fresh produce, and overall physicochemical properties, phytochemical content and consumer acceptability. Depending on the raw material, the e-beam dose delivered varies. However, in the majority of the instances the doses are of medium level and below the international acceptable limits.

In one of the studies reported by Boyton *et al.* (2006) combination of EBI of fresh-cut cantaloupe with modified-atmosphere packaging resulted in an extension of shelf life by up to 2–3 weeks. This is just an example to show the effectiveness of combination treatments. Conducting a detailed quantitative microbial risk assessment with modelling, maintaining good agricultural and manufacturing practices with a Hazard Analysis Critical Control Point (HACCP) approach, along with the application of EBI throughout the processing chain, might provide more insight and be useful for commercializing the novel technology of using e-beams with fresh produce.

17.3.2 Cereals, legumes and seeds

Cereal grains and legumes form an integral part of the regular human diet all over the world. They are recognized to be a vital source of dietary proteins, carbohydrates, vitamins, minerals and fibre for people residing in developing and underdeveloped parts of the world (Blandino *et al.*, 2003; Bhat and Karim, 2009). Some of the popular cereals and grains, such as rice, wheat, millets (pearl millet, finger millet), maize and sorghum, and underutilized legumes

like *Mucuna*, *Canvalia* and *Sesbania* spp. are of major significance in these regions. These cereals, grains or legumes also contain substantial amounts of phenolics and other bioactive compounds, known to provide potential health benefits (Dykes and Rooney, 2007; Bhat and Karim, 2009).

E-beam treatments have been successfully used in cereals, grains and legumes for decontamination purposes, to achieve insect disinfestation, sprout inhibition, reductions in antinutrient contents and improvement of functional properties (Hayashi, 1998; Hayashi and Todoriki, 2001; Kottapalli *et al.*, 2006; Bhat *et al.*, 2007, 2008). Dose requirements vary among legumes, seeds and cereals, depending on variety and species. Not enough information is available on the application of e-beam technology in cereals, except for a few scattered reports. For example, a dose of 3.2 kGy is reported to effectively reduce bacteria and moulds in unhusked rice samples, with a total decontamination achieved at 7.5 kGy (Sarrías *et al.*, 2003). In addition, a reduction in microbial contaminants (bacteria, fungi and yeasts) in nutraceutically valued lotus seeds was reported on exposure to e-beams from a microtron source (0, 2.5, 5, 7.5, 10, 15 and 30 kGy) (Bhat *et al.*, 2010b).

Nutritionally rich raw sprouts are increasingly becoming popular in households all over the world. However, raw sprouts contaminated with *Salmonella* spp., *E. coli* O157:H7 and *Bacillus cereus* (during sprouting, harvesting, packaging and transport) have been related to outbreaks of foodborne illness (National Advisory Committee on Microbiological Criteria for Foods, 1999; Taormina *et al.*, 1999; Tournas, 2001; Waje and Kwon, 2007). Previously, γ-irradiation treatments of sprouts of alfalfa, radish and mung bean have been shown to effectively reduce foodborne pathogens to undetectable levels (Rajkowski and Thayer, 2000; Bari *et al.*, 2004). Also, irradiation treatment of sprouts (maximum 1 kGy dose) and seeds intended for sprout production (up to 8 kGy) has been approved by the US Food and Drug Administration (FDA; Code of Federal Regulations, 2000). Schoeller *et al.* (2002) reported successful application of e-beams at 3.3 or 5.3 kGy in eliminating *Listeria monocytogenes* on alfalfa sprouts.

The use of soft electrons, with an electron energy of 300 kV or lower, and capable of ensuring the surface decontamination of cereals, grains and pulses, has been proposed by some researchers (Hayashi *et al.*, 1997; Todoriki and Hayashi, 2000; Todoriki *et al.*, 2002). However, in the case of these studies uniform and homogeneous irradiation of the surface of the food product with soft electrons was required to achieve complete decontamination. The major disadvantage of using soft electrons might be their inability to deeply penetrate the food to remove endophytic microbes or insect larvae (i.e. present inside the food). Kikuchi *et al.* (2003) reported soft-electron doses of 9 and 17 kGy to be insufficient to eliminate the contaminant microbes in soybeans and thus recommended higher doses of EBI (26 kGy) to achieve total decontamination.

Additionally, soft-electron treatments for disinfestation of grains has been reported, wherein soft electrons at 60 keV were found to inactivate eggs, larvae, pupae and adults of red flour beetle (*Tribolium castaneum*) and Indian meal moth (*Plodia interpunctella*), and eggs and adults of adzuki bean weevil (*Callosobruchus chinensis*) (Imamura *et al.*, 2004a). Soft electrons at 150 kV were also observed to effectively disinfest 'brown rice' grains pre-infested with maize weevil (*Stiophilus zeamais*) and Indian meal moth and adzuki beans with adzuki bean weevil (Imamura *et al.*, 2004b).

EBI also possesses great potential to reduce the antinutritional factors in legumes. EBI at varying doses (0–30 kGy) has been shown to reduce some of the antinutritional factors in wild legume seeds or in seed meals, which is considered to be useful for increasing their consumption (Bhat *et al.*, 2007). Reduction of antinutrient compounds like phytate has

been reported in sorghum grains treated with e-beams (Shawrang *et al.*, 2011a). Removal of free gossypol and reducing the total gossypol content to permissible levels in poultry feed by EBI (10, 15, 20, 25 and 30 kGy doses) has also been reported recently (Shawrang *et al.*, 2011b).

Improvement in functional properties such as foaming, water and oil absorption capacities, and gelation properties along with increases in crude protein and crude carbohydrates, linoleic acid (a polyunsaturated fatty acid) and *in vitro* protein digestibility has been reported in *Mucuna pruriens*, an underutilized, nutraceutically valued legume seed, on exposure to EBI from a microtron source (0–30 kGy) (Bhat *et al.*, 2008). Similar improvements in the nutritional qualities in e-beam-irradiated lotus seeds (*Nelumbo nucifera*) have also been reported (Bhat and Sridhar, 2008).

17.3.3 Poultry, meat and seafood

Some selected examples on the use of e-beams in poultry, meat and seafoods are given in Table 17.2.

In addition to beef and pork, poultry meat is highly popular in several parts of the world. Irrespective of the avian sector facing several sanitary problems and issues (like bird flu/avian influenza), consumption of poultry meat has been predicted to increase in the coming years, which is mainly due to low production costs (compared to beef and pork), religious issues, consumer preference, wholesomeness and nutritious qualities, and increased demand for low-price proteins in the international market (Vandeplas *et al.*, 2008; Anang *et al.*, 2010). Safety of poultry has been an issue of concern due to the presence of spoilage and foodborne pathogens like *Streptococcus*, *Staphylococcus*, *Micrococcus*, *Bacillus*, *Salmonella*, *Listeria* and *Campylobacter*. Foodborne outbreak of salmonellosis and campylobacteriosis in humans is due to the consumption of raw or undercooked poultry meats and/or by-products (Suzuki and Yamamoto, 2009; Anang *et al.*, 2010). According to Engvall (2001) *Campylobacter* contamination is considered to be a major problem in both conventional and organic farms. The contamination by these pathogens occurs either during processing or certain types of storage. Various methods of inhibiting growth of both pathogenic and spoilage microorganisms in poultry have been suggested, such as the use of organic acids, salts, etc. (Luber, 2009; Anang *et al.*, 2010).

Irradiation has also been reported to be an effective means to decontaminate pathogenic microorganisms. Application of γ irradiation for decontamination of poultry viscera has been reported by Jamdar and Harikumar (2008). The effects of ionizing radiation on egg and egg products have also been reported in detail by various researchers (Narvaiz *et al.*, 1992; Pinto *et al.*, 2004; Min *et al.*, 2005; Badr, 2006; Bakalivanov *et al.*, 2008).

Non-significant changes in the physicochemical or functional changes in the properties of egg yolk or cleavage of egg yolk protein was recorded on exposing 1 day-old raw yolk of eggs to linear EBI at 2.5 kGy (Huang *et al.*, 1997). The use of linear accelerators (linacs) for management of *Salmonella* in poultry has also been reported (Sadat and Volle, 2000). Application of high-energy (10 MeV) EBI for decontamination of poultry products (egg and meat) and reduction in virus load resulted in the inactivation of avian influenza virus (Brahmakshatriya *et al.*, 2009). E-beam-irradiation of turkey rolls combined with use of antimicrobial compounds such as potassium benzoate and sodium lactate, or sodium diacetate and sodium lactate, has been shown to be effective in suppressing the growth of *L. monocytogenes* (Zhu *et al.*, 2009). The effectiveness of EBI (at 1.0 and 1.8 kGy) in eliminating contaminant bacteria from boneless, skinless chicken breasts has been reported

by Lewis *et al.* (2002). The application of nanosecond e-beams for sterilization in industrial poultry farming that could be technically feasible and more economical has been reported by Kotov *et al.* (2003). A 4 log reduction in *Salmonella* for chicken breasts inoculated and treated with 1, 2 or 3 kGy of EBI has been reported by Sarjeant *et al.* (2005). However, some reports (Lee and Ahn, 2005) indicate that application of irradiation to meat is limited, since it can produce changes in the sensory attributes such as colour and flavor, which could substantially affect consumer acceptance.

Two major problems which the food industry had to face in the past few years for beef was the outbreak of bovine spongiform encephalopathy and foot-and-mouth disease. Managing the rate of contamination along with inactivation of residing pathogenic bacteria has been considered to be important for achieving the safety of meat and meat products (Borch and Arinder, 2002). The most common microbial contaminants of beef include *Staphylococcus aureus*, *L. monocytogenes*, *E. coli* O157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica*, and contamination by these organisms has led to serious foodborne illness and recall of contaminated meat (Elliot *et al.*, 2004; Levanduski and Jaczynski, 2008). The presence of these organisms is an indication of improper processing, and use of poor hygiene in handling, distribution and storage. Several novel techniques have been tried in the decontamination of beef or its related products, such as spray-wash methods, hide-on decontamination, combinations of non-thermal and thermal preservation technologies, and use of bio-preservatives (Elliot *et al.*, 2004; Aymerich *et al.*, 2008; Fung *et al.*, 2008; Juneja *et al.*, 2010). Radiation processing has been widely accepted as an effective method for reducing microbial population in raw meat and meat products for shelf-life improvement and for achieving overall safety. Low-dose irradiation ranging between 2 and 3 kGy has been reported to either eliminate or reduce pathogenic and spoilage bacteria such as *E. coli* O157:H7, *Salmonella* (up to 2 log cycles) pseudomonads and lactic acid bacteria in beef (Radomyski *et al.*, 1994; Wong *et al.*, 2005). The US Department of Agriculture (USDA, 1999) has permitted the application of 4.5 and 7.0 kGy radiation for refrigerated meat and frozen meat products, respectively. Several recent studies have indicated the effectiveness of EBI in improving the overall quality of meat and meat products (Miyahara and Miyahara, 2002; Arthur *et al.*, 2005; Kim *et al.*, 2010b; Park *et al.*, 2010).

Quality deterioration of meat during storage is mainly dependent on the levels of lipid oxidation and associated changes. Radiation processing is capable of enhancing the onset of lipid oxidation (due to generation of superoxide and hydroxyl radicals) resulting in the development of off-flavours, adverse colour changes and reduction of shelf life (Wong *et al.*, 2005; Kanatt *et al.*, 2007). According to Ahn *et al.* (1998), development of lipid oxidation in irradiated cooked meat might be the outcome of processing conditions, packaging or storage environment employed, during either pre- or post-irradiation stages. Hence, it is essential that proper and appropriate methods be adopted along with EBI to minimize these deleterious changes for better stability of the products. Combination of e-beams with natural preservatives might provide more eco-friendly alternatives in preserving the quality of meat (Kim *et al.*, 2004; Kwon *et al.*, 2008).

Aquaculture is considered as one of the fastest growing food-production systems in the world. Increased demand for fishery/seafood products is predicted to pose serious threats to the environment and human health, mainly due to the possible risks of contamination of harvested products by chemical, microbial or other biological agents (United Nations Development Programme, 1994; Huss *et al.*, 2000; FAO, 2005). According to FAO (2009), nearly 520 million people all over the world depend on seafood as a source of protein and as a major source of family income.

Being highly perishable, seafoods provide favourable conditions for the growth of various spoilage and pathogenic microorganisms. Almost all the types of seafood are known to harbour a wide array of pathogenic and spoilage microorganisms (bacteria, viruses and protozoan parasites). The principles of bacterial spoilage and other associated parameters leading to reduced shelf life in fish and fish products have been detailed by Gram and Huss (1996). Previously, Sumner and Ross (2002) published an excellent review of semi-quantitative risk assessment associated with 10 seafood hazards or product combinations. Various foodborne pathogens have been reported in raw and cooked seafoods (mainly fish and shellfish) that include *Aeromonas* spp., *Bacillus* spp., *Campylobacter* spp., *Vibrio* spp., *Listeria* spp., *Shigella* spp., *Y. enterocolitica*, *E. coli* O157:H7, norovirus, astrovirus, rotavirus, hepatitis A virus, and others (Novotny *et al.*, 2004; Gillespie *et al.*, 2010; Walker and Winton, 2010). According to the US Center for Disease Control and Prevention, fish and shellfish accounts for nearly 5% of the individual cases and 10% of all foodborne illness outbreaks, with the majority of outbreaks resulting from consumption of raw molluscan shellfish (Flick, 2008).

Chemical-based decontamination techniques as well as modern preservation techniques such as vacuum packaging, modified-atmosphere packaging, ultra low freezing temperature, and use of natural antimicrobials, organic acids and their salts (malic acid, citric acid, sodium acetate, sodium citrate, potassium sorbate) are routinely used for controlling the growth of pathogenic and spoilage microorganisms. However, each of these treatments has its own limitations.

With regard to seafoods, the major objectives of using e-beams have been for decontamination purposes and for improving textural attributes. Successful applications of EBI in various types of seafood have been reported. Low-dose e-beams (1 kGy) have been reported to reduce *L. monocytogenes* counts with 2.0 kGy being effective in eliminating this pathogen in cold-smoked salmon (Su *et al.*, 2004). Improvement in textural quality attributes such as firmness in Alaskan pollock surmi gel on exposure to e-beams (6–8 kGy) has been reported (Jaczynski and Park, 2004). The efficacy of employing γ irradiation and EBI (0, 0.5, 1, 2 and 5 kGy) against foodborne pathogens including a three-strain cocktail of *L. monocytogenes*, *Staph. aureus* and *Vibrio parahaemolyticus* in salted, seasoned and fermented oyster has been reported by Song *et al.* (2009). EBI at 2 kGy was efficient in controlling the microbial population on smoked salmon (<6 log colony-forming units (CFU)/g) even after 35 days of storage at 5°C, with insignificant changes observed in its odour (Medina *et al.*, 2009).

Reports on quality attributes of e-beam-irradiated shrimps, prawn, mussels (crustaceans and molluscs) and other seafood are scarce. Additionally, the effects of e-beams on the microbial and functional qualities of fish gelatin needs to be explored, as fish gelatin has been in focus as an alternative to bovine or pig gelatin.

17.4 ELECTRON-BEAM IRRADIATION AND MICROORGANISMS

Irrespective of the radiation source (γ, e-beams, X-rays, UV rays), raw material or products, the inactivation of microorganisms or insect pests revolves around the direct damage of DNA (Urbain, 1986). According to Tauxe (2001), EBI utilizes a stream of high-energy electrons which directly damage the DNA of exposed organisms by introducing cross-linkages, preventing them from growing or reproducing. Apart from damage to DNA or reproductive cells, e-beams also induce changes in the chemical and molecular bonds. Water has been shown to play a significant role in the inactivation of microbes, leading to their death due to

water radiolysis or production of free radicals (El Assis *et al.*, 1997; Black and Jaczynski, 2006; Bhat, 2007). Additionally, the efficacy of EBI is considered to be higher than for γ irradiation, as e-beams are directed at the product containing the microorganisms, while in the case of γ-radiation sources the radiation is emitted in all the directions (Diehl, 1990).

Certain microorganisms tend to survive low does of radiation and require higher-dose treatments to be eliminated (e.g. *Deinococcus radiodurans*). However, there is a chance that high-dose treatments might significantly affect the organoleptic characteristics and nutritional quality of the food (Valero *et al.*, 2006). Monk *et al.* (1995) provided details on the relative resistance and inactivation of foodborne microbes to irradiation. The effect of accelerated electrons, alone or followed by heat treatment, on *B. cereus* and *Bacillus subtilis* spore counts and spore heat resistance was investigated by Lara *et al.* (2002), who showed radiation treatment to exhibit a heat-sensitizing effect on bacterial spores. The *D*T values (>3.3 kGy) were reduced more than three times for both *B. cereus* and *B. subtilis* spores. An increase in the resistance of *E. coli* O157:H7 to e-beams following repetitive irradiation at sub-lethal doses in ground beef has been reported by Levanduski and Jaczynski (2008). However, the authors could not conclude the exact mechanism observed for the increased radio-resistance of *E. coli* to e-beam, but attributed this to potential genetic mechanisms of resistance.

As there are still wide gaps in the field, such as evidence provided by molecular studies, further studies on the effects of EBI on resistant microbes, especially yeasts and moulds, need to be undertaken.

17.5 CONCLUSION AND FUTURE OUTLOOK

EBI is a novel, non-thermal, economical, fast, effective, environmentally safe, physical method of food processing that is useful to achieve sterilization, microbial decontamination and insect disinfestation of various food and agricultural commodities. Owing to several advantages, EBI can be an effective alternative to chemical fumigants and to γ irradiation. EBI can be considered as an effective mode of food preservation along with other common conventional techniques such as drying, canning, freezing, atmospheric packaging and use of biopreservatives to prevent spoilage of perishable food products. There are still broad gaps prevailing in the body of research as we strive for successful application of e-beams in food products, such as the need to study their effects on induced enzymatic changes, combination treatments and shelf-life improvement, standardizing the appropriate dose level for a single product, assuring uniform dose distribution and evaluation of sensory quality. Effects of e-beams on herbal products (natural botanicals), spice powders and organically produced food or agricultural commodities need to be investigated.

Future studies should be initiated on the employment of e-beams for decontaminating as well as inducing cross-linking in biopolymer-based biodegradable films (especially fish gelatin) developed for food packaging purposes. The effects of e-beams on preserving local and traditional foods, intended for export purposes, need to be investigated. Overall, it should be noted that without employing proper good manufacturing practice and good agricultural practice with a HACCP approach, EBI will not be a stand-alone solution to the problems outlined above. Good agricultural and manufacturing practice along with EBI can potentially benefit food-processing and -distribution chains. The benefits of nuclear energy and use of radiation technology for food preservation has been popularized from time to time by the programme 'Atoms for Peace', as well as through a series of monographs and lectures held the

Figure 17.1 The Radura logo, which is required for food labelling as an indication of e-beam irradiation.

world over. Educating consumers on the benefits of employing radiation technology, especially those of machine-generated e-beams, is vital in today's world. A recent review by Arvanitoyannis (2010) assessed consumer behaviour and attitudes towards irradiated food. Today, consumers are more knowledgeable and are in a state of acceptance when ample evidence on the advantages of this novel technology is provided. One vital step towards educating consumers is displaying the Radura logo (see Figure 17.1) on the packaging of irradiated products, which should include details on the date of irradiation and the batch, and confirm the safety of the product. It is all the more important to conclusively prove that the e-beam-irradiated foods are not negatively affected in terms of nutritional quality, and are not harmful in any way, in order to attain consumer acceptance.

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Part III Modelling

18 Treatment of Foods using High Hydrostatic Pressure

Sencer Buzrul and Hami Alpas

Abstract: High hydrostatic pressure (HHP), which was started to be used over a century ago, is now a well-established technology and has become a reality in the food industry. In many countries fruit juices, meat, seafood, and vegetable products are treated with HHP on a large scale to improve flavor, color, and taste, and to eliminate pathogenic or spoilage microorganisms, similar to heat treatment. HHP has several advantages, such as conveying less energy to the food and even application to all sides of the food, unlike thermal treatment. Surveys have revealed that consumers who are aware of HHP technology may accept and eat HHP-treated foods.

Keywords: consumer awareness; food quality; high hydrostatic pressure; microorganisms

18.1 INTRODUCTION

Many of the food-preservation methods that are used at present have several drawbacks. Traditional food-processing methods mostly rely on high temperature as a way to ensure prolonged shelf-life and food safety. The use of heat (pasteurization, sterilization, and bleaching, for example) can destroy nutrients such as thermally labile vitamins and also components responsible for product flavor and taste. It can also produce some undesirable compounds which originate from the Maillard reaction and caramelization (Cheftel, 1995a). All these changes result in products that are far from similar to the original fresh product (San Martín, 2002).

In modern times, the quality and safety of food products are among the most important factors that influence consumer choice (Considine *et al.*, 2008). Today's consumers demand more "fresh" and "natural" food products and this has led the food industry to investigate alternative processing methods (Balasubramaniam *et al.*, 2004).

Among these alternative methods high hydrostatic pressure (HHP) is, perhaps, the most popular. Currently this method is successfully applied on a commercial scale for pasteurization of a whole range of food products such as fruit juices, seafoods, and meat products (Matser *et al.*, 2004). HHP treatment at refrigeration, ambient or moderate heating temperatures allows inactivation of pathogenic and spoilage microorganisms in foods with fewer changes in texture, color, and flavor compared to conventional techniques (Knorr, 1993;

Progress in Food Preservation, First Edition. Edited by Rajeev Bhat, Abd Karim Alias and Gopinadhan Paliyath.

Cheftel, 1995b; Velazquez *et al.*, 2002). HHP technology has been also quoted as being one of the best innovations in food processing in 50 years (Balasubramaniam *et al.*, 2008).

18.2 PRESSURE AND THE EARTH

Just like temperature, pressure is an important thermodynamic parameter that can profoundly influence molecular systems. However, compared to heat, the effects of high pressure on living systems and biomolecules have historically not received the same attention. Nevertheless, the pressure levels that are imposed on biological systems, either in natural ecosystems or industrial processing, span almost four orders of magnitude (Aertsen *et al.*, 2009).

HHP is a characteristic parameter of the biosphere when viewed in terms of the volumes occupied by the Earth's major terrestrial (land) and aquatic components. Terrestrial habitats, where the pressure is close to 1 atm (0.101 MPa) or lower, account for less than 1% of the total volume of the biosphere. The oceans, which cover approximately 70% of the Earth's surface, have an average depth of 3800 m and consequently an average pressure of 381 atm (38.5 MPa). Approximately 79% of the volume of the marine component of the biosphere lies below 1000 m (Somero, 1992; Buzrul, 2008).

HHP treatment of foods uses a pressure-transmitting fluid such as water exactly as we experience it in deep-sea regions, where pressures of up to 110 MPa (the Challenger Deep in the Marianas Trench is nearly 11 000 m deep) can be reached.

18.3 MAIN FACTORS CHARACTERIZING HIGH HYDROSTATIC PRESSURE

18.3.1 Energy

When energy developed by high pressure is compared to the average value of the energy of chemical bonds, it can be seen that energy developed by high pressure is quite low. Consequently, high pressure affects only weak chemical bonds (Buzrul, 2008). Thus small molecules such as amino acids, vitamins, and flavor components, which are major contributors to the sensory and nutritional quality of a food, remain unaffected by the application of HHP since they have relatively simple structures (Balci and Wilbey, 1999). Table 18.1 shows a comparison of the energy conveyed by pressure in different media (gas, liquid, and solid) and the average energy of a chemical bond. Table 18.2 gives a comparison of the energy conveyed by pressure and temperature in the same medium (water). This comparison

Table 18.1 Energy developed by compression in different media is compared to the average energy of a chemical reaction (source: Demazeau *et al.*, 2006).

Pressure (bar)	Medium	Energy (cal/mol)
1000	Gas	3000
1000	Solid	1
10 000	Solid	5
100 000	Iron	20
100 000	H_2O	1000
1	Chemical reaction	20 000

Table 18.2 Increasing the temperature and pressure of 1 L of water with the same amount of energy (E).

	1 L of H_2O
Temperature (T)	$P = 0.1$ MPa $T = 20 \rightarrow 25°C$ $E \approx 20$ kJ
Pressure (P)	$T = 20°C$ $P = 1 \rightarrow 400$ MPa $E \approx 20$ kJ

may underline that the energy developed by pressure is very small compared to that developed by temperature; consequently the phenomena induced by both parameters in food sciences are completely different (Buzrul, 2008). The high capital investment costs of HHP equipment can be overcome by operating HHP plants at full capacity (Torres and Valezquez, 2005).

18.3.2 Densification effect

Under high pressures the difference between final and initial volumes (ΔV value) is always negative. This leads the formation of new structural forms such as HHP denaturation of proteins or the dissociation of water (Buzrul, 2008). Oligomeric proteins dissociate to subunits with negative ΔV. After dissociation, subunits may reaggregate or denature. At pressures above 200 MPa chains start unfolding and subunits of dissociated oligomers start reassociating (Tauscher, 1990). The breaking of ionic bonds leads to a volume decrease due to electrostriction of water in the proximity of ions and as a result is enhanced by HHP. Hydrogen bonds are also favored by HHP, since their formation involves a decrease in volume (Mertens, 1992).

18.3.3 Isostatic (Pascal) principle

When HHP is exerted it is the same in all directions of a space. HHP applied to foods being processed is transmitted isostatically and instantaneously; thus the process is not dependent on the shape or size of the food (Smelt, 1998). The major advantage of this is that the food is treated evenly throughout, which can often be problematic in thermal processing of large or bulky food products (Considine *et al.*, 2008).

18.4 HISTORICAL PERSPECTIVE

HHP treatment to kill bacteria such as *Escherichia coli* and *Staphylococcus aureus* was first described by Royer (1895); however, in food science and technology the most important work involving microbial inactivation was that by Hite (1899). Hite showed that the shelf life of raw milk could be extended by about 4 days after HHP treatment at 600 MPa for 1 h at room temperature. Souring was delayed for about 24 h after treatment at 200 MPa. Hite *et al.* (1914) found that most pressure-treated fruits remained commercially sterile for at least 5 years after HHP treatment at 400–820 MPa. Hite's last contribution to the field was in 1929 (Giddings *et al.*, 1929), in which tobacco mosaic virus was treated at pressures above 930 MPa which produced inconsistent inactivation (Farkas and Hoover, 2000).

Bridgman (1914) investigated the coagulation of albumen under pressure. He reported "a fact of possible biological interest": "If the white of an egg is subjected to hydrostatic pressure at room temperature, it becomes coagulated, presenting an appearance much like that of a hard boiled egg" (Balny, 2006). This phenomenon was elucidated by Grant *et al.* (1941) as the protein denaturation phenomenon.

Larson *et al.* (1918) showed that HHP can inhibit microbial growth and cause cells to die. Vegetative types were killed after 14 h at 607 MPa. It was recognized that spores of bacteria were extremely resistant to HHP, but could be killed at 1214 MPa. Basset and Macheboeuf (1932) reported the survival of spores of *Bacillus subtilis* exposed to more than 1724 MPa for 45 min. The effect of HHP on viruses (Basset and Macheboeuf, 1933a; Basset *et al.*, 1935, 1956; Atanasiu *et al.*, 1951), and on antigens, antibodies, and their influence on immunogenicity (Basset and Macheboeuf, 1933b) were also studied.

Timson and Short (1965) pressurized milk at 1034 MPa and 35°C for 90 min and learned that approximately 0.05% of the bacterial population was capable of surviving this pressure. Microbial analysis identified the survivors as spores of *B. subtilis* and *Bacillus alvei* (*Paenibacillus alvei*).

Gould and Sale (1970) showed the germination of bacterial spores under hydrostatic pressure. Wilson (1974) showed that low pressures (around 140 MPa) combined with temperatures (82–103°C) were effective for sterilization of low-acid foods in sealed containers. Charm *et al.* (1977) studied use of pressure for long-term refrigerated storage of foods. During the 1980s and the beginning of the 1990s, research involving HHP in food processing evolved, with the work of Elgasim and Kennick (1980) on the effects of pressure on beef proteins, Morild (1981) on the effects of high pressure on enzymes, Heremans (1982) on high-pressure effects on proteins and other biomolecules, Hoover *et al.* (1989) on the biological effects of HHP on food microorganisms, Popper and Knorr (1990) on the applications of high-pressure homogenization for food preservation, and Farr (1990) on the high-pressure technology in the food industry.

Strong efforts for setting up new food processes have been conducted in Japan (Hayashi, 1989, 1990; Ogawa *et al.*, 1990; Horie *et al.*, 1991; Tanaka and Hatanaka, 1992). On 23 April 1990, the Meijiya Food Company introduced three kinds of jam (strawberry, kiwi fruit, and apple) onto Japanese market which were made using HHP treatment without application of heat. The products were vivid and natural in color and taste. These jams were the first pressure-processed foods in history (Hayashi, 1995).

Grapefruit juice treated with HHP appeared on the Japanese market in 1991. A high pressure of 200 MPa is applied to freshly squeezed grapefruit juice for 10 min at 5°C. The shelf life of this product is 3 months at room temperature (Suzuki, 2002).

Over the last 15 years HHP technology in food processing has steadily increased and several products are now available on the market in different countries (Figure 18.1).

18.5 HIGH HYDROSTATIC PRESSURE PROCESS AND EQUIPMENT

HHP is as a batch process where prepackaged food products are treated in a chamber surrounded by a pressure-transmitting fluid (generally water). Semi-continuous systems have been developed for pumpable foods where the product is compressed without a container and subsequently packaged aseptically (Campus, 2010).

The primary components of an HHP system include a pressure vessel; closure(s) for sealing the vessel; a device for holding the closure(s) in place while the vessel is under

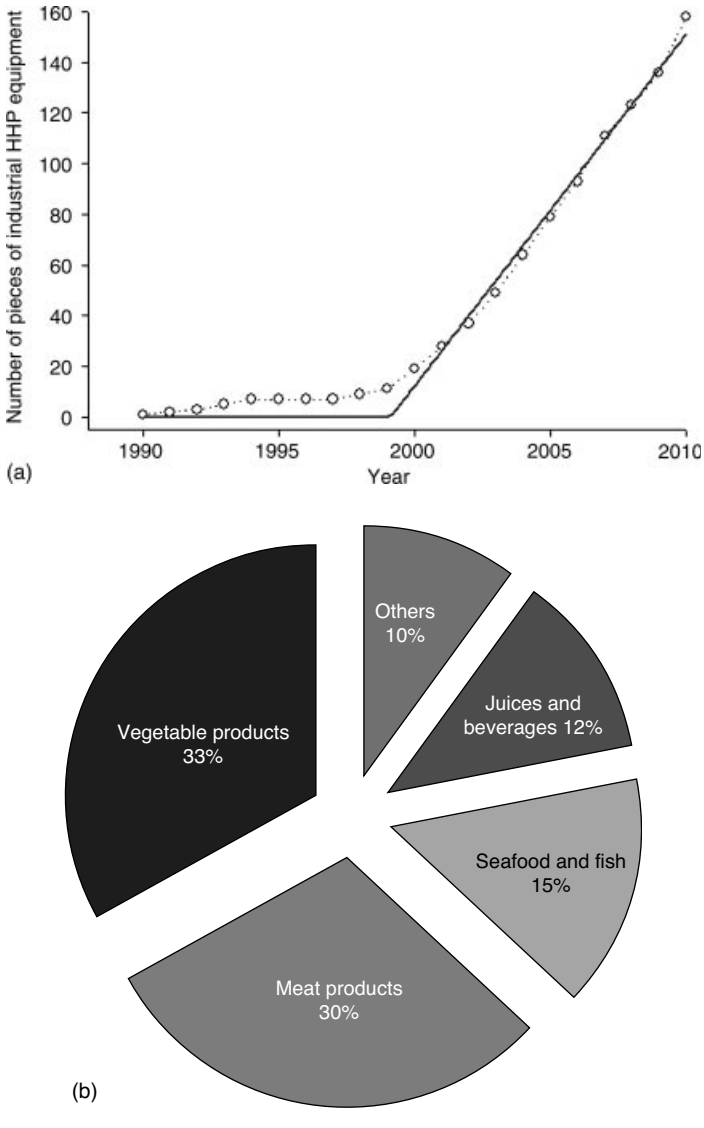

Figure 18.1 Graph showing (a) the amount of industrial HHP equipment installed around the world; (b) use of industrial HHP equipment in different food industries (adapted from Campus, 2010).

pressure; high-pressure intensifier pump(s); a system for controlling and monitoring the pressure and temperature; and a product-handling system for transferring product to and from the pressure vessel (Campus, 2010).

The generation of pressure inside the vessel may be achieved by one of three ways, as follows:

(i) Direct compression: the volume of the treatment chamber is reduced by the action of hydraulic pressure applied with a piston.

(ii) Indirect compression: in these systems an intensifier or high-pressure pump is used to pump the pressurizing medium directly into the vessel to reach a given pressure. This is the method generally used for the application of HHP in food processing.

(iii) The third pressurization method, which has not been used in the food industry so far, involves heating the pressure-transmitting medium inside the vessel to cause expansion by an increase in temperature (San Martín, 2002).

Food products are generally held for 3–5 min at 400–600 MPa. Approximately 5–6 cycles/h are possible, allowing time for compression (to the target pressure value), holding, decompression (expansion to atmospheric pressure), loading, and unloading. After HHP treatment, the processed product is removed from the vessel and stored/distributed in a conventional manner (see Balasubramaniam *et al.*, 2008).

Liquid foods can be processed in a batch or semi-continuous mode. In the batch mode, the liquid product is prepackaged and HHP-treated as described above for packaged foods. Semi-continuous operation requires two or more pressure vessels, each equipped with a free-floating piston that allows each vessel to be divided into two chambers. One chamber is used for the liquid food; the other for the pressure-transmitting fluid. The basic operation involves filling one chamber with the liquid food to be treated. The fill valve is closed and then pressure-transmitting fluid is pumped into the second chamber of the vessel on the opposite side of the floating piston. Pressurization of the fluid in this second chamber results in the compression of the liquid food in the first. After an appropriate holding time, the pressure is released from the second chamber. The product discharge valve is opened to discharge the contents of the first chamber, and a low-pressure pump injects pressure-transmitting fluid into the second chamber, which pushes on the piston and expels the contents of the product chamber through the discharge valve. The treated liquid food is directed to a sterile tank from which sterile containers can be filled aseptically (Farkas and Hoover, 2000; Campus, 2010).

Avure Technologies (USA), NC Hyperbaric (Spain), and Uhde (Germany) are major suppliers of commercial-scale pressure equipment. Both horizontal and vertical pressure-vessel configurations are available (commercial size from 30 to 600 L capacity) for batch HHP equipment. Avure Technologies also make semi-continuous systems for the processing of liquid beverages such as juices. While commercial pressure vessels have a pressure limit of 700 MPa, research machines can go up to 1400 MPa. A commercial-scale high-pressure vessel costs between approximately US$500 000 and $2.5 million dollars depending on capacity and the extent of automation (Balasubramaniam *et al.*, 2008).

Currently, HHP treatment costs are quoted as ranging from 8.8 to 22.1 US cents/kg, including operating cost and depreciation, and are not higher than for thermal food processing (Sáiz *et al.*, 2008). With two 215 L HHP units operating under typical food-processing conditions a throughput of approximately 9000 tonnes per year is achievable. High throughput is accomplished by using multiple pressure vessels. Factory production rates beyond 18 000 tonnes per year are now in operation. As demand for HPP equipment grows, capital costs and operating costs will continue to decrease. Consumers benefit from increased shelf life, quality and availability of value-added and new types of foods, which are otherwise not possible to make using thermal processing methods (http://grad.fst.ohio-state.edu/hpp/faq.html; Balasubramaniam *et al.*, 2008).

18.6 COMMERCIAL HIGH HYDROSTATIC PRESSURE-TREATED FOOD PRODUCTS AROUND THE WORLD

One can find numerous state-of-the-art reviews on HHP and its effects on biological systems such as microorganisms, proteins, and enzymes (Hoover *et al.*, 1989; Cheftel, 1992, 1995a; Hoover, 1993; Knorr, 1993; Farkas and Hoover, 2000; Patterson, 2000, 2005; San Martín, 2002; Matser *et al.*, 2004; Mañas and Pagán, 2005; Considine *et al.*, 2008). These will not be repeated here. Instead, in this section we will summarize the commercial HHP-treated food products that are available.

18.6.1 Meat products

The application of HHP to fresh meat products is a powerful tool to control risks associated with *Salmonella* spp. and *Listeria monocytogenes* (Campus, 2010). Commercial applications of HHP to meat products include several ready-to-eat pork and poultry products, as listed in Table 18.3.

Table 18.3 HHP-treated meat products on the world market (adapted from www.nchyperbaric.com).

Country (year)	Product	Treatment	Achievements
Spain (1998)	Delicatessen: cooked sliced ham and "tapas" (pork and poultry cuts)	400 MPa 10 min at 8°C	Sanitization without modifying color and taste
USA (2001)	Cooked sliced ham, pork meat products, and Parma ham		Sanitization without modifying color and taste Destruction of *Listeria*
USA (2001)	Ready-to-eat poultry products		Sanitization without modifying color and taste Destruction of *Listeria*
USA (2002)	Spicy sliced precooked chicken and beef for fajitas		Sanitization without modifying color and taste Destruction of *Listeria*
Spain (2002)	Thick sliced ham, chicken and turkey products, cooked and Serrano ham, chorizo	500 MPa 4–10 min at 8°C	Sanitization without modifying color and taste Destruction of *Listeria* Increase in shelf life and reduction of additives
Italy (2003)	Parma ham (prosciutto), salami, mortadella	600 MPa 10 min at 7°C	Sanitization without modifying color and taste Destruction of *Listeria* Increase in shelf life Products for US and Japanese export
Japan (2005)	Cooked pork meat products, nitrite-free ham, sausages, and bacon	600 MPa 5 min at 5°C	Microbial inactivation Increase in shelf life
Germany (2005)	Smoked German ham: whole, sliced, and diced products	600 MPa 2 min at 5°C	Destruction of *Listeria* Products for US export

Table 18.4 HHP-treated seafood products on the world market (adapted from www.nchyperbaric.com).

Country (year)	Product	Treatment	Achievements
USA (1999)	Oysters sauce for oyster dishes	200–350 MPa 1–2 min	Opening of the shells (kept closed by a plastic band) Destruction of *Vibrio vulnificus* Marketing of fresh and frozen opened oysters
USA (2001)	Oysters	240 MPa 90 s	Opening of the shells (kept closed by an elastic band) Destruction of *Vibrio*
Canada (2004)	Seafoods		Opening of the shells
Spain (2004)	Ready-to-eat fish: salmon, hake	500 MPa	Reconstituted sanitized sliced fish without modifying color and taste Destruction of *Listeria* Increase in shelf life and reduction of additives Ready to eat after 1.5 min in a microwave
Italy (2004)	Desalted cod	600 MPa	Increase in shelf life, sanitization
South Korea (2006)	Oysters	Indirect	Opening of shells, destruction of *Vibrio*

18.6.2 Seafood and fish products

HHP is a valid process for reducing pathogenic *Vibrio*, coliform bacteria, and viruses in fish products. Table 18.4 summarizes the commercial HHP-treated seafood and fish available throughout the world.

18.6.3 Vegetable products

HHP is a valuable tool for the production of microbiologically safe and shelf-stable vegetables without change in flavor (Guerrero-Beltrán *et al.*, 2005). Commercial HHP-treated vegetable products are listed in Table 18.5.

18.6.4 Juices and beverages

HHP leads to microbial inactivation while keeping the organoleptic properties of juice mostly intact. Commercial HHP-treated juices and beverages are listed in Table 18.6.

18.7 CONSUMER ACCEPTANCE OF HIGH HYDROSTATIC PRESSURE PROCESSING

Consumers form opinions about new technologies, especially when they are applied to food production. Some methods, like organic production, are warmly welcomed by many consumers, whereas others, like genetic modification and irradiation, have been firmly rejected. Novel processes like the use of HHP are probably somewhere between these extremes, but it is important to understand how consumers will form opinions about these technologies before attempting large-scale introductions onto the market (Olsen *et al.*, 2010).

Table 18.5 HHP-treated vegetable products on the world market (adapted from www.nchyperbaric.com).

Country (year)	Product	Treatment	Achievements
Japan (1990)	Jams: apple, strawberry, blueberry Coatings: apple, strawberry, blueberry Sauces: apple-onion, orange, grapefruit Fruit jellies: orange, pineapple, grapefruit, mandarin	Indirect 400 MPa 10–30 min without temperature regulation	Preservation of fruit color and fresh taste Vitamin C content is unmodified
Japan (2000)	Precooked rice, hypoallergenic rice	400 MPa	Products for hospitals
Italy (2001)	Fruit desserts: apple, pear, strawberry	Indirect 3–5 min at 600 MPa and 17°C	Enzyme inactivation; keeping sensorial properties of fresh fruit purees Shelf-life increase without the help of chemicals
USA (2002)	Avocado-based products	Indirect	Enzyme inactivation; keeping sensorial properties of fresh avocado Shelf-life increase without the help of chemicals
Mexico (2002)	Avocado-based products	Indirect	Enzyme and microbial inactivation; keeping sensorial properties of fresh avocado Shelf-life increase without the help of chemicals
USA (2003)	Sliced onions	Indirect	Microbial inactivation No bitterness; fresher and crunchier Increase of shelf life
Canada (2003)	Apple-based purees and sauces		Microbial inactivation Preservation of sensorial properties of fresh apple Increase in shelf life
USA (2004)	Soya products, tofu		Microbial inactivation Increase in shelf life
Spain (2005)	Ready-to-eat vegetable dishes	500 MPa	Microbial inactivation Increase in shelf life

Table 18.6 HHP-treated juices and beverages on the world market (adapted from www.nchyperbaric.com).

Country (year)	Product	Treatment	Achievements
Japan (1993)	Sake (rice wine)	Indirect 400 MPa 30 min, 15°C	Yeast inactivation without thermal treatment Retaining the specific taste of raw sake
France (1994)	Orange, lemon, grapefruit juice	Indirect 400 MPa 1 min, ambient temperature	Microbial inactivation Retaining the sensory qualities of fresh juice
Mexico (2000)	Citrus juices, smoothies	Indirect and direct 500 MPa	Microbial inactivation Retaining sensory qualities of fresh juice
Lebanon (2001)	Fruit juices (54 different varieties or blends)	Indirect 500 MPa	Microbial inactivation Retaining sensory qualities of fresh juice
USA (2001)	Organic apple juice	Direct, semi-continuous	Microbial inactivation Retaining sensory qualities of fresh juice
Portugal (2001)	Apple juice, citrus/apple juices	Indirect 450 MPa 20–90 s 12°C	Microbial inactivation Retaining sensory qualities of fresh juice
Italy (2001)	Apple, pear, strawberry, carrot juices	Indirect 600 MPa 3–5 min 17°C	Microbial inactivation Retaining sensory qualities of fresh juice
USA (2002)	Orange juice, lemonade, smoothie	Direct 2 min	Microbial inactivation Retaining sensory qualities of fresh juice
Czech Republic (2004)	Broccoli/apple juice	Indirect	Microbial inactivation Retaining sensory qualities of fresh juice

A study in which personal interviews with 3000 consumers in France, Germany, and the UK were conducted (Butz *et al.*, 2003) revealed that HHP was acceptable to the majority of consumers in France and Germany. It was important that the product price does exceed not that of conventional products and that there is a health benefit. Those who perceived the greatest personal advantage from the technology were most likely to buy the product. This group tended to include a higher proportion of young, educated people (Olsen *et al.*, 2010).

Surveys were administered by Cardello *et al.* (2007) to 225 potential consumers of foods processed by innovative and emerging food technologies (HHP, pulsed electric fields (PEFs), irradiation, ionizing energy, genetic modification) to assess the factors contributing to their interest in using such products. Respondents rated their interest in 49 different food-product concepts that varied in food type, processing or production technology, costs, benefits, risks, endorsing agencies, and product information. Among the emerging technologies assessed, irradiation and genetic modification resulted in the greatest negative effect on likely use, while HHP processing produced the most positive effect.

A study was carried out with 96 consumers of fruit juice between 18 and 66 years of age living in Brazil (Deliza *et al.*, 2005). Results showed that when the advantages of HHP technology were presented on pineapple juice labels, participants understood the benefits and expressed a higher intention to purchase the product. A qualitative study on consumer attitudes towards HHP and PEF processing of food was carried out (Nielsen *et al.*, 2009). Ninety seven adults between 20 and 71 years of age participated in Slovenia, Hungary, Serbia, Slovakia, Norway, and Denmark using a common guideline. The results showed that environmental friendliness and the more natural state of the product were seen as the main advantages of PEF and HHP processing, while there were concerns about body and health, the higher price of the products, and the lack of information about the technologies, along with a general scepticism. The study also showed that Northern European participants were a bit more sceptical towards PEF and HPP products than Eastern European participants.

An online consumer survey in the USA was implemented by Hicks *et al.* (2009) to assess the awareness of alternative food-processing technologies, consumer food safety attitudes and knowledge, and the willingness to pay for HHP-treated products. The survey was completed by 1204 adults. Results revealed that while traditional methods – that is, canning, freezing, and microwaving – were all well recognized by over 80% of respondents, only 8% recognized HHP. Trends indicated that an increase in age, education, and income reflected greater food safety knowledge. Given an explanation of HHP and its benefits, 39% of respondents indicated they would be willing to pay an additional cost for such foods, with higher income and education having the most impact.

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19 Role of Predictive Microbiology in Food Preservation

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Abstract: The levels of microorganisms in food ecosystems can be controlled by diverse factors such as temperature, pH, water activity or preservatives, which determine their growth, survival or inactivation. Predictive microbiology is a multidisciplinary area of food microbiology which is devoted to studying and predicting, by means of mathematical models, the effects of the mentioned factors on microbial behaviour. The present chapter shows an overview of the successive steps to follow for the successful development and implementation of a predictive model, as well as the most common types of secondary models used in food microbiology. The chapter continues with a general review of the most powerful predictive microbiological tools that are currently available on the web, and finalizes by commenting on the direct implications and applications of predictive microbiology in food preservation.

Keywords: food safety; food spoilage; mathematical modelling; predictive microbiology

19.1 MICROORGANISMS IN FOODS

19.1.1 Why is it necessary to control microbial growth in foods?

Food microbiology is a branch of microbial ecology concerned with studying the occurrence and growth of microorganisms in foods. Diverse yeast, bacterial and filamentous fungal genera are associated with food and beverage ecosystems, where they can play a double role.

Many types of beverage (beer, wine and cider) and foods (bread, meats and vegetables) are produced industrially through the favourable action of microorganisms (ICMSF, 2005; Querol and Fleet, 2006). For example, the preservation of foods by fermentation is a widely practised and ancient technology. Fermented foods are more stable over time, either by the production of antimicrobial compounds (bacteriocins, lactic acid, ethanol, etc.) or by the reduction of the initial sugar concentration provided by the raw material. Throughout fermentation, diverse compounds are also produced or transformed due to the metabolism of microorganisms, making some foods more digestible and considerably influencing the flavour and aroma characteristics of the final product. A second favourable aspect of microorganisms in foods is that they can also be used as probiotic or biocontrol agents, either to encourage the natural human defenses (Williams, 2010) or to control the growth of undesirable species (Barnby-Smith, 1992; Querol and Fleet, 2006) respectively.

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However, microorganisms can also represent an important risk for consumers. Pathogen species such as *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes* or *Clostridium botulinum*, among others, cause thousands of outbreaks of food diseases each year around the world due to poorly processed foods, both in developing and developed countries (Much *et al*., 2009; Minami *et al*., 2010; Newell *et al*., 2010). In addition, spoilage microorganisms can alter the appearance and flavour of foods and beverages, which originate the rejection of the product by consumers with the consequent loss of money. Thus, unwanted microbial growth in a food-processing environment can be disastrous. The economic and social consequences of microorganisms in foods depend not only on the species present but, most importantly, on their quantitative populations. Populations in foods are not static and can change both qualitatively and quantitatively throughout production or packaging. It is the number of microbial cells that ultimately determines whether or not the product will cause an outbreak of disease or develop off-flavours (Fleet, 1999).

For all these reasons, it is essential to control the microbial life cycle in food ecosystems, favouring the growth of desirable microorganisms and inhibiting spoilage and pathogens.

19.1.2 Main factors affecting microbial growth and survival in food ecosystems

An important factor that affects the behaviour of microorganisms in foods is the composition and characteristics (broth, solid or semi-solid) of the matrix. Food and beverages have a very heterogeneous composition compared to laboratory media. Much of our knowledge on microbial growth is derived from pure culture studies carried out in laboratory or well-defined media. However, real foods are more complex, and the ability of microorganisms to grow and proliferate and, consequently, to consume carbon and nitrogen sources, vitamins, trace metals or other essential compounds provided by the raw material will depend of the form in which these substrates are available (Boddy and Wimpenny, 1992; Fleet, 1999). Each genus has its own particular nutritional requirements, and each one will be affected in predictable ways by the composition of the food. Moreover, some foods have natural compounds that end up being toxic for many microorganisms (essential oils, polyphenols, etc.).

The growth, survival and activity of any species or strain, whether it be an unwanted or a desirable organism, will, in most cases, be determined by the presence of other species. With the exception of highly controlled processed products, most foods may carry a complex mixture of microorganisms which includes diverse species of bacteria, yeasts and filamentous fungi. Moreover, large populations of microorganisms can be formed by different subpopulations or strains of the same species, which can present different degrees of adaptability or responses to environmental factors. Interactions (antagonism, commensalism or synergism) between microorganisms in the food matrix can determine the dominance or inhibition of the present species (Fleet, 1999). Antagonism is probably the best known microbial interaction because it can be applied to enhance food quality and safety. For example, lactic acid bacteria are widely exploited in the biopreservation of foods by the production of lactic acid and bacteriocins (Boddy and Wimpenny, 1992; De Vuyst and Vandamme, 1994), which inhibits the growth of many Gram-negative bacteria. Somewhat analogous to the production of bacteriocins by bacteria is the production of killer toxins by yeasts. These are proteins that inhibit the growth of other killer-sensitive yeasts (Fleet, 1999).

There are also a certain number of factors, so-called extrinsic factors, which affect the microorganism response in foods. Among the most important, pH, temperature, water

activity or diverse types of preservatives (acetic acid, sorbate, benzoate, sulphites, etc.) can be mentioned. The effects of these extrinsic factors on microorganism response have been widely studied by food microbiologists, especially for foodborne pathogens (ICMSF, 1996), since they can easily be modified and controlled by the food industry during processing. Each microorganism presents an optimum value of pH, temperature and water activity for growth. A substantial growth reduction can be achieved when conditions are displaced away from these optimal values, reaching even regions where the microorganism is not able to grow. In food environments, rarely is a single stress present, and microbial cells are simultaneously exposed to a combination of stresses (the hurdle effect). The effect of the combined stresses on growth and survival may be additive or synergistic, which originates a significantly greater influence on microorganism (Leistner and Gorris, 1995).

The study of all these effects on microbial response in food ecosystems are the bases of predictive and risk assessment strategies. Reliable, confident decisions concerning public health and spoilage risks require quantitative ecological data that take into consideration the combined effects mentioned above. In this way, predictive microbiology is a powerful tool for food industries because it may help the people in charge of them to make such decisions.

19.2 PREDICTIVE MICROBIOLOGY

19.2.1 Origin and concept

Fermentation, drying or salting have been used by humans for thousands of years to preserve foods, representing an empirical approach to the control of microbial populations. On the contrary, predictive microbiology currently offers a quantitative and objective approach to food preservation, emerging as a new and crucial element of food microbiology.

Predictive microbiology probably arose in 1922 with the appearance of the first model that described the thermal inactivation of *C. botulinum* type A spores (Esty and Meyer, 1922). References to the potential use of predictive microbiology to describe microbial growth can also be found from literature published in the 1930s, when Scott understood the benefit of accumulating kinetic microbial growth data to predict the shelf life and safety of foods (Scott, 1937). The origin of 'modern' predictive microbiology can be traced to the 1960s and 1970s, when kinetic and probability models were used to address both food spoilage and poisoning problems (Spencer and Baines, 1964; Nixon, 1971; Genigeorgis, 1981). However, it was not until the 1980s, with the development of computer technology and statistical software (which considerably reduced the complexity of calculus for model development), together with the appearance of diverse outbreaks of food poisoning, when predictive microbiology really had an important expansion. Research articles and reviews of predictive microbiology quickly began to appear (Roberts and Jarvis, 1983; Farber, 1986; McMeekin and Olley, 1986; Baird-Parker and Kilsby, 1987). Since then, hundreds of papers, book chapters and general reviews have been written on this topic.

Predictive microbiology is an interdisciplinary area where statisticians, food microbiologists, mathematicians, food technologists and computer scientists all collaborate. In the first book on the subject, published by McMeekin *et al.* (1993), predictive microbiology was defined as a quantitative science that enables users to objectively assess the effect of processing, distribution and storage on the microbiological safety and quality of foods. A later book on the field, published by McKellar and Lu (2003), define it as the quantitative description of the microbial response in food ecosystems by mathematical models. Thus, as

can be directly deduced from both definitions, a first and important step in the development of a predictive model is the accumulation of data on microbial behaviour in foods. The increasing importance and usefulness of predictive microbiology in foods favoured the publication of a third book on the matter, recently edited by Brul *et al.* (2007).

A model can be defined as 'the description of a system, theory, or phenomenon that accounts for its known or inferred properties and may be used for further study of its characteristics'. The model is an often simplified description of relationships between observations of the system (dependent variables) and the factors that are believed to cause the observed responses (independent variables). Such a description can be expressed quantitatively in one or more mathematical relationships or equations. A mathematical model can simply describe a collection of data, or can represent a hypothesis or series of hypotheses about underlying relationships among the independent variables. The first approach is often termed an empirical model, while the latter is described as mechanistic (McMeekin *et al.*, 2008). Both types of model can be used to assess the risks of food processing and consequently to implement control measures to protect the microbiological quality and safety of foods, anticipating the behaviour of pathogen and spoilage microorganisms. It is clear that predictive microbiology has a major role to play for industry, government and consumers as a modern and essential tool for food microbiology. Below, the steps to follow in the development and implementation of a predictive model in foods are presented. The most important types of secondary models which have been used in food microbiology are also addressed. Due to the huge expansion that predictive food microbiology has shown in recent years, compiling all the current methodologies available is a vast task.

19.2.2 The modelling process

All models seek to link observations to the variables believed to control them. The successful development and implementation of a predictive model in foods and beverages involves a series of steps that include, in this order, a detailed study of the matrix, choice of an appropriate experimental design, data collection, primary modelling, secondary modelling and, finally, model validation (Figure 19.1). The final result is obtaining a safe and useful tool to evaluate the applications of corrective actions during food processing.

19.2.2.1 Study of the food matrix

Foods and beverages are heterogeneous media. Foods provide all the substrates necessary to support microbial growth (sugars, nitrogen compounds, vitamins, oligoelements, etc.) but sometimes can also have toxic compounds for microorganisms. Thus, a first and vital step for model development is detailed physicochemical information on the characteristics of the matrix to establish the levels of the most important compounds presents. In the food industry it is possible to manipulate the levels of many preservatives in foods. However, the control of the compounds present in the raw material is more difficult because their levels can vary depending of many factors. Thus, three alternatives appear when choosing the medium for model development. The first option is to use a standard laboratory medium the components and constants of which are well known, and which is modified by the factors that the industry can govern (salt, pH, temperature, etc.). The second alternative, and a better approximation to authentic conditions, is to obtain a simulated food ecosystem where many of the food components are added in known concentrations. For example, in wine studies, a synthetic must that mimics the natural must composition is widely used (Rossignol *et al.*, 2003). A third

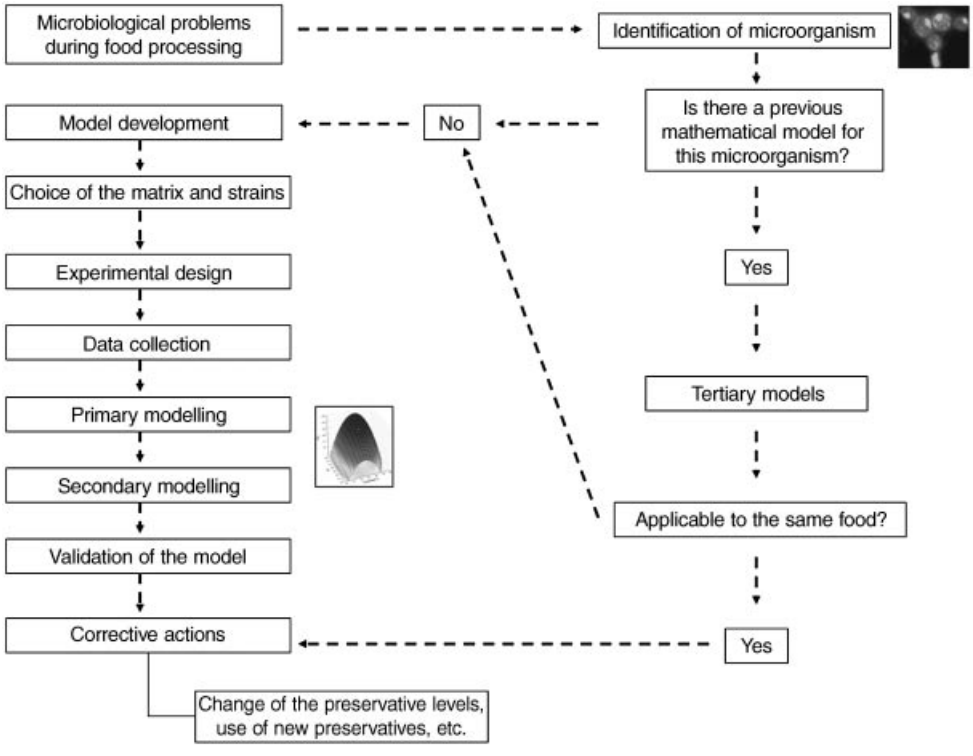

Figure 19.1 Development of a predictive model.

and more laborious option is to work directly with the food. In this case, the food has to be previously sterilized (by immersion in sodium hypochlorite, irradiation, etc.) before inoculation. Inoculation can be carried out with only a single strain of the species, or, more appropriately, with a cocktail of strains which leads to a better estimation of the general behaviour of microorganisms (Panagou and Nychas, 2008; Valero *et al.*, 2009).

19.2.2.2 Experimental design

This is an important step because the choice of the experimental design will determine the number of experiments to be carried out, the combination of factors and how data will be analysed and processed. In this step, one must establish the range and number of environmental factors to study. The experimental design must be chosen as a function of the final objectives. For instance, if we can build a polynomial model, the application of central composite or D-optimal designs will be useful. These designs considerably reduce the total number of experiments to be performed with respect to a factorial design, and consequently save cost and time. They have already been satisfactorily applied to model different *Lactobacillus* and yeast species related with foods (Tsapatsaris and Kotzekidou, 2004; Arroyo *et al.*, 2005; López *et al.*, 2006). On the contrary, a full or fractional factorial design is most appropriate to estimate the growth/no-growth boundaries of microorganisms, as was proved recently for *Monascus ruber*, *Saccharomyces cerevisiae* and *Staph. aureus* (Panagou *et al.*, 2003; Arroyo-López *et al.*, 2007b; Valero *et al.*, 2009). A third type of design with a great application in the food industry is the mixture of designs, where the response is

associated to the proportion of the components in the mixture. In this way, Arroyo-López *et al.* (2008a) used a simplex-centroid mixture design to evaluate the combination of different proportions of sodium chloride, ascorbic acid and sodium metabisulphite on the microbiological profile of Manzanilla–Aloreña table olive fermentation.

19.2.2.3 Data collection

Microorganism response over time can be determined in several ways, but the most common procedures are plate-count and optical density (OD) measurements. Data collection is a laborious and expensive step which can be facilitated by the use of an automatic apparatus (spiral plate maker, spectrophotometer, bactometer, etc.). Each method presents its own limitations and advantages. Plate count provides a direct estimation of the total viable cells, but is extremely laborious and time-consuming. On the contrary, OD is faster and cheaper, but it is an indirect method that does not offer information on cell vitality. Other techniques such as flow cytometry have also been successfully used in predictive microbiology to collect growth data (Sørensen and Jakobsen, 1997). An important advantage of this methodology is that cells can be marked individually with different fluorochromes, providing real-time information of cell vitality.

19.2.2.4 Primary modelling

Microbial population density changes with time in a specified environment can be segmented into four phases: lag phase, growth phase, stationary phase and finally death phase. Many primary models have been developed for microbial growth, which include the first three phases, but few have been built in the case of inactivation or survival (death phase).

Essentially, the function of a primary model is to obtain the growth/inhibition parameters of the microbial population for each of the treatments established in the experimental design. In the case of microbial growth, diverse sigmoid equations (modified Gompertz, Baranyi–Roberts, logistic, etc.) are used to fit the experimental data and obtain the growth parameters (lag phase, maximum specific growth rate and maximum population level). This task is accomplished by curve-fitting with appropriate software. A non-linear regression procedure is usually used for this purpose. Some of the most widely used primary models are described in Table 19.1. While indirect methods, such as turbidimetry, are widely used in the

Table 19.1 Several primary models used to fit the growth phase of microorganisms in foods.

Model	**Equation**
Modified Gompertz (Zwietering *et al.*, 1990)	$Y = A^{*}\exp(-\exp\{(\mu_m{}^{*}e^{*}(\lambda - t))/A + 1\})$ where $A = \ln(N_\infty/N_0)$, upper asymptote
Modified Logistic (Zwietering *et al.*, 1990)	$Y = A/(1 + \exp\{(4\,\mu_m(\lambda - t))/A + 2\})$ where $A = \ln(N_\infty/N_0)$, upper asymptote
Modified Richards–Stannards (Zwietering *et al.*, 1990)	$Y = A\{1 + \nu^{*}\exp(1+\nu)^{*}\exp[(\mu_m/A)^{*}(1+\nu^{(1+(1/\nu))})^{*}(\lambda - t)]\}^{(1/\nu)}$ where $A = \ln(N_\infty/N_0)$, upper asymptote; ν = parameter of the model
Baranyi–Roberts (Baranyi and Roberts, 1994)	$\ln N = N_0 + \mu_m{}^{*}A(t) - \ln\{1 + [\exp(\mu_m{}^{*}A(t) - 1)/\exp(N_\infty - N_0)]\}$ where $A(t) = t + (1/\mu_m)\ln\{[\exp(-\mu_m{}^{*}t) + 1/(\exp(\lambda^{*}\mu_m) - 1)]/[1 + (1/(\exp(\lambda^{*}\mu_m) - 1))]\}$

General symbols: $Y = \ln(N_t/N_0)$; N_t = colony-forming units at time t; N_0 = colony-forming units at $t = 0$; N_∞ = colony-forming units at $t = \infty$; μ_m = maximum specific growth rate; λ = lag phase; t = time.

Table 19.2 Several primary models used to simultaneously fit both the growth and decay phases of microorganisms in foods.

Model	Equation
Peleg (1996)	$N(t) = (N_s{*}(1 + \exp(k_1(t - t_{cl}))))/(1 + \exp(k_g(t_{cg} - t)))$ where $N(t)$, population at time t; N_s, maximum population density; k_g, growth rate constant; t_{cg}, time to reach half the maximum population; k_1, decline rate constant; t_{cl}, time to reach 50% survival.
Two-term Gompertz equation (Bello and Sánchez-Fuertes, 1995)	$\log N_t = \log(N_0) + k_1{*}\exp(-\exp(-k_2(t - k_3))) - k_4{*}\exp(-\exp(-k_5(t - k_6)))$ where N_t, population at time t; N_0, initial population; k_1, increase of microorganisms from the initial level to the maximum; k_2, relative growth rate; k_3, time at which growth rate is maximum; k_4, decrease from the maximum to a minimum level; k_5, relative death rate; k_6, time at which death rate is maximum.
Simplified Churchill model (Membré *et al.*, 1997)	$\log N_t = (1/k_1{*}\exp(-\lambda_1{*}t) + 1/k_2{*}\exp(\lambda_2{*}t))^{-1}$ where N_t, population at time t; k_1 and k_2, constants; λ_1 and λ_2, growth and decline rates, respectively.

development of growth models, only viable counts data are valid for inactivation or survival studies. The inactivation of microorganisms may reflect an initial 'shoulder', a linear reduction or sometimes the presence of a tail. Van Boekel (2002) developed an inactivation model based on the Weibull distribution which can be used to determine the shape of the inhibition curve as well as the time necessary to take the first decimal reduction. The Weibull model was recently applied to determine the inactivation parameters of the olive-spoiling yeast *Issatchenkia occidentalis* as a function of citric and sorbic acids (Arroyo-López *et al.*, 2007a). In another inactivation survey, the survival of the pathogen microorganism *E. coli* O157:H7 during the fermentation of green olives was studied by Spyropoulou *et al.* (2001) by calculating the death rate of the bacteria. There are other types of primary model which are used to simultaneously fit both the growth and decay phases of microorganisms in foods, such as the quasi-chemical primary model (Taub *et al.*, 2003), the Peleg model (1996) or the two-term Gompertz equation proposed by Bello and Sánchez-Fuertes (1995). Mathematical equations for some of these are shown in Table 19.2.

Finally, for cases where the probability of growth is the only relevant factor, which is useful to determine the growth/no-growth boundaries of microorganisms as a function of environmental factors, data may be scored simply as growth (scored as 1) or no-growth (scored as 0), representing a simple binary response.

19.2.2.5 Secondary modelling

Secondary models are built with parameters estimated from primary modelling, and they are used to quantitatively characterize these parameters as a function of the environmental factors included in the experimental design. Sometimes, primary model parameters need to be transformed ($\log_{10}$, square root, etc.) before the modelling process in order to homogenize the variance of data and to improve the quality prediction of the secondary model, but once the secondary model has been built, this can be used to predict the response of microorganisms against new combinations of environmental variables not included originally in the experimental design.

The type of secondary model used is closely related to the experimental design chosen. Secondary models can be easily obtained by non-linear regression, or conversely be more

complex polynomial, probabilistic or artificial neural network models that require sophisticated computational software for data processing and analysis. Fortunately, a wide range of powerful regression software tools are now readily available for use on laptop computers to assist in the development of reliable and robust secondary mathematical models. By means of the mathematical analysis of secondary models, the environmental variables with the highest influence on the response can be identified. Below, some of the most habitual predictive models used in food microbiology are highlighted.

There is a first type of secondary model which is exclusively related to explaining the effects of a single environmental factor. In this way, McMeekin *et al.* (1993) mentioned different types of secondary models for temperature based on the Bělehrádek and Arrhenius models. The Bělehrádek-type model can be written in its general form as:

$$k = (a(t-t_0))^d \tag{19.1}$$

where k = rate, and a, d and t_0 are parameters to be fitted. The parameter t_0 is regarded as the temperature below which no growth is possible. Based on this equation, the most successful has been the so-called square root model proposed by Ratkowsky *et al.* (1983), which has the following expression:

$$\sqrt{k} = b(T-T_{\min}) \tag{19.2}$$

where $T_{\min}$ is calculated from the regression line derived from the plot of $\sqrt{k}$ as a function of T, and b is a parameter to be fitted. An extension of equation 19.2 is the four-parameter square root model developed to include the whole biokinetic temperature range. Its equation is as follows:

$$\sqrt{k} = b(T-T_{\min})\{1-\exp[c(T-T_{\max})]\} \tag{19.3}$$

where b and c are parameters of the model with no biological meaning, and $T_{\max}$ is the temperature above which growth is not possible.

On the contrary, the Arrhenius-type models are based on works related to chemical reactions. The general form is:

$$k = A\exp(-E_a/RT) \tag{19.4}$$

where k is the specific reaction rate constant, E_a is the activation energy of the 'reaction' system, T is the absolute temperature, R is the gas constant and A is a 'collision factor'. Models derived from the Arrhenius-type are less known, although Davey's modified Arrhenius model (Davey, 1989) is the most applied. It takes the form:

$$\mathrm{Ln}\, k = C_0 + \frac{C_1}{T} + \frac{C_2}{T^2} \tag{19.5}$$

where k is the rate, T is the temperature in degrees Kelvin, and C_0, C_1 and C_2 are the parameters to be fitted.

However, in real foods there are more factors that influence microbial growth. For this reason, diverse approaches have been developed to include them in the previous models.

Chandler and McMeekin (1989) enlarged the square root model to incorporate the effect of water activity (a_w):

$$\sqrt{k} = b(T - T_{min})\sqrt{(a_w - a_{w\,min})} \tag{19.6}$$

where T_{min} and $a_{w\,min}$ represent values below which growth is not possible. The combined effect of pH and temperature was also studied by Adams *et al.* (1991) using the following equation:

$$\sqrt{k} = C(T - T_{min}) \cdot \sqrt{pH - pH_{min}} \tag{19.7}$$

where pH_{min} has the same meaning as above.

A relevant drawback of these types of models is the presence of some parameters without biological interpretation, as well as the increasing complexity as the number of factors to be studied increases. An important step in model development was the introduction of the cardinal parameter models (CPMs), in which the parameters have a biological or graphical interpretation. An obvious advantage of these models is that appropriate starting values for non-linear fitting can be easily found. Their applicability is strongly related to the simultaneous development of the gamma (γ) concept by Zwietering *et al.* (1992), which relies on the concept that many factors that affect microbial growth rate act independently and that the effect of each measurable factor on growth rate can be represented by a discrete term, a fraction of the maximum growth rate (the rate when it is at the optimum level). The overall effect can be represented by the multiplication of all factors affecting growth rate. Then the cumulative effect of many factors at suboptimal levels can be estimated from the product of the relative inhibition of growth rate due to each factor, which is described by a growth factor (γ), a dimensionless measure that has a value between 0 and 1. In this model, the reference growth rate is μ_{max} and the reference levels for temperature (T_{opt}), water activity ($a_{w\,opt}$), pH (pH_{opt}) or other factors are those that are the optimum for growth rate. The combined effect of several environmental factors is then determined by the multiplication of their respective γ factors (McKellar and Lu, 2003):

$$\mu_{max} = \mu_{max\,opt} \cdot \gamma(T) \cdot \gamma(a_w) \cdot \gamma(pH) \tag{19.8}$$

Other known types of secondary model for simultaneously studying the combined effect of several factors are the polynomial or response surface (RS) models. The general equation of an RS model is the following:

$$Y = a + b_1S + b_2T + b_3P + b_4S^2 + b_5T^2 + b_6P^2 + b_7ST + b_8SP + b_9TP + b_{10}STP + \varepsilon \tag{19.9}$$

where Y is the biological parameter or its transformations, S, T and P are the environmental variables, and a and b_i are the parameters to be determined by the use of least squares. In case of more independent variables the model should be properly enlarged. The structure of an RS model is flexible enough to incorporate even very strong interactive effects by stating the order of the model. For this reason, RS models can provide reasonable predictions of the microbial response in food ecosystems. However, RS models are purely empirical and have certain limitations. A higher-order polynomial model, such as third order, with a great number of coefficients, can be expected to show a better fit to primary model parameters, but it often

produces greater topographic complexity. According to the principle of parsimony, a model should contain as few terms as possible. The decision to remove or include a term in the final polynomial equation only depends on whether or not the regression coefficient for the term has a significant effect on the predictive capability of the model. The sign and value of the coefficients in the regression show their influence on the modelled parameter. In this way, a negative sign is indicative that the parameter decreases when the environmental factor increases, or vice versa.

Probabilistic models are used to determine the growth/no-growth boundaries of microorganisms as a function of environmental variables. The general model can be represented by:

$$\text{Logit}\, p = a + b_1 S + b_2 T + b_3 P + b_4 S^2 + b_5 T^2 + b_6 P^2 + b_7 ST + b_8 SP + b_9 TP + b_{10} STP + \varepsilon \tag{19.10}$$

where $\text{Logit}\, p = \ln(p/1-p)$ and the other symbols have the same meaning as in the RS model. These models incorporate growth/no-growth data (binary response), which are processed by means of a logistic regression model that relate the probability of growth (p) and no growth ($1-p$) with the environmental factors assayed. Logistic regression requires a higher number of experiments in comparison with polynomial models, but the great advantage is that they can be easily automatized by means of OD measurements. An important feature of probabilistic models is that the level of probability can be set depending on the level of stringency required, obtaining different growth/no-growth boundaries as a function of the risk that the modeller can assume. Obviously, probabilistic models have a direct application to formula packaging and processing conditions that inhibit the growth of spoilage and pathogen microorganisms (Genigeorgis, 1981; Arroyo-López *et al.*, 2007a, 2007b; Valero *et al.*, 2009).

There are also models focused on the estimation of the susceptibility and resistance of microorganisms against inhibitory compounds. Lambert and Pearson (2000) developed a simple method for the estimation of the minimum inhibitory concentration (MIC) and non-inhibitory concentration (NIC) of a determined compound using turbidimetry. The procedure relates the area under the OD/time curves to the degree of inhibition observed, using the ratio of control (absence of inhibitor) to that of the tests (progressive concentrations of inhibitor), termed as fractional area (*fa*). As the amount of inhibitor in the well increases, the effect on the growth of the organism also increases. This effect on the growth is expressed by a reduction in the area under the OD/time curve relative to the positive control (optimal conditions) at any specified time. The plot of the *fa* versus the decimal logarithm of the inhibitor concentration produces a sigmoid-shaped curve, which can be fit by a modified Gompertz function for decay (Lambert and Pearson, 2000):

$$fa = A + C^{*}\exp[-\exp(B(x-M)] \tag{19.11}$$

where A is the lowest asymptote of *fa* (approximately zero), B is a slope parameter, C is the distance between the upper and lower asymptote (approximately 1) and M is the log concentration of the inflexion point. The NIC and MIC values can then be estimated as:

$$\text{NIC} = 10^{\left(M - \frac{1.718}{B}\right)} \quad \text{MIC} = 10^{\left(M + \frac{1}{B}\right)} \tag{19.12}$$

The whole sigmoid-shaped curve is divided into three regions: zone below the NIC (concentrations of the compound at which no inhibitory effects are observed), concentrations

between the NIC and MIC (within which growth inhibition progressively occurs) and a third section, above the MIC, where no growth relative to the control is recorded. With this simple idea in mind, Lambert and Pearson (2000) calculated the MIC and NIC values for a large number of compounds against the pathogens *E. coli*, *Staph. aureus* and *Pseudomonas aeruginosa.* Bautista-Gallego *et al*. (2008) determined the MIC and NIC values of diverse chloride salts on the widely extended table olive fermentative microorganisms *S. cerevisiae* and *Lactobacillus pentosus.* A similar methodology was also recently used by Arroyo-López *et al*. (2008b) to estimate the MIC and NIC values of the preservatives sorbic and benzoic acids at selected pH values on a native spoilage yeast cocktail isolated from table olives.

Neural network (NN) models have also been widely used in predictive microbiology to model microbial response (Hajmeer *et al*., 1997; García-Gimeno *et al*., 2003; Esnor *et al*., 2006). An NN model is a computer algorithm which learns or is trained from examples through iteration and automatically derives the mathematical formulae to map the relationships between the input and output data, without any prior knowledge of their relationships. The basic construction of NN consists of input, hidden and output layers, which are composed of neuros that transmit information among the layers. NN is capable of operating with a large number of neurons at the same time, and for this reason it has been employed in predictive microbiology as an alternative to conventional regression models because of its ability to describe highly complex non-linear problems. Because of the peculiarites mentioned above, these empirical models are also known as 'black boxes'. The advantage of the use of the NN model is derived from its remarkable information-processing characteristics, such as: (i) non-linearity; (ii) noise intensity; (iii) learning and adaptativity; (iv) high parallelism; and (v) generalization. An NN model normally has no restriction on the type of relationship between the growth parameters (input patterns) and the desired outputs. Compared with RS models, the NN model is considered more versatile, flexible and less restrictive because it does not impose any assumptions pertaining to the form of functions. When NN is trained on the appropriate data set (supervised data learning) it can then be used to predict values for unseen cases (generalization) within the experimental region assayed.

The secondary models mentioned above are used to estimate the effects of environmental factors at predetermined initial levels. However, it its reasonable that dynamic food conditions should be also considered when modelling. In this way, more information has to be incorporated into the existing models, so the physiological response of microorganisms and their microbial dynamic could be explained under varying conditions. The growth process could be modelled by a differential equation, as was proposed by McKellar and Lu (2003):

$$\frac{dN_i(t)}{dt} = \mu_i(N_i(t), < N_j(t) >_{i \neq j}, < env(t) >, < P(t) >, < phys(t) >, \ldots) \cdot N_t \qquad (19.13)$$

where $i,j = 1,2\ldots$ is the number of microbial species involved in the process, $N_i(t)$ is the cell density of species i; μ_i is the overall specific rate, $<env>$ is the physicochemical environmental conditions, $<P>$ is the microbial metabolite concentrations, and $<phys>$ is the physiological state of the cells. The equation may be enlarged with any other possible influential factor.

19.2.2.6 Validation of the model

Regardless of the type of secondary model used, it is necessary to check that the model makes good predictions before it can be used for food safety and quality decisions. Sometimes,

the model is built under laboratory conditions, so validation with real food is essential to see how the model works in practical situations. A set of new experiments, not included originally in the experimental design, is carried out and the observed responses are compared with those predicted by the models. The accuracy (A) and bias (B) factors (Baranyi *et al*., 1999) have been satisfactorily used in the case of polynomial model validations (Arroyo *et al*., 2005; López *et al*., 2006). Accuracy (A) is the sum of absolute differences between predictions and observations and measures the overall model error. On the contrary, bias (B) is a multiplicative factor that is used to determine whether the model over- or under-predicts the growth response. Under-prediction of growth (or risk) is dangerous for consumers and an undesirable characteristic for the model. For probabilistic models, validation can be carried out determining the growth/no-growth limits and formulating diverse combinations of the variables where the microorganism is not able to grow (Arroyo-López *et al*., 2007b). The percentage of correct predictions is then obtained. In any case, a model is valid only to make predictions in the environment for which it was formulated, and cannot be used to extrapolate the response of microorganisms outside these limits. If the model was built only for a single strain of microorganism, it would also be convenient to corroborate that the obtained results can be extrapolated to other strains of the same species.

19.3 SOFTWARE PACKAGES AND WEB APPLICATIONS IN PREDICTIVE MICROBIOLOGY

The successful and routine use of mathematical models by the food industry, and governmental or educational agencies, will depend on the development of appropriate and useful applications for their management. Initiatives to develop microbiological modelling programs have been ongoing in the USA, the UK, Belgium, Denmark, France, Australia and other countries. These programs have resulted in the availability of a wide range of microbiological modelling software packages, to either facilitate the fit of experimental data to primary models or provide automated predictions for diverse microorganisms in specific food ecosystems. In both cases the presence of a trained operator is necessary to correctly interpret the microbiological significance of the results. Some of the more commonly used applications are mentioned below.

DMFIT is an application developed by the Institute of Food Research (IFR) in the UK to fit bacterial curves where a linear phase is preceded and followed by a stationary phase, calculating such parameters as lag phase, growth rate and maximum population level. Experimental data are directly fitted by the program to diverse primary models, mainly to the Baranyi and Roberts model (1994). A web edition can be used directly on www.combase.cc/index.php/en/about-combase/2-uncategorised/136-dmfit-web-edition, but a Microsoft Excel version is also available for downloading from the IFR web page (www.ifr.ac.uk/safety/DMfit/default.html).

GInaFiT (the Geeraerd and Van Impe Inactivation Model Fitting Tool) is also a freeware add-in application for Microsoft Excel aim to bridge the gap between developers of predictive modelling approaches and end users in the food industry who are not familiar with or have not had access to advanced non-linear regression analysis tools. The program is able to fit 10 different types of microbial survival model on user-specific experimental data relating the evolution of microbial populations over time. In this way GInaFiT can help the end user to communicate the performance of food-preservation processes in terms of the number of log cycles of reduction rather than the classical D value. It is downloadable via

the Katholieke Universiteit Leuven BioTeC homepage (http://cit.kuleuven.be/biotec/downloads.php).

Software packages and web applications that predict microorganism response versus environmental variables in real or simulated food ecosystems are also known as *tertiary models*. Many of these programs are sponsored by government agencies of different countries. These include, for example, the Pathogen Modeling Program (PMP) in both PC (http://ars.usda.gov/Services/docs.htm?docid=11550) and online versions (http://pmp.arserrc.gov/PMPOnline.aspx). PMP, developed by the US Department of Agriculture (USDA) Agricultural Research Service, is a package of models that can be used to predict the growth and inactivation of different foodborne bacteria, primarily pathogens, under diverse environmental conditions. Another program is the Seafood Spoilage and Safety Predictor (SSSP), which was developed by the Danish National Institute of Aquatic Resources to predict shelf life and bacterial growth in different fresh and lightly preserved seafood. The software, which is available in different languages, can be downloaded for free from the website of this institution (http://sssp.dtuaqua.dk/). A third and well-known piece of microbiological modelling software is Growth Predictor (www.ifr.ac.uk/safety/growth-predictor/), which provides a set of models for predicting the growth of organisms as a function of environmental factors, including temperature, pH and a_w. The predictions are based on data generated in various laboratories in the UK, under the funding of the UK Food Standards Agency.

For risk assessments, Risk Ranger (www.foodsafetycentre.com.au/riskranger.php) is a simple food safety risk-calculation tool intended as an aid to determining relative risk from different product, pathogen and processing combinations. The program, developed to work in Microsoft Excel, provides a simple and quick means to develop a first estimate of relative risk. Risk Ranger helps to focus the attention of the user on the interplay of factors that contribute to foodborne disease. Another Microsoft Excel tool is the Risk Assessment Calculator (RAC), which was specially developed for meat products within the EC Fifth Framework Programme (SMAS project). It allows for the estimation of microbial concentration and probability of illness of different pathogens in this type of food (http://smas.chemeng.ntua.gr/miram/).

Finally, there are also online web platforms exclusively devoted to predictive microbiology. Sym'Previus (www.symprevius.org) is an extensive French decision-support system that includes a database of microorganism responses in foods and diverse predictive models for growth and inactivation of pathogenic bacteria and some spoilage microorganisms. The database contains growth, survival and thermal destruction kinetics obtained in foodstuffs for the main pathogens. Data are mainly bibliographic and are progressively enriched from national and international research programmes. Sym'Previus also has diverse online tools (probabilistic modules, growth interfaces, growth curve fitting, etc.) which are available by simply taking out a subscription to the operational cell. However, the most ambitious project carried out on a global scale concerning predictive microbiology is undoubtedly ComBase (www.combase.cc/). This initiative emerged from a collaboration between the UK Food Standards Agency and IFR, the USDA Agricultural Research Service and its Eastern Regional Research Center in the USA and the Food Safety Centre in Australia. ComBase contains thousands of microbial growth and survival curves (currently more than 50 000 data records), for both spoilage and pathogen organisms, in different food matrixes (cheese, vegetables, seafood, etc.). These are freely available and grouped into numerous microbial models. The main purpose of ComBase is to be a useful and easy tool for industry, academia and regulatory agencies, providing online information about how microorganisms respond to environmental condition changes over the course of time.

19.4 APPLICATIONS OF PREDICTIVE MICROBIOLOGY IN FOOD PRESERVATION

Mathematical models can be used to optimize fermentative conditions by favouring the growth of desirable microorganisms or their metabolites (bacteriocins, ethanol, aromatic compounds, etc.). In this way, mathematical modelling can contribute to a better understanding and control of the fermentative process, helping to clarify in what manner, and to what degree, the food environment will interfere with the functionality of microorganisms.

But the use of mathematical models also opens a wide range of possibilities to improve food safety and quality. The availability of quantitative information on the growth and survival of diverse spoilage and pathogen organisms in food ecosystems will help to make more reliable decisions on the best practices to develop during processing, as well as in the determination of the appropriate levels or preservatives (pH, salt, manufacturing, etc.) applicable to a specific food. This will considerably reduce the incidence of food poisoning outbreaks, caused by the production of toxins by foodborne pathogens and, consequently, decrease health expenditure. The reduction of food spoilage will allow industries to increase the shelf life of products using lower concentrations of preservatives while offering more natural products. By means of mathematical models, food technologists will be able to objectively evaluate the influence of new technological treatments or combinations of preservatives on the food microbiota before market launch, obtaining safer and more stable products. Nowadays, the consumer demands, among other things, products with lower amounts of calories and sodium levels, but the formulation of new products that satisfy these needs, for example adding other lower-energy sugars or salts different from sodium chloride, must be accompanied by studies that determine their influence on microbial response. For all these reasons, predictive microbiology is currently recognized as an important and necessary subdiscipline in expansion within food microbiology.

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20 Factors Affecting the Growth of Microorganisms in Food

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Abstract: When microorganisms grow in food they cause varying degrees of change in the food's characteristics as a result of metabolic activity. Some of these changes, like those taking place during fermentation, are desirable, while others, like those resulting in food spoilage and food poisoning, are undesirable. The role of the food technologist is to encourage the desirable and prevent the undesirable changes. The most important factors that affect microbial growth in foods can be summarized in the following categories: (i) factors related to the food itself, the "intrinsic factors," which include nutrient content, water activity, pH value, redox potential, and the presence of antimicrobial substances and mechanical barriers to microbial invasion; (ii) factors related to the environment in which the food is stored, the "extrinsic factors," including the temperature of storage, and the composition of gases and relative humidity in the atmosphere surrounding the food; (iii) factors related to the microorganisms themselves, the "implicit factors," including interactions between the microorganisms contaminating the food and between these microorganisms and the food, e.g., their abilities to utilize different nutrient sources, tolerate stresses, and produce promoters or inhibitors of growth of other microorganisms, etc.; (iv) processing factors, which include treatments such as heating, cooling, and drying that affect the composition of the food and also affect the types and numbers of microorganisms that remain in the food after treatment; and (v) interaction between the above-described factors can also affect the growth of microorganisms in foods in a complicated way; the combined effects may be additive or synergistic.

Keywords: contamination; extrinsic factors; freezing; implicit factors; intrinsic factors; microorganisms; processing factors

20.1 INTRODUCTION

When microorganisms grow in foods they cause varying degrees of change in the characteristics of these foods as a result of their metabolic activities. Some of these changes, like those taking place during fermentation, are desirable, while others, like those resulting in food spoilage and food poisoning, are undesirable. Some food microbiologists have nicknamed microorganisms according to the types of change that their growth causes in food. Those used in fermentation of food to bring about desirable changes are called "the good," the food spoilers that cause economic losses are called "the bad," and those which cause foodborne diseases that endanger human life are called "the ugly." The role of the food technologist is to encourage the desirable and prevent the undesirable changes resulting from microbial growth

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in foods. The most important factors that affect microbial growth in foods include intrinsic, extrinsic, implicit, and processing factors as well as interactions between these factors.

20.2 INTRINSIC FACTORS

Intrinsic factors are related to the food itself, including:

- water activity;
- pH value;
- nutrient content;
- antimicrobial substances and mechanical barriers to microbial invasion;
- redox potential.

20.2.1 Water activity

Living organisms need water to grow. The food taken by microorganisms must be dissolved in water to pass through the membranes and get into the cell. The presence of water is also necessary for the chemical reactions in the cell and for the transport of nutrients and wastes into and out of the cell.

The water requirement of microorganisms in foods is generally described in terms of water activity (a_w) of the food. It is defined as the ratio of water vapor pressure of the food to the vapor pressure of pure water at the same temperature:

$$a_w = p/p_o$$

where p is the vapor pressure of the food and p_o is the vapor pressure of pure water. The a_w of pure water is 1 and that of a completely dry food is 0. Addition of solutes leads to a decrease in water activity because these solutes tie up water with their molecules. Sodium chloride is more effective in the reduction of water activity than sucrose (Table 20.1). For example, to reduce the water activity of pure water to 0.990 we need to add 1.74% NaCl or 15.45% sucrose, and to reduce it to 0.860 we need 18.18% NaCl or 65.63% sucrose (Table 20.1).

Table 20.1 Water activity at various concentrations of sodium chloride and sucrose at 25°C. Reproduced from Christian (2000), with kind permission of Springer Science+Business Media.

a_w	NaCl (%; w/w)	Sucrose (%; w/w)
1.000	0	0
0.990	1.74	15.45
0.980	3.43	26.07
0.960	6.57	39.66
0.940	9.38	48.22
0.920	11.90	54.36
0.900	14.18	58.45
0880	16.28	62.77
0.860	18.18	65.63
0.840	19.94	
0.820	21.59	
0.800	23.13	
0.753	26.5	

The optimum water activity for the growth of the large majority of food spoilage microorganisms is above 0.90, and the minimum is in the range 0.80–0.90. Each organism has a maximum, an optimum, and a minimum water activity for growth. In general, bacteria need higher water activity for growth than yeasts and molds. The water activity value of the food therefore determines to a considerable extent the type of organism that can grow in it.

There are some groups of microorganisms that can grow at water activity values of 0.60–0.75. These groups are termed xerophiles (dry-loving), halophiles (salt-loving), and osmophiles (preferring high osmotic pressure). Most halophiles are bacteria, xerophiles are molds, and osmophiles are yeasts (see Table 20.3, below). Gram-negative bacteria are the most water-loving microorganisms, followed by Gram-positive bacteria, followed by yeasts and then molds. Bacteria are generally fast growing and hence they are the most important spoilers of fresh foods with high water content.

20.2.1.1 Halophiles

Microorganisms that require a minimum concentration of salt for growth are called halophiles. They are categorized in groups according to their requirement and tolerance for salt in their growth media. Margesin and Schinner (2001) grouped them into:

- slight halophiles (optimal growth at about 3% (w/v) NaCl);
- moderate halophiles (optimal growth at 3–15% (w/v) NaCl);
- extreme halophiles (optimal growth at 25% (w/v) NaCl);
- borderline extreme halophiles (requirement of at least 12% (w/v) NaCl).

According to Baross and Lenovich (1992) slight halophiles grow optimally at 0.5–3% salt, moderate halophiles at 3–15% salt, and extreme halophiles at 15–30% salt. These authors also include a halotolerant group that can grow in a sodium chloride concentration exceeding 5% as well as in media containing no sodium chloride. Slightly halophilic microorganisms such as *Pseudomonas*, *Moraxella*, *Acinetobacter*, and *Flavobacterium* contribute to the spoilage of marine fish and shellfish. Moderately halophilic microbes significant in food spoilage include species of *Bacillus*, *Vibrio*, *Micrococcus*, *Pseudomonas*, *Moraxella*, and *Acinetobacter*. They spoil salted herring, other seafoods, and bacon-curing brines. Extreme halophiles such as *Halobacterium* and *Halococcus* have been involved in the spoilage of fish, bacon, and hides preserved in salt (Baross and Lenovich, 1992).

20.2.1.2 Xerophiles

Xerophilic, or dry-loving, microorganisms are mainly molds. They are important in the spoilage of dried fruits because they can grow at water activity values down to about 0.60. *Xeromyces bisporus* has the lowest minimum requirement for water known for a microorganism; it can grow at water activities down to 0.605 (Pitt and Christian, 1968). It is an important spoilage agent of prunes, chocolate, syrup, and other similar foods. *Wallemia sebi* can grow at a minimum water activity of 0.69 and spoils dried and salted fish (Jay, 2000). *Chrysosporium* species are known spoilers of dried foods, they were isolated from chocolate, commercial milk crumb, and Ghanaian cocoa beans (Kinderlerer, 1997). *Chrysosporium fastidium* was shown to grow at water activity as low as 0.686, and *Chrysosporium xerophilum*, *Chrysosporium inops*, and *Chrysosporium farinicola* were isolated from commercial chocolate bars with a water activity of approximately 0.28 (Pitt and Christian,

1968; Kinderlerer, 1997). *Aspergillus* species are of special importance because some of them, such as *Aspergillus flavus* and *Aspergillus parasiticus*, can produce aflatoxins in foods. *A. flavus*, *Aspergillus oryzae*, and *A. parasiticus* have minimum water activity values for germination and growth of 0.82, 0.81, and 0.80, respectively (Pitt and Miscamble, 1995). These molds have great affinity for growing on peanuts, corn, and cottonseed.

20.2.1.3 Osmophiles

The most important osmophilic and osmotolerant microorganisms in food spoilage are the yeasts. They can cause spoilage of honey, jams, syrups, and other similar foods. *Saccharomyces bisporus* var. *mellis* (*Zygosaccharomyces mellis*) is an obligate osmophilic yeast that requires high sugar concentrations for growth, with optimum growth at 60% glucose (Munitis *et al*., 1976). *Saccharomyces rouxii* (*Zygosaccharomyces rouxii*), *S. mellis* (*Z. mellis*), and *Torulopsis* spp. are osmophilic yeasts found in intermediate sugar refinery products (Scarr and Rose, 1965) and in spoiled date fruits (Hamad, 2008).

Table 20.2 shows water activity values for selected foods at their normal water contents, and Table 20.3 shows the minimal water activities required for growth of some foodborne microbes.

As can be seen from these tables, microbial growth needs a minimum water activity of about 0.6 in foods. According to their water content, foods are divided as follows (Jay, 2000; Montville and Matthews, 2005; Food and Drug Administration, 2010):

- high-moisture foods (a_w above 0.85);
- intermediate-moisture foods, (a_w 0.60–0.85);
- low-moisture foods, (a_w below 0.60).

High-moisture foods with water activity above 0.85 are highly perishable foods because they are susceptible to the growth of spoilage and pathogenic microorganisms, especially the

Table 20.2 Approximate water activity values for selected foods at their normal moisture contents (sources: Christian, 2000; Banwart, 2004; Montville and Matthews, 2005).

Range of a_w	Foods
0.95–1.00	Meat (fresh and cooked), fresh poultry, fresh fish, milk, eggs, fruit (fresh and canned in syrup), vegetables (fresh and canned in brine), bread, liver, sausage, pudding, fresh fruit and vegetable juices, some types of cheese
0.90–0.95	Cured meat products, some hard cheese, mayonnaise, low-calorie jams, baked cake, refrigerated biscuit dough
0.85–0.90	Aged hard cheeses, dry ham, fruit juice concentrates, maple syrup (saturated sucrose solution; a_w 0.86)
0.80–0.85	Sweetened condensed milk, parmesan cheese, fruit cake, high-moisture prunes, rice, beans, peas
0.75–0.80	Heavily salted fish, molasses, jams, marmalades, conserves, marzipan, glace fruits, prunes, fondants (saturated NaCl solution; a_w 0.75)
0.65–0.75	Some dried fruit (dates, figs, sultanas) and nuts, rolled oats, malt extract, chocolate candies
0.60–0.65	Some dried fruits, honey, caramels, toffee
Below 0.60	Dry pasta, spices, biscuits, crackers, dried milk, dried whole egg, dried vegetables, flour, instant coffee, cereals, sugar, noodles

Table 20.3 Minimal water activity required for growth of foodborne microbes at 25°C (sources: Jay, 2000; Banwart, 2004; Montville and Matthews, 2005).

Group of microorganisms	Minimal a_w required
Most bacteria	0.88–0.91
Most yeasts	0.87–0.94
Most molds	0.70–0.80
Halophilic bacteria	0.75
Xerotolerant molds	0.71
Osmophilic yeasts	0.60–0.78
Xerophilic molds	0.60–0.70
Specific spoilage organisms	
Pseudomonas aeruginosa	0.96–0.98
Pseudomonas fluorescens	0.94–0.97
Lactobacillus	0.90–0.96
Leuconostoc	0.96–0.98
Hansenula	0.89–0.90
Zygosaccharomyces rouxii	0.62–0.81
Aspergillus niger	0.80–0.84
Xeromyces bisporus	0.60–0.61
Specific pathogenic organisms	
Escherichia coli	0.94–0.97
Clostridium botulinum	0.90–0.98
Staphylococcus aureus	0.83–0.92
Vibrio parahaemolyticus	0.94–0.98
Salmonella	0.93–0.96
Bacillus cereus	0.92–0.95
Aspergillus flavus	0.78–0.90

fast-growing bacteria. To avoid spoilage by microbial growth, such foods must be cooled, dried, or treated with other inhibitors of microbial growth. Microbial spoilage of intermediate-moisture foods is a relatively slow process, caused mainly by yeasts and molds. Low-moisture foods are not spoiled by microorganisms unless their moisture content is raised by any means. Microbial growth stops at water activity below the minimal value, but the microbes don't die immediately and may remain dormant in the food for prolonged periods of time.

20.2.2 pH value

Microorganisms grow over a wide range of pH values extending from below pH 1 to about pH 11. Molds have the widest range, followed by yeasts, while bacteria have a narrower pH range of growth (Jay, 2000). In general, yeasts and molds grow better in acid foods than bacteria, although some bacteria can grow and spoil such foods. The respective minimum, optimum, and maximum pH values for the growth are (Jay, 2000; Lund and Eklund, 2000):

- pH 4.5, 6.5–7.5, and 9.0 for most bacteria;
- pH 1.5–3.5, 4.5–6.8, and 8.0–8.9 for yeasts;
- pH 1.5–3.5, 4.5–6.8, and 8.0–11.0, respectively for molds.

Non-pathogenic bacteria are more acid-tolerant than pathogenic ones, with a minimum pH for growth of 2.0–4.4 for the non-pathogenic group and 3.8–5.0 for the pathogenic group

Table 20.4 Minimum pH values for the growth of some microorganisms associated with foods (sources: Jay, 2000; Lund and Eklund, 2000; Ray, 2004).

Microorganism	Minimum pH
Pathogenic bacteria	
Escherichia coli O157:H7	4.5
Salmonella spp.	3.8–4.05
Campylobacter jejuni/coli	4.9
Vibrio parahaemolytics	4.8
Yersinia enterocolitica	4.2
Clostidium botulinum	4.6–5.0
Clostridium perfringens	5.0
Staphylococcus aureus	4.0
Listeria monocytogenes	4.1–4.5
Bacillus cereus	4.9
Non-pathogenic bacteria	
Pseudomonas spp.	5.6
Bacillus stearothermophilus	5.2
Clostridium pasteurianum	4.2
Clostridium butyricum	4.2
Lactobacillus brevis	3.2
Yeasts	
Candida krusei	1.3
Saccharomyces cerevisiae	1.6
Pichia membranifaciens	1.9
Zygosaccharomyces bailii	1.8
Molds	
Aspergillus spp.	1.6
Penicillium spp.	1.6–1.9
Fusarium spp.	1.8

(Lund and Eklund, 2000). As can be seen from Table 20.4 and Table 20.5, in general fruits have lower pH values than vegetables, meat, poultry, fish, and dairy products. Hence fruits are more susceptible to spoilage by yeasts and molds while bacteria are the most important spoilage agents of the other groups of food.

According to their pH values, foods are generally divided into four groups:

- *Group 1:* low-acid foods with a pH more than 5.3 include corn, meat, fish, and milk. All types of microorganisms can grow on them, especially bacteria.
- *Group 2:* medium-acid foods with a pH range of 5.3–4.5 include bananas, yogurt, and pumpkin. All types of microorganisms can grow on them.
- *Group 3:* acid foods with a pH range of 4.5–3.7 include tomatoes, orange juice, sugar beet, and grapes; they are susceptible to the growth of yeasts and molds, and a few types of bacteria. Most pathogenic bacteria do not grow on such foods.
- *Group 4:* high-acid foods with a pH below 3.7 include apples, grapefruit juice, and limes; they are susceptible to the growth of yeasts and molds. Most bacteria, especially the pathogenic ones, do not grow on such foods.

In the practices of food processing, foods with pH values below 4.5 (UK) or 4.6 (USA) are termed acid foods and those with pH above these levels are termed low-acid foods. The heat treatment applied to acid foods is pasteurization, and to low-acid foods the treatment is

Table 20.5 Approximate pH values of some foods (sources: Jay, 2000; Lund and Eklund, 2000).

Product	pH
Vegetables	
Beans (lima)	5.4–6.2
Cabbage (green)	5.4–6.3
Carrots	4.9–6.3
Potatoes	5.6–6.2
Peppers	5.0–7.0
Lettuce	6.0
Tomatoes, ripe	3.4–4.9
Fruits	
Apples	2.9–3.4
Bananas	4.5–4.7
Grapefruit (juice)	3.0
Grapes	3.4–4.5
Limes	1.8–2.0
Meat	
Beef (ground)	5.1–6.2
Ham	5.9–6.1
Veal	6.0
Chicken	6.2–6.4
Fish and shellfish	
Fish (most species)	6.6–6.8
Crabs	7.0
Clams	6.5
Shrimp	6.8–7.0
Dairy products	
Milk	6.3–6.5
Cream	6.5
Butter	6.1–6.4
Yogurt	4.6–5.0

commercial sterilization. Both types of foods may contain living spores of bacteria after heat treatment; those present in commercially sterilized foods are the spores of thermophilic bacteria. The low pH of acid foods prevents the germination of the bacterial spores, while the spores of the thermophilic bacteria in low-acid foods are unable to grow at temperatures below about 30°C. Therefore commercially sterilized low-acid foods must be stored at temperatures below 30°C.

The pH of foods can be natural, due to microbial fermentation, or result from acids added to the food. Microbial growth leads in most cases to a change in the pH of the food. When lactic acid bacteria for example grow in milk they consume its lactose and produce lactic acid, which lowers the pH of the milk. Conversely, when *Pseudomonas* grows in meat it consumes its proteins and produces ammonia, which raises the pH of the meat.

Weak organic acids such as benzoic, sorbic, and propionic acids are added to foods as preservatives. The antimicrobial effect of these organic acids results partly from lowering the food pH and partly from the toxicity of these acids themselves to certain microorganisms. The toxicity is caused by the undissociated form of the acid. Because of their relatively high dissociation constants (4.2 for benzoic, 4.8 for sorbic, and 4.9 for propionic acids), the greater portions of the acids added to the food remain undissociated. The undissociated molecules of

the acid can easily cross the membranes of the microbial cells and enter the cytoplasm (non-polar molecules, hence, can pass through the phospholipid bilayer of the membrane). Once inside the cytoplasm these molecules dissociate because the pH of the cytoplasm of most microbial cells is more than 6. This will lead to a lowering of the pH of the cytoplasm, but the cell will try to maintain the pH at its normal level. In doing so, it will keep using its energy to pump H^+ions out of the cytoplasm. Eventually a point will be reached where the cell fails to keep the required pH and dies.

20.2.3 Nutrient content

Like other living organisms, microorganisms need nutrients for growth and maintenance. These nutrients are used as sources of energy, carbon, nitrogen, minerals, vitamins, and other growth factors. The ability of a food item to support microbial growth depends on its content of these nutrients. The major nutrients used by microorganisms as sources of energy, carbon, and nitrogen are the carbohydrates, proteins, and lipids. Almost all microorganisms use simple sugars, present in most foods, such as glucose, fructose, sucrose, and maltose, as sources of energy and carbon. Also amino acids, alcohols, and free fatty acids are used by many microorganisms as sources of energy and carbon. Only a few specialist microorganisms that produce specific extracellular enzymes (amylases, lipases, and proteases) can utilize complex polysaccharides such as starch and cellulose, in addition to lipids and proteins, as carbon and energy sources. These macromolecules must first be enzymically broken down into their simple constituents outside the cell before they can pass through the cell membrane into the cytoplasm.

Amino acids are the primary sources of nitrogen used by almost all microorganisms. Some microorganisms with the required enzymes can also use proteins and nucleotides as nitrogen sources. Thus foods containing simple sugars and free amino acids in high concentrations are more suitable for microbial growth. These nutrients are normally consumed first by microorganisms before they move to the other complex nutrients like starches and proteins. Many bacterial species, especially Gram-negative rods such as *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Shewanella*, and *Aeromonas*, as well as pathogenic spore formers like *Clostridium botulinum*, are proteolytic and can grow well in protein-rich foods and spoil them quickly or cause disease (International Commission on Microbiological Specifications for Foods, 2005). Foods rich in carbohydrate can be spoiled by carbohydrate-fermenting microorganisms, particularly by yeasts and molds. Bacterial species of the genera *Bacillus*, *Clostridium*, *Aeromonas*, *Pseudomonas*, *Leuconostoc*, and *Enterobacter* are saccharolytic and can also attack carbohydrates (Banwart, 2004; Ray, 2004). Relatively few types of microorganisms, such as molds, yeasts, and a few Gram-negative bacteria, are capable of fat metabolism. Spoilage microorganisms of fatty foods like butter and margarine include lipolytic species such as *Pseudomonas* spp., *Flavobacterium* spp., *Saccharomycopsis lipolytica*, *Candida lipolytica*, *Penicillium* spp., and *Aspergillus* spp. (Banwart, 2004; International Commission on Microbiological Specifications for Foods, 2005).

Most microorganisms need one or another vitamin as growth factors, normally in small amounts, and almost all natural human foods contain enough of such vitamins to support microbial growth. In general, Gram-positive bacteria are more fastidious in their needs for vitamins, while Gram-negative bacteria and molds can synthesize most or all of their needs from these growth factors (Jay, 2000).

Foods contain the nutrients required for the growth of microorganisms in varying quantities. Milk contains all nutrients in sufficient amounts that support the growth of almost all types of microorganisms. Meats are rich in proteins, lipids, minerals, and vitamins, but

poor in carbohydrates, while foods of plant origin are generally rich in carbohydrate, but relatively poor in proteins, minerals, and some vitamins (Ray, 2004).

20.2.4 Antimicrobial substances and mechanical barriers to microbial invasion

20.2.4.1 Biological structure

Most raw foods derived from plant or animal origins normally have one or another type of natural biological structure that may hinder microorganisms from entry into the cells and tissues of the food (Frazier and Westhoff, 1988; Jay, 2000; Margesin and Schinner, 2001). These structures include such physical barriers as skin of fruits and vegetables, testa of seeds, shell of nuts, animal hide, and the shell, cuticle, and membrane of egg. The skin does not favor microbial growth because it has low water activity, is deficient in readily available nutrients and, often, contains antimicrobial compounds such as short-chain fatty acids in the case of animal skins or essential oils in the case of plant coatings (Bohra and Parihar, 2006). Fats covering meat may protect the flesh, and also scales of fish can give some degree of protection. In some cases an artificial coating of, for example, plastic or wax is added to the food item as an extra barrier.

The susceptibility of the natural protective physical barriers to penetration by microorganisms is influenced by many factors. The effective protection of the barriers decreases with progressing maturity of fruits and vegetables. Physical damage that may occur during harvest, transport, processing, and storage or that caused by insect, bird, or rodent attack can make the way free for microbial penetration. These physical and artificial barriers not only protect the food from microbial invasion, but also can determine the kinds of microorganisms that may grow and the rate and course of expected spoilage. Some processing practices like slicing, grinding, chopping, skinning, and comminuting increase the exposed surface area, distribute contaminating microorganisms all over the food, and release juices containing nutrients for the invaders. Similar results may also be accomplished by damage to the food tissues caused by freezing. Washing of eggs, especially when using abrasives, can damage the cuticle layer and make microbial penetration easier.

20.2.4.2 Antimicrobial substances

Foods contain many natural antimicrobial substances, while others are added to the food or are formed in it during processing. These substances inhibit or delay microbial growth in foods.

Antimicrobials formed during processing

Smoking of fish, meat, poultry, and cheese results in the deposition of antimicrobial substances such as formaldehyde, phenols, and cresols onto the product surface (Ray, 2004). These chemicals act as bacteriostatic and bactericidal agents. Maillard compounds such as melanoidins are formed during heating of certain foods as a result of reactions between sugars and amino acids or peptides in the food. They are found to have antimicrobial activities against some Gram-positive and Gram-negative foodborne pathogens like *Staphylococcus aureus* and *Escherichia coli* (Rufian-Henares and Morales, 2007).

Many species of bacteria and yeasts used in food fermentation, especially lactic acid bacteria, produce antimicrobial substances such as bacteriocins, organic acids, diacetyl, hydrogen peroxide, and enzymes.

Bacteriocins

Bacteriocins are the most widely studied and used antimicrobials. They have bacteriostatic, bactericidal, fungistatic, and fungicidal actions against microorganisms that cause food spoilage and foodborne disease. Well-known producers of bacteriocins in food fermentation include many species of the genera *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Bifidobacterium* (Ray, 2004). Nisin produced by strains of *Lactococcus lactis* is used in the food industry to prevent spoilage of cheese by *Clostridium butyricum*. A combination of nisin and pediocin (produced by *Pediococcus acidilactici*) was found to inhibit the growth of different spoilage and pathogenic bacteria when applied to hot dog, roast beef, turkey roll, and ham. The bacteria inhibited were *Leuconostoc mesenteroides*, *Lactobacillus viridescens*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *E. coli* O157:H7 (Ray, 2004).

Antimicrobials added to foods

Many organic and inorganic chemicals are added to foods as preservatives (Jay, 2000; Ray, 2004). Addition of these chemicals to foods is regulated by strict rules of safety all over the world. The term GRAS stands for generally recognized as safe, and describes the chemicals permitted for use in food preservation.

Propionic acid and the propionates are used to preserve bread, cakes, jam, jellies, and some cheese against molds and bacteria at concentrations of 1000–3200 ppm. Yeast and mold growth in syrups, salad dressings, mayonnaise, cakes, and jellies can be controlled by the addition of sorbic acid and sorbates at concentrations of 500–2000 ppm. Benzoic acid and the benzoates are applied to inhibit the growth of yeasts and molds in margarine, soft drinks, tomato ketchup, pickles, confectioneries, mayonnaise, and salad dressings. They are normally used at concentrations of 500–2000 ppm. Parabens are methyl-, ethyl-, butyl-, propyl-, and heptyl-esters of *p*-hydroxybenzoic acid. They are used as preservatives against bacteria, yeasts, and molds in confectionary, soft drinks, pickles, salad dressings, jams, and jellies at concentrations of 100–1000 ppm (heptyl-ester is used at concentrations of 12–20 ppm). The most widely used inorganic preservatives in the food industry are the nitrites, sulfur dioxide, and the sulfites. Sodium and potassium nitrite are used as part of curing formulas for meat, poultry, and fish products to control *C. botulinum* at concentrations of about 120 ppm. They are also used to control spoilage of cheese by *C. butyricum* and *Clostridium tyrobutyricum*. Nitrites are also found to have some inhibitory effects on *Staph. aureus*, *Escherichia*, *Pseudomonas*, and *Enterobacter* spp. when applied at 200 ppm. Sulfur dioxide, sodium sulfite (Na_2SO_3), sodium bisulfite ($NaHSO_3$), and sodium metabisulfite ($Na_2S_2O_5$) are used for the control of molds, yeasts, and insects in dried fruits, fruit juices, soft drinks, wines, sausages, pickles, and fresh shrimp. These chemicals are applied to foods in concentrations of 200–300 ppm.

Antimicrobials naturally present in foods

A variety of natural components in plant tissues such as phenolics, alkaloids, pigments, and resins have antimicrobial activities. The flavoring components of herbs and spices, the volatile essential oils, contain antimicrobial compounds such as allicin in garlic, eugenol in allspice and cinnamon, thymol in thyme and oregano, and cinnamic aldehyde in cinnamon and cassia (Bohra and Parihar, 2006). Al-Zoreky (2009) isolated phenolic compounds from pomegranate fruit peels which showed high inhibitory effects against *L. monocytogenes*, *Staph. aureus*, *E. coli*, and *Yersinia enterocolitica*.

Raw milk contains many antimicrobial compounds intended to give protection to neonates and also to the mammary glands of the animal species from which milk is drawn. These compounds remain after milking for varying periods of time and play a role in protecting milk from microbial spoilage.

The lactoperoxidase system

Lactoperoxidase is a peroxidase enzyme that functions as a natural antibacterial agent in milk. It catalyzes the oxidation of thiocyanate in the presence of hydrogen peroxide (both naturally present in milk) to form the antimicrobial agent hypothiocyanite that causes structural damage to the cytoplasmic membrane of bacteria. Lactoperoxidase, thiocyanate, hydrogen peroxide, and hypothiocyanite are known together as the lactoperoxidase system (Griffiths, 2000). The system is effective against many pathogenic microorganisms such as *E. coli* (including *E. coli* O157:H7), *Salmonella* spp., *Campylobacter jejuni*, *Staph. aureus*, *L. monocytogenes*, *Y. enterocolitica*, *Streptococcus mutans*, *Aeromonas hydrophila*, *Candida albicans*, *Helicobacter pylori*, *Brucella melitensis*, and *Pseudomonas* spp. (Gaya *et al.*, 1991; Food and Agriculture Organization/World Health Organization, 2005). The system can be activated in milk by the addition of about 10 ppm of thiocyanate (preferably in powder form) to the raw milk to increase the overall level to 15 ppm (around 5 ppm is naturally present in milk). The solution is thoroughly mixed for 30 s and then an equimolar amount (8.5 ppm) of hydrogen peroxide is added (generally in the form of a granulated sodium carbonate peroxyhydrate). This activation has a bacteriostatic effect and can extend the shelf life of raw milk for 7–8 h under ambient temperatures of around 30°C, or longer at lower temperatures. This allows adequate time for the milk to be transported from the collection point to a processing center without refrigeration (Food and Agriculture Organization/World Health Organization, 2005).

Lactoferrin

Lactoferrin, also known as lactotransferrin, is a globular glycoprotein of the transferrin family. It is naturally present in milk and other body fluids such as saliva, tears, and nasal secretions (Brock, 2002). Lactoferrin is one of the components of the immune system of the body with antimicrobial activity against bacteria, viruses, and parasites. Two different mechanisms are involved in the antimicrobial activity of lactoferrin (Conneely, 2001). The first mechanism is bacteriostatic, resulting from the high iron-binding affinity of the protein. It deprives the iron-requiring bacteria of this essential growth nutrient. Because of the iron-binding ability, lactoferrin can retard the growth of a broad range of microorganisms, including Gram-positive and Gram-negative bacteria and certain yeasts. The second mechanism by which lactoferrin acts as antimicrobial agent is due to its ability to damage the outer membrane of the cell wall of Gram-negative bacteria by causing the release of lipopolysaccharides. Lactoferrin is also known to have antiviral and antiparasitic activities. It can inhibit disease formation by the herpes and hepatitis C viruses, the protozoans *Toxoplasma gondii* and *Eimeria stiedae*, and the fungus *Pneumocystis jirovecii* (Jay, 2000). Although the mechanism of the antiviral activity is not well known, but it is thought that the protein blocks the cell–virus interactions. The activity against the protozoan is attributed to effects on the membrane integrity of the cells or interaction with the host tissues. With respect to the fungus, the inhibitory effect is thought to be associated with iron binding (Brock, 2002).

Lysozyme

Lysozyme, also known as muramidase or N-acetylmuramide glycanhydrolase, is an enzyme capable of damaging the cell wall of bacteria. Gram-positive bacteria are highly sensitive to

the enzyme because the peptidoglycan layer in their cell walls is exposed, while the Gram-negative bacteria are less sensitive because their peptidoglycan is protected by an outer membrane. The enzyme breaks the β-1,4-glycosidic bonds between *N*-acetylmuramic acid and *N*-acetylglucosamine of the peptidoglycan of the cell wall (Madigan *et al.*, 2002). It is naturally present in egg white and milk in addition to other body secretions like tears, saliva, and mucus. The enzyme can survive high-temperature/short-time (HTST) preservation (Griffiths, 2000). Egg white is particularly rich in lysozyme, containing about 3.5% on a dry-weight basis (Banwart, 2004). Industrial methods have been developed for the economical recovery of lysozyme from egg white, and its use in food preservation have been approved in Europe, Japan, and the USA (Hughey and Johnson, 1987; Montville and Matthews, 2005). It is used in Europe to prevent gas formation in cheeses, and in Japan to preserve seafood, vegetables, pasta, and salads. The enzyme is active against foodborne pathogenic bacteria including *L. monocytogenes*, *Bacillus cereus*, and certain strains of *C. botulinum*. It has also high antimicrobial activity against the food spoilage bacteria *Clostridium thermosaccharolyticum*, *C. tyrobutyricum*, and *Bacillus stearothermophilus* (Hughey and Johnson, 1987; Montville and Matthews, 2005).

20.2.5 Redox potential

The oxidation-reduction or redox potential (usually written Eh) of a substance can be defined as the ease with which the substance loses electrons and becomes oxidized or gains electrons and becomes reduced. A substance that readily donates electrons is a good reducing agent while that which readily accepts electrons is a good oxidizing agent. When electrons are moved from the donor compound to the acceptor, a potential difference is generated between the two compounds. This difference represents the redox potential (Eh) of these two compounds. It can be measured using special electrodes and expressed in millivolts. A relative scale of redox potential for different pairs of compounds has been constructed, with the standard hydrogen pair set to have an Eh value of 0 (Morris, 2000). Pairs showing positive values of Eh are more oxidizing than the standard hydrogen couple, and those with negative Eh values are more reducing than the standard hydrogen couple, and the more positive or negative the more oxidizing or reducing is the couple. Being a strong oxidizing agent, the presence of oxygen in a medium will shift its Eh value in a positive direction. Thus, media with positive Eh values are more likely to be aerobic (hence support the growth of aerobic microorganisms) while those with negative Eh values will be anaerobic (and support the growth of anaerobes). Therefore, the redox potential of a foodstuff will determine its ability to support the growth of either aerobic or anaerobic microorganisms. The Eh is affected by the pH of the substrate; normally it is measured at pH 7.0, and if measurement is made at another pH this must be indicated (Morris, 2000).

Foods of plant origin have in general positive Eh values; hence they are normally spoiled by aerobic bacteria and fungi. Fruit juices have Eh values ranging between +300 and +400 mV, cherries +179 mV, peaches +175 mV, spinach +74 mV, barley +225 mV, and wheat germ −470 mV. Solid meats have negative Eh values of about −200 mV, minced meats positive values of about +200 mV, while cheese of various types have Eh values ranging from −20 to around −200 mV. The Eh of canned foods ranges from −18 mV for sliced carrots packed in glass to −446 mV for beef stew packed in cans (Jay, 2000; Banwart, 2004).

The growth of aerobic microorganisms leads to the lowering of the Eh value of a food because they consume oxygen and produce reducing metabolic products such as hydrogen sulfide and carbon dioxide. When the Eh of the food reaches negative values due to the growth

of aerobes, then the growth of anaerobes present as contaminants in the food may start, leading to further spoilage. The rate at which the Eh value of a food changes due to microbial growth depends on its redox-buffering capacity (also known as the poising effect), i.e., its resistance to change in redox potential. The buffering capacity in turn is determined by the concentration and the ratio in which the oxidizing and reducing compounds are present in the food (Morris, 2000). The most important reducing agents (antioxidants) naturally present in plant and animal tissues include reducing sugars, enzymes (catalase, superoxide dismutase, peroxidase), thiols (mercaptans), ascorbic acid, and the polyphenols (tannins, lignin, flavonoids). As long as the plant or animal cells respire and remain active, these reducing agents tend to poise the redox system of the tissues at low levels against the effect of oxygen diffusing from outside. Some processing practices may alter this situation. Heating may reduce the poising power of the food by destroying the reducing agents. Also, it can affect the poising capacity of the food by allowing more rapid diffusion of oxygen inward because of destruction of food structures. Clear fruit juices lose reducing substances by removal during extraction and filtration, and become more favorable for yeast growth than the original juice containing the pulp (Frazier and Westhoff, 1988).

The ranges of Eh values at which aerobic and anaerobic microorganisms can grow are approximately as follows: +500 to +300 mV for aerobes, +300 to −100 mV for facultative anaerobes, and +100 to −250 mV or lower for anaerobes (Ray, 2004). The range varies greatly with concentrations of reducing components in a food and the presence of oxygen. Examples of aerobic microorganisms important to foods are the molds (*Aspergillus*, *Penicillium*), and many bacteria (*Pseudomonas*, *Flavobacterium*). Many facultative anaerobic microorganisms are important spoilage agents and foodborne pathogens, such as the yeast *Saccharomyces cerevisiae*, and the bacteria *Salmonella* and *E. coli*. The strict anaerobes important to foods are the clostridia, e.g., the food poisoning agent *C. botulinum*, and the food spoilage organism *C. thermosaccharolyticum*.

20.3 EXTRINSIC FACTORS

The factors related to the environment in which the food is stored are referred to as the extrinsic factors, and they include:

- the temperature of storage;
- the composition of gases and the relative humidity in the atmosphere surrounding the food.

20.3.1 Impact of storage temperature

Microbial growth is a result of a series of chemical reactions; hence it is strongly affected by temperature. For these chemical reactions to take place, the cell membranes and the enzymes, which are also strongly affected by temperature, must remain intact, and water must be available in liquid form. Microorganisms in general grow over a wide range of temperatures, extending from below 0 to above 100°C, but the range for each individual microorganism is much narrower. Also, each individual microorganism has its defined cardinal temperatures of growth, namely the maximum, minimum, and optimum. As the temperature at which the organism grows rises above the minimum growth temperature, the rates of chemical reactions in the cell increase and growth becomes faster, reaching its maximum at the optimum growth temperature. When the temperature exceeds the optimum, metabolic reactions start to slow

down due to damage to enzymes and membranes of the cell. As a result, the growth rate declines quickly and reaches zero at the minimum growth rate. Thus, the optimum temperature for growth is the temperature at which microbial growth is maximum at the prevailing conditions. The minimum temperature is the temperature at which microbial growth ceases, and the maximum is the one above which microorganisms die because of irreversible damage to enzymes and membranes.

With respect to their cardinal growth temperature, microorganisms can be placed into five groups. The definitions given by various authors to each group vary; the most commonly used are the following:

- *Psychrophilic:* these are microorganisms with an optimum temperature for growth of 15°C or lower, a maximum below 20°C, and a minimal temperature for growth of 0°C or lower. There are doubtful reports in the literature of molds that can grow at −20°C, and yeasts at −34°C, but bacterial growth at −12°C is clearly demonstrated (Herbert and Sutherland, 2000). Psychrophiles are naturally found in waters and soils of Arctic and Antarctic regions. Most psychrophiles belong to the bacterial genera *Vibrio*, *Aeromonas*, *Alcaligenes*, *Pseudomonas*, and *Flavobacterium.* Psychrophilic yeasts include *Candida gelida*, *C. nivalis*, *Leucosporidium scottii*, and *Cryptococcus vishniacii* (Herbert and Sutherland, 2000). Psychrophiles may be important in the spoilage of frozen foods.
- *Psychrotrophic:* these are organisms that grow at 0°C, but their temperature optimum is in the range 20–40°C, and the maximum about 43°C. Psychrotrophs are also defined as those microorganisms able to grow at temperatures between 0 and 7°C and produce visible colonies (or turbidity) within 7–10 days. This group of microorganisms is naturally found in the soil and water in temperate regions. Many important food spoilage and food poisoning microorganisms belong to this group (Jay, 2000). Examples of psychrotrophic food spoilage bacteria include *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Leuconostoc mesenteroides*, and *Lactobacillus sake*. Strains of *Cladosporium* and *Aspergillus* were isolated from spoiled date fruits at the rutab stage which were kept at temperatures of 5°C (Hamad, 2008). The yeast species *Yarrowia lipolytica*, *Debaryomyces hansenii*, and *Pichia membranifaciens* were isolated from various chilled foods (Deák, 2008). Examples of psychrotrophic microorganisms causing foodborne illnesses include *L. monocytogenes*, *Y. enterocolitica*, and *Aeromonas hydrophila.*
- *Mesophilic:* these microorganisms have adapted themselves to live in the bodies of humans and warm-blooded animals and in soils and water in tropical and temperate climates. The optimum growth temperature of mesophiles is 30–40°C, and their range of growth is between 10°C minimum and 45°C maximum. Most microorganisms that cause food spoilage and foodborne diseases are in this category. Food spoilers include bacteria (e.g., strains of *Lactobacillus*, *Bacillus*, *E. coli*), molds (e.g., *Aspergillus*, *Penicillium*, *Mucor*), and yeasts (e.g., *Candida*, *Kluyveromyces*, *Saccharomyces*). The mesophilic foodborne pathogens include *Salmonella* spp., *E. coli*, *Staph. aureus*, and *Vibrio parahaemolyticus*.
- *Thermoduric:* these are microorganisms that can survive exposure to relatively high temperatures, e.g., pasteurization at 63°C for 30 min, but may not grow at these temperatures. The range of growth temperatures of this group lies within that of the mesophiles. Examples of non-spore-forming thermoduric bacteria that can survive milk pasteurization are strains of *Streptococcus*, *Micrococcus*, and *Lactobacillus.*
- *Thermophilic:* thermophilic organisms are those that require relatively high temperatures for growth. The optimum growth temperature for this group is 45–65°C, the minimum is

Table 20.6 Cardinal growth temperatures of mesophilic food poisoning bacteria (sources: Herbert and Sutherland, 2000; Banwart, 2004).

Organism	Minimum (°C)	Optimum (°C)	Maximum (°C)
Escherichia coli	7	35–40	46
Salmonella spp.	5–10	35–37	45–49
Staphylococcus aureus	5–10	35–40	44–48
Clostridium perfringens	12–20	30–47	45–51
Clostridium botulinum (proteolytic strains)	12.5	35	50
Campylobacter jejuni	30	42–45	47
Vibrio parahaemolyticus (mesophilic strains)	13	35–37	42–44
Vibrio cholerae	10	37	43
Bacillus cereus (mesophilic strains)	10–15	35–40	47–55
Shigella	7	37	45–47

Table 20.7 Cardinal growth temperatures of psychrotrophic foodborne pathogenic bacteria (sources: Herbert and Sutherland, 2000; Banwart, 2004).

Organism	Minimum (°C)	Optimum (°C)	Maximum (°C)
Bacillus cereus (psychrotrophic strains)	4–5	28–35	30–35
Yersinia enterocolitica	−1 to 4	28–30	37–42
Listeria monocytogenes	0–4	30–37	45
Aeromonas hydrophila	0–4	28–35	42–45
Clostridium botulinum (non-proteolytic strains)	3.0–3.3	30	45
Vibrio parahaemolyticus (psychrotrophic strains)	3–5	30–37	40–42

35–45°C, and the maximum is 60–90°C. Thermophilic microorganisms important in food spoilage and poisoning are contained in the bacterial genera *Clostridium* and *Bacillus.* Vegetative cells and spores of most thermophilic microorganisms that can cause spoilage of low-acid canned foods are killed by commercial sterilization of foods, but the spores of a few bacterial species can survive this treatment. These include the facultative anaerobic producers of flat-sour spoilage (the spore-formers *B. stearothermophilus*, and *Bacillus coagulans*), the causative agent of thermophilic anaerobe (TA) spoilage (the anaerobic spore-former *C. thermosaccharolyticum*), and the causative of sulfide stinker spoilage (the anaerobic Gram-negative spore-former *Desulfotomaculum nigrificans*).

Table 20.6 and Table 20.7 contain examples of some spoilage and pathogenic microorganisms and their cardinal temperatures of growth.

20.3.1.1 Effect of heat treatment of foods on contaminant microorganisms

If microorganisms are heated to temperatures beyond their optimum growth temperature, the rate of growth will start to decrease because of increasing damage to the enzymes and membranes of the cell. When the maximum growth temperature is reached growth stops and the cells die because of irreversible damage to the enzymes and membranes. The heat treatments normally applied to foods to control microbial growth are pasteurization and commercial sterilization.

Pasteurization treatments are normally designed to kill all vegetative cells of foodborne pathogenic microorganisms and also all vegetative cells of psychrotrophic and mesophilic spoilage microorganisms, including bacteria, yeasts, and molds. Vegetative cells and spores of thermoduric and thermophilic food spoilage microorganisms as well as spores of some pathogens (*C. botulinum*) may survive this treatment. The growth of the survivors can be stopped by refrigeration of the pasteurized foods, but if the food is kept at ambient temperatures growth of these microorganisms might occur, leading to food spoilage or food poisoning. Milk can be pasteurized following different regimes of heating: the low-temperature/long-time (LTLT) treatment is at 63°C for 30 min. The high-temperature/short-time (HTST) treatment can be done at 72°C for 16 s, 89°C for 1.0 s, 90°C for 0.5 s, 94°C for 0.1 s, or 100°C for 0.01 s. Such treatments are enough to kill all vegetative cells of the most heat-resistant non-spore-forming pathogenic microorganisms expected to contaminate milk, namely *Mycobacterium tuberculosis* and *Coxiella burnetii*.

Commercial sterilization is applied to canned foods of low acidity (pH higher than 4.5 or 4.6). The treatment should be enough to inactivate all potential spoilage and pathogenic microorganisms that may be present in a particular food item. A more accurate definition of the process is to reduce the probability of survival and/or growth of microorganisms in a particular food to an acceptably low level (Pflug and Gould, 2000). The acceptability level for pathogenic microorganisms in foods is set at very low levels in comparison to that for food spoilage organisms. An acceptably low level of survival probability for safety from a public health standpoint is reached when the process can achieve a probability of a viable *C. botulinum* spore surviving in a container of food to the order of 10^{-9}; i.e., one spore in 10^9 containers. For preservation against spoilage by mesophilic spore-forming organisms, the probability of a mesophilic organism surviving the process should be of the order 10^{-6}. In case of preservation against spoilage by thermophilic spore formers, if the canned food is to be stored at temperatures below the growth range of thermophilic organisms (30°C), then the probability of a thermophilic organism surviving the process should be equal to or less than 10^{-2}, but if the canned food is stored at temperatures higher than 30°C, the probability of survival should be 10^{-6}.

20.3.1.2 Effect of food freezing on contaminant microorganisms

Microorganisms in frozen foods may remain alive for different periods of time, and the survival rates are affected by many factors (Frazier and Westhoff, 1988; Jay, 2000; Lund, 2000):

- The kind of microorganism and its phase of growth: bacterial spores and Gram-positive-bacteria are more resistant to freezing than Gram-negative bacteria. About 90% of spores of *Bacillus mesentericus* remained viable after freezing to −70°C. Survival of vegetative bacteria under the same treatment were 18% for *P. aeruginosa*, 58% for *E. coli*, 96% for *Staph. aureus*, and 11% for *S. cerevisiae*. Microorganisms in the logarithmic phase of growth are more sensitive to freezing than those in other phases.
- The freezing rate: response to cooling rate varies, even for the same organism. Survival of *E. coli* frozen to −70°C at a rate of 6°C/min was about 70%. This value dropped to about 40% at a cooling rate of 1°C/min and to about 20% at a rate of 100°C/min. The cooling rates that resulted in highest survival of *Azotobacter chroococcum* and *P. aeruginosa* were 7°C/min and 11°C/min, respectively.

- Freezing temperature: higher freezing temperatures are more damaging. Inactivation rate is higher at −1 to −10°C than at −15 to −30°C. The decrease in viability is usually very small at temperatures of −60°C and below. About 50% of the spores of *B. mesentericus* survived storage in frozen water at −1°C for 133 days, while almost 100% of these spores survived storage at −20°C for the same period of time. Similar trend was observed in *Staph. aureus*, *E. coli*, and *P. aeruginosa.*
- The composition of freezing medium: the damaging effects of increased concentrations of extra solutes on microbial cells during freezing are minimized if the cells are cooled in distilled water. Survival of cells of *E. coli* frozen in distilled water to −70°C at different cooling rates ranged between about 20 to over 80%, while the survival of the cells of the same bacterium frozen in 0.85% saline under otherwise similar conditions did not exceed 10%. Various compounds like sugars, proteins, fats, glycerol, and other substances can act as cryoprotectants and protect the microbial cells against the damaging effect of freezing in water and saline. Addition of 3% glycerol to water or to 0.85% saline increased the amount of survivors of *E. coli* cells frozen to −70°C to more than 90% in both cases, irrespective of the freezing rate. The pH of the medium also affects the rate of survival, which decreases with increased acidity of the medium. Lowering the pH of the growth medium of *Staph. aureus* from pH 7.3 to 3.8 decreased survival of the bacterium during freezing to −30°C by eight-fold.

20.3.2 Impact of storage atmosphere of the food

The composition of gases and the relative humidity in storage have great effect on the microorganisms contaminating foods. Carbon dioxide, ozone, and oxygen have varying degrees of toxicity to different microorganisms. Modifying the content of air from oxygen and carbon dioxide is used for the control of microbial growth in foods during storage. Technologies used include modified-atmosphere packing (MAP), controlled-atmosphere packaging (CAP), controlled-atmosphere storage (CAS), direct addition of carbon dioxide (DAC), and hypobaric storage. Controlled-atmosphere and modified-atmosphere packaging of certain foods can dramatically extend their shelf life. The preservation principle of antimicrobial atmospheres has been applied to fruits and vegetables, raw beef, chicken, and fish, dairy foods including milk and cottage cheese, eggs, and a variety of prepared, ready-to-eat foods.

20.3.2.1 Effect of oxygen on microorganisms

Oxygen is needed by aerobic microorganisms for energy generation because it is a powerful oxidant and an excellent electron acceptor for respiration; hence its presence is necessary for this type of microorganism to grow. On the other hand, oxygen is toxic to anaerobes, and its presence in their growth medium can lead to death. Oxygen in its normal ground state (lowest-energy state) is referred to as triplet oxygen ($^3O_2$). Higher-energy forms of oxygen exist, which are more reactive than the ground form and hence hazardous to cells. These include singlet oxygen ($^1O_2$), superoxide anion ($\bullet O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH). If oxygen is present around microbial cells, it will automatically diffuse inside the cells irrespective of whether it is needed for respiration or not. There it will gain electrons from the various reducing

agents in the cell to form the higher-energy forms of superoxide and hydrogen peroxide.

$$O_2 + e^- \rightarrow \bullet O_2^- (\text{superoxide})$$
$$O_2 + e^- + 2\,H^+ \rightarrow H_2O_2 (\text{hydrogen peroxide})$$

These forms of oxygen can react with vital cell components like membranes and destroy them, leading to cell death. Aerobic and facultative anaerobic microorganisms and those which tolerate oxygen like lactic acid bacteria contain the enzyme superoxide dismutase which catalyzes a reaction that converts the superoxide to hydrogen peroxide:

$$\bullet O_2^- + \bullet O_2^- + 2H^+ + \text{superoxide dismutase} \rightarrow H_2O_2 + O_2$$

Hydrogen peroxide is removed by the enzyme catalase present in aerobic and facultative anaerobes or by the enzyme peroxidase in lactic acid bacteria:

$$H_2O_2 + H_2O_2 + \text{catalase} \rightarrow 2H_2O + O_2$$
$$H_2O_2 + NADH + H^+ + \text{peroxidase} \rightarrow 2H_2O + NAD$$

Anaerobic microorganisms do not have the enzymes superoxide dismutase, catalase, or peroxidase, and hence the presence of oxygen in their growth medium is toxic to them.

20.3.2.2 *Effect of carbon dioxide on microorganisms*

Carbon dioxide has inhibitory effect to certain microorganisms if its concentration in the growth medium exceeds certain critical levels. Sensitivity to inhibition varies greatly among different microbial species (Bennik *et al*., 1995). In general, among bacteria more inhibition is observed in pseudomonads, while lactobacilli were found to be relatively resistant. The maximum specific growth rate of *Pseudomonas fragii* 72 growing in batch culture aerated with pure air was about $0.4\,h^{-1}$. When the batch culture was aerated with 50% carbon dioxide, the specific growth rate of the bacterium decreased to $0.1\,h^{-1}$ (Molin, 1983a). On the other hand, the maximum specific growth rate of a *Lactobacillus* sp. grown in batch culture was $0.17\,h^{-1}$ under pure air, and decreased slightly to $0.12\,h^{-1}$ under 100% carbon dioxide (Molin, 1983b). Carbon dioxide can affect the action of enzymatic decarboxylation and block the metabolism of some microorganisms like *P. aeruginosa*. It may also affect the permeability of cell membrane as observed in the spores of *Clostridium sporogenes*, *C. perfringens*, and *B. cereus* (Jay, 2000). Germination of spores of *C. sporogenes* and *C. perfringens* was stimulated by 1 atm CO_2, whereas germination of spores of *B. cereus* was inhibited. In general, the inhibitory effect of carbon dioxide increases as storage temperature decreases, due in part to increased solubility of carbon dioxide in water at lower temperatures. Inhibition also increases as the pH of the food is decreased into the acid range.

20.3.2.3 *Effect of relative humidity*

Relative humidity of the storage environment of foods may affect their quality, because it can lead to change in their water activity. Foods will eventually come to moisture equilibrium with their surroundings, hence evaporation from or condensation of moisture on the surface of food may occur. If water activity of a food is important for its safety or shelf life, then this food will

need to be stored in an environment which does not markedly change this characteristic. Packaging plays a major role in determining the degree to which foods will be affected by the relative humidity of storage. Sometimes water migration may take place in sealed containers due to temperature fluctuations of the environment. This may lead to conditions where water activity on surfaces can reach values that allow microbial growth, leading to food spoilage or even poisoning. As a general guideline, the product should be held such that environmental moisture, including that within the package, does not have an opportunity to alter the water activity of the product in an unfavorable way. Microorganisms that may cause spoilage under humid storage conditions include molds, yeasts, and certain bacteria. Improperly wrapped beef cuts and whole chicken often undergo surface spoilage in the refrigerator due to elevated relative humidity.

20.4 IMPLICIT FACTORS

The implicit factors are the factors related to the microorganisms themselves, including interactions between the microorganisms contaminating the food and between these microorganisms and the food. Some species predominate in certain foods depending on their abilities to utilize different nutrient sources, tolerate stresses, or produce inhibitors of growth to other competitors. At conditions that favor the growth of different types of microorganisms, bacteria usually grow faster than yeasts, and yeasts faster than molds. Microorganisms differ in their metabolic activities. A microorganism with amylase activity (e.g., *Aspergillus niger*, *Bacillus licheniformis*) can grow in starchy foods, whereas one with proteolytic activity (e.g., *A. oryzae*, *P. fluorescens*) will grow in foods that contain proteins. They also differ in their ability to survive stresses, e.g., high or low temperature, low water activity, high osmotic pressure, or presence or absence of oxygen. Thermophilic microorganisms (e.g., *C. thermosaccharolyticum*, *D. nigrificans*) may survive heat treatments and cause spoilage of canned foods, whereas psychrotrophs (e.g., *P. aeruginosa*, *L. monocytogenes*) endure cold stresses and may grow in cooled foods causing spoilage or disease. Aerobic microorganisms will grow faster and spoil fresh foods held in open containers, while anaerobes can grow in and spoil canned foods with low oxygen concentration. Accumulation of metabolic products may limit or promote the growth of particular species, i.e., either antagonistic or synergistic interactions. Antagonism may be due to competition for nutrients or due to production of growth inhibitors. Synergism may be a result of production of growth promoters or change of food characteristics such as pH or redox potential to more favorable levels. If a metabolic product can be used as a substrate by other species, these may take over and create microbial succession. Hence, foods may have different characteristic microbial associations at different points in time, each association succeeding the other in what is called microbial succession.

Interactions between microorganisms in a food system continue as long as these microorganisms remain active. Based on their growth-promoting or inhibiting nature, these interactions are either antagonistic or synergistic.

20.4.1 Antagonism

In food systems, antagonistic processes usually include competition for nutrients and/or unfavorable alterations of the environment through production of growth inhibitors such as bacteriocins, hydrogen peroxide, and organic acids. When other growth conditions are optimal, microorganisms with high specific growth rates will be the best competitors for nutrients in a

food system. In mixed populations they can dominate quickly and overgrow others. Raw meats are normally contaminated with many types of microorganisms including different genera of bacteria, yeasts, and molds. However, spoilage of meats such as ground beef is caused by only a few genera, dominated by the fast-growing *Pseudomonas* spp. Staphylococci are particularly sensitive to nutrient depletion. Coliforms and *Pseudomonas* spp. have generally higher growth rates than *Staphylococcus* spp., hence they may utilize amino acids necessary for staphylococcal growth and make them unavailable. Other genera of Micrococcaceae can also utilize nutrients more rapidly than staphylococci. Streptococci inhibit staphylocci by depletion of the supply of nicotinamide or niacin and biotin in the growth medium.

A well-studied example of microbial antagonism is the lactic antagonism. More than 70 years ago it was observed that lactic acid bacteria inhibit or kill certain types of food poisoning and food spoilage organisms when present in mixed cultures (Jay, 2000). The phenomenon is commonly referred to as lactic antagonism. Although the precise mechanism of lactic antagonism is not yet well known, many factors are identified as potential causatives. These include antibiotics, hydrogen peroxide, lowered pH, diacetyl, nutrient depletion, and bacteriocins. *Propionibacterium freudenreichii* subsp. *shermanii* produces an inhibitory system effective against Gram-negative bacteria and molds in cottage cheese. *Lactobacillus reuteri* forms reuterin (3-hydroxypropionaldehyde), which inhibits the growth of *E. coli* (including the strain O157:H7) and *L. monocytogenes*.

Inhibition due to production of organic acids and reduction of pH is common in food fermentation. In spontaneous sorghum flour fermentation remarkable microbial succession has been observed (Hamad *et al.*, 1997). The dominant microflora in the flour before fermentation (pH 6.4) was made of different species of Gram-negative, catalase-positive rods with no detectable presence of lactic acid bacteria. After 24 h of fermentation, where the pH dropped to 3.5, only *Lactobacillus fermentum*, *Enterococcus faecalis*, and *L. lactis* were present in the dough. At the end of fermentation after 42 h the pH was 3.35 and the dominant microflora was made of *L. fermentum* and *L. reuteri*.

20.4.2 Synergism

The growth of certain microorganisms can lead to changes in the characteristics of a food system that may promote the growth of other foodborne microorganisms. Many types of synergistic interactions are known, particularly in food fermentation (Deák, 2008). Different microorganisms in association can provide each other with nutrients and other essential growth factors. In kefir fermentation the yeasts provide the lactic acid bacteria with vitamins, and the bacteria provide the yeasts with lactate. In red wine, fermentation activity of the bacterium *Leuconostoc oenos* is increased by vitamins and amino acids produced by yeasts. Changes in pH may promote the growth of certain microorganisms. An example is the growth of molds on high-acid foods which has been found to raise the pH, thus stimulating the growth of *C. botulinum*. Changes in Eh or a_w in the food can influence symbiosis. At warm temperatures, *C. perfringens* can lower the redox potential in the tissues of freshly slaughtered animals so that even more obligately anaerobic organisms like *C. botulinum* can grow.

20.5 PROCESSING FACTORS

Processing factors include treatments such as heating, cooling, and drying that affect the composition of the food and also affect the types and numbers of microorganisms that remain

in the food after treatment. A food contaminated with a mixture of heat-sensitive and heat-tolerant microorganisms may be spoiled by the heat-sensitive ones if it is kept as raw food at room temperature, because these conditions favor the fast growth of mesophiles. Heating, however, will kill the temperature-sensitive microorganisms while the temperature-tolerant ones may remain unaffected. Milk is a good example. Unpasteurized raw milk is normally spoiled by the fast-growing bacteria from the family Pseudomonadaceae, including *P. fluorescens*, *P. fragii*, and *Psuedomonas putida*. If pasteurization is not adequate, it may kill the vegetative cells of the spoilage microflora leaving the spores of some bacterial species behind without competitors. These species, e.g., spores of *B cereus*, start germination soon after the heat treatment, indicating that they were heat activated. Spoilage of fresh and processed vegetables is another good example. Spoilage of fresh vegetables can be caused by any of the contaminants from bacteria, yeasts, or molds, but spoilage of commercially sterilized and canned vegetables is due to the growth of the thermophilic spores of few bacterial species such as *B. stearothermophilus*, *B. coagulans*, *C. botulinum*, or *D. nigrificans*. Similarly, anaerobic microorganisms like *Clostridium* will not grow in fresh foods kept in containers open to air, but if these foods were processed and kept under anaerobic conditions in cans, foodborne *Clostridium* may grow and spoil or poison them. The pH of raw milk is more than 6, which is ideal for the growth of almost all types of foodborne spoilage and pathogenic microorganisms. The pH of fermented dairy products such as yoghurt, cultured buttermilk, cultured sour cream, and cottage cheese is below 5, which does not favor the growth of most foodborne pathogenic microorganisms. Condensed and evaporated milk products, because of reduced water activity, can only be spoiled by a few microbial genera including species of the yeast *Torulopsis* in the case of sweetened condensed milk and *Bacillus* species such as *B. stearothermophilus*, *B. coagulans*, *B. licheniformis*, *B. subtilis*, and *B. macerans* in the case of evaporated milk. Unlike fresh fruits which can be spoiled by yeasts, molds, and some species of bacteria, pasteurized and canned fruits are mainly spoiled by spore formers of the species *Clostridium pasteurianum*, *C. butyricum*, and *B. coagulans.*

20.6 INTERACTION BETWEEN FACTORS

Interaction between the various factors described above can also affect the growth of microorganisms in foods; the combined effects may be additive or synergistic. This interaction is used in the food-preservation technology known as the hurdle concept. The most important factors used in the hurdle technology are the intrinsic factors (a_w, pH, Eh, and chemicals) the extrinsic factors (temperature of storage and gas atmosphere), and the processing factors (heating, drying, fermentation). The hurdle concept is based on the fact that although many inhibitory factors (hurdles) are individually unable to inhibit microbial growth, they can, nevertheless, be effective if combined. The technology is used in place of harsh treatments needed when only one control parameter is applied. The well-informed consumers want their foods to look natural and fresh, to be healthy and convenient, and to contain less fat, salt, and sugar. A food preserved solely by high salt or low acidity may not be organoleptically acceptable, but if low salt is combined with less acidity then the consumer acceptability level will be higher. Examples of foods in which the hurdle concept applies include jams and jellies. Here, heating, low pH, low water activity, and anaerobic packaging are used to inhibit the growth of the majority of microorganisms even when the product is kept at room temperature. Another example is the preservation of

high-moisture fruits suggested by Alzamora *et al.* (1989, 1993). The suggested treatment includes:

- mild heating to inactivate enzymes and to lower microbial load (heating with saturated steam for 1–3 min);
- slight reduction of water activity by addition of sucrose or glucose (a_w to 0.98–0.94);
- adjustment of pH in fruits with high pH by addition of citric or phosphoric acid (pH adjusted to 3.0–4.1);
- addition of two preservatives: potassium sorbate (or sodium benzoate, 1000 ppm) and sodium sulfite (or sodium bisulfite, 150 ppm).

Interactions between pH and water activity are used in the control of *C. botulinum* (Christian, 2000). At 37°C and pH 7, the minimum water activity for the growth of type A was 0.94, but at 37°C and pH 5.3 the minimum water activity for growth increased to 0.99. The US Department of Agriculture (USDA; Food and Drug Administration, 2010) recognizes the hurdle concept in the preservation of semi-dry sausages. Such food products are designated as shelf-stable when their moisture/protein ratio is less than or equal to 3.1:1 and the pH less than or equal to 5.0. In salad dressings and mayonnaises, the acid/moisture ratio combined with pH adjustment is used as the governing factor for pathogen control. An acid/moisture ratio greater than 0.70 in combination with a pH less than 4.1 is often used as the target level for the control of pathogens in these food products. The times needed for a 3 log increase in the concentration of *Staph. aureus*, grown at 25°C (predicted by a modeling program) were, at a_w 0.92: 171.3 h at pH 4.62, 113.1 h at pH 5.0, and 80.7 h at pH 5.5. When water activity was raised to 0.96 the times needed were 86.3, 53.4, and 35.9 h, for the three pH values, respectively (Food and Drug Administration, 2010).

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21 A Whole-Chain Approach to Food Safety Management and Quality Assurance of Fresh Produce

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Abstract: Raw vegetables can become contaminated with bacteria that cause foodborne illness, degrade food quality or reduce product shelf life. Since fresh produce can become contaminated at any point from cultivation to consumption along the food supply chain, it is essential to apply a whole-chain approach to managing food safety and quality. The Institute for Microbial Control of the Food Chain (iMIK) was established in Belgium to examine the different processes and stakeholders in the entire food supply chain, to identify critical points concerning food safety and quality, and to provide the food industry with an evidence-based approach to food safety management that is supported by scientific data. In this chapter, case studies of food safety controls at the different stages of the supply chain are presented, such as the impact of harvest procedures on fresh produce microbiology and the evaluation of fresh produce in the cold chain. Based on the results obtained, critical points were identified and processes were modified to improve food safety and quality. The data presented in this chapter clearly demonstrate the usefulness of a whole-chain approach to effective food safety management and quality assurance of fresh produce.

Keywords: cold chain; foodborne illness; food quality; food safety; food supply chain; fresh produce; hand hygiene; process integrated microbiology; spoilage bacteria

21.1 INTRODUCTION: THE MANAGEMENT OF FOOD SAFETY REQUIRES A HOLISTIC APPROACH

The world per-capita consumption of fresh produce has increased significantly in the last decade (European Commission, 2007). Although stricter standards, quality and food control procedures are being applied to the food industry, there has also been an increase in the incidence of foodborne illnesses, which Houghton *et al.* (2008) called 'the paradox of progress'. Moreover, the globalization of the food trade has increased the risk of the rapid spread of infectious agents (Käferstein *et al.*, 1997; Redmond and Griffith, 2003; European Union, 2010). To ensure that high-quality and safe fresh food products reach consumers, appropriate measures must be implemented throughout the entire food supply chain.

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Fresh produce is particularly vulnerable to the detrimental effects of microorganisms, because of the absence of an inactivation step prior to consumption (Bean *et al.*, 1997; Burnett and Beuchat, 2001; Beuchat, 2004; Lynch *et al.*, 2006; Sapers *et al.*, 2006). For example, raw vegetables can become contaminated with human pathogens or spoilage bacteria at any point from cultivation to consumption in the supply chain (James 2006). During cultivation, vegetables can become contaminated with manure, soil microorganisms or untreated sewage or irrigation water. Postharvest contamination occurs mainly by contaminated harvesting equipment or during transport and processing (Beuchat and Ryu, 1997). Finally, epidemiologic surveillance of foodborne diseases has clearly indicated that consumer behaviour can be a major contributor to disease incidence (Patil *et al.*, 2004).

In the past, food safety and quality assurance focused mainly on individual stages of the food supply chain, e.g. during processing. However, it is becoming increasingly important to coherently manage food safety at all stages of the food supply chain and not simply focus on a particular stage (Desmarchelier *et al.*, 2007; Stringer and Hall, 2007). Each stakeholder plays a key role in food safety management during its particular stage of the food supply chain. Because contamination can occur at any step of the food supply chain, it is clear that a 'whole-chain approach' is essential for successfully managing food safety and quality. In today's world there are several reasons why an integrated food chain approach is necessary, which are now driving the different stakeholders in the food supply chain to recognize the advantages of working together as partners in maximizing the benefits of a safe, wholesome and efficient food supply chain (Stringer and Hall, 2007).

In this regard, the Institute for Microbial Control of the Food Chain (iMIK) was founded in Flanders, Belgium. iMIK is a consortium of three research organizations (the Laboratory for Process Microbial Ecology and Bioinspirational Management, Scientia Terrae Research Institute and the Research Centre for Vegetable Production), each with their own research expertise. iMIK has focused mainly on the microbiology of fresh produce because this is inherently important to food safety and quality (Claes and Rediers, 2007). Contamination of fresh produce by spoilage bacteria greatly affects food quality and product shelf life, while contamination with microbial pathogens directly contributes to the risk of foodborne illnesses.

In a whole-chain approach, the fresh-produce supply chain can be divided into the following 5 major stages: primary production (on the farm), processing, distribution, wholesaling, and retail/catering. In adopting a holistic food safety approach, post-retail plays an ultimate role in the management of food safety. Dividing the whole chain into stages enables identification of potential key weaknesses in the chain, diagnosis of relevant breakdowns and trend analyses, and most importantly provides a mechanism for targeting research on the issues that will accrue the greatest benefit. It is evident that this holistic approach will assist food safety risk management and the Hazard Analysis and Critical Control Points (HACCP) system decision-making process, and will help to identify the critical control points where safety is a potential issue (Stringer and Hall, 2007).

The primary objective of iMIK is to support the stakeholders at the different stages of the food supply chain (i.e. the producer, processor, distributor, wholesaler, caterer/retailer and consumer) with scientific evidence-based solutions. These solutions are provided through research projects as well as through mining, consolidating and interpreting pertinent information from the literature. This chapter demonstrates with relevant cases that scientific review of food safety controls and subsequent (minor) modifications at the different stages of the food supply chain can lead to considerable improvements in both food quality and food safety (Figure 21.1).

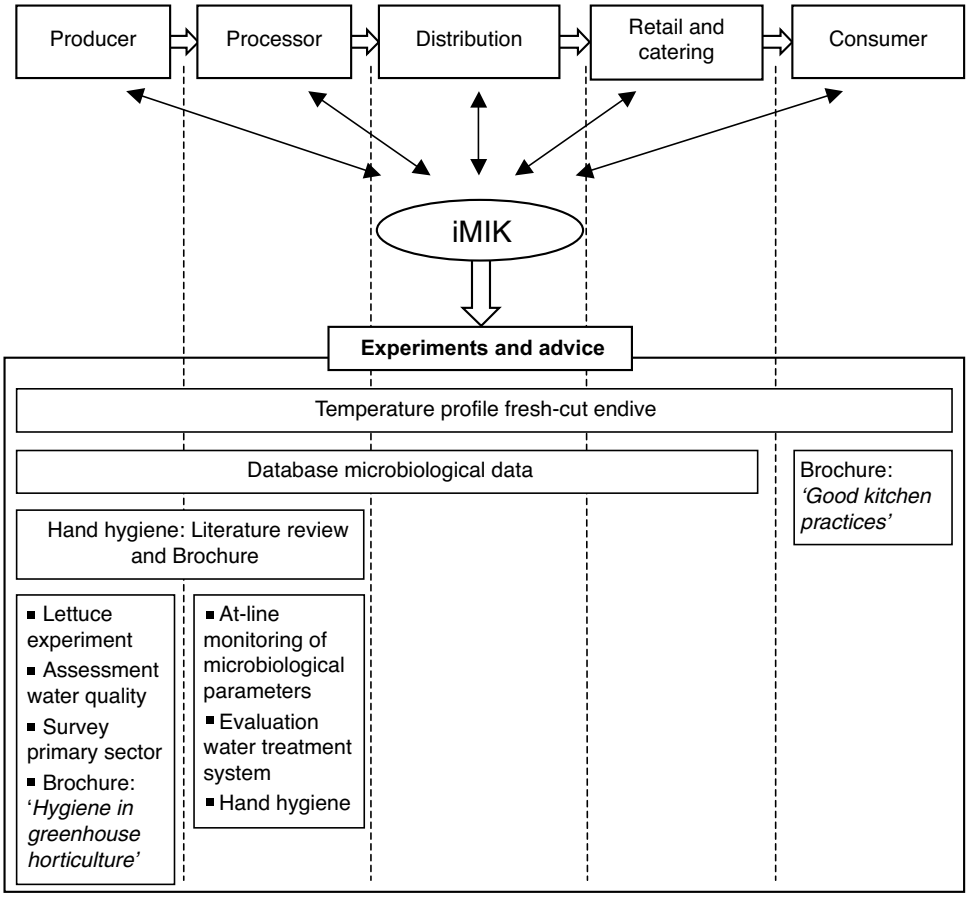

Figure 21.1 Diagrammatic overview of the whole-chain approach of iMIK. The different stakeholders in the food supply chain, i.e. from producer to consumer, are supported with the output from small research projects and literature review.

21.2 MICROBIAL QUALITY MANAGEMENT STARTS IN PRODUCTION

As stated in the introduction, ineffective food safety controls at the cultivation and postharvest steps can contribute considerably to the contamination of fresh produce with spoilage and pathogenic microorganisms.

To ensure food quality and food safety, various codes of Good Agricultural Practices (GAP), such as FlandriaGAP (www.flandriagap.be) and GlobalGAP (www.globalgap.org), are frequently implemented in the primary production sector. These codes of practice include guidelines for maintaining good hygiene standards during the cultivation and harvest of fresh produce that reduce the risk of contamination with spoilage and pathogenic microorganisms. It is generally recognized that efforts such as promoting awareness of microbiological problems and acknowledging the reasons for good hygiene practices result in greater motivation towards complying with and implementing the codes of good practice.

In 2004, iMIK conducted a survey among nearly 200 vegetable growers to assess the adoption of codes of practice and subsequently analyse the utilization of quality systems in the

primary production sector (Hanssen *et al.*, 2005a, 2005b). A possible correlation between grower awareness of problems that result from microbiological contamination and grower perception of microbiological criteria or GAP implementation was also investigated. This enquiry showed that most of the vegetable growers surveyed (92%) already implemented a code of practice, which was predominantly FlandriaGAP. Interestingly, two-thirds of the vegetable growers were convinced that food safety had increased due to the implementation of such a code of practice. Almost three-quarters of the vegetable growers surveyed shared the opinion that microbiological quality of the product is important, and this for the following reasons: increased food safety (46%), increased shelf life (23%), higher food quality (11%), image of the primary sector (7%), visual aspects of the product (5%) and consumer satisfaction (5%).

The survey also demonstrated that vegetable grower knowledge about microbiology is rather limited. Furthermore, approximately 40% of the vegetable growers surveyed indicated that supplementary advice regarding good hygiene practices would be useful. Nevertheless, the survey demonstrated that vegetable growers recognize the contribution of microbiological knowledge towards effective food safety controls in food production. Moreover, the growers were able to identify critical points in the process and intuitively apply good practices regarding microbiological food safety and hygiene.

In addition to the survey described above, iMIK conducted a few small experiments in order to explore the microbiological aspects of some harvest and early postharvest procedures. In the first experiment, the microbiological quality of irrigation and rinse water used by Flemish vegetable growers was examined (Luyten *et al.*, 2006). A total of 99 water samples, originating from three different sources (i.e. ground water, municipal water and rainwater) were analysed. Overall, it was concluded that the microbiological quality of ground water and stored rainwater was satisfactory. Not surprisingly, municipal water was found to possess the highest microbiological quality.

In the second experiment, the extent to which standing water in piping systems is prone to the proliferation of resident microorganisms was examined (Luyten *et al.*, 2006). Water samples were taken either immediately or after the water had run for 2 min. The microbiological status of standing water and running water samples was analysed using microbiological indicators such as the assessment of total mesophilic aerobic bacteria, total coliforms, fecal coliforms, and fecal enterococci. Results from this experiment (Figure 21.2)

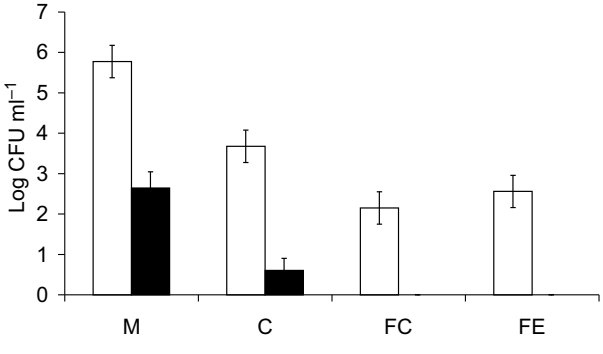

Figure 21.2 Microbiological parameters of standing water in a piping system (white bars) and of water after it was left running for 2 min (black bars). The levels of total mesophilic aerobic bacteria (M), total coliforms (C), fecal coliforms (FC), and fecal enterococci (FE) are expressed as log colony-forming units (CFU)/ml. Error bars indicate standard error of the mean.

clearly demonstrated that after 2 min the levels of indicator microorganisms in the water were reduced considerably. Nearly 1000-fold reductions in total mesophilic and total coliform bacteria levels were observed. The levels of fecal coliforms and fecal enterococci did not reach the lower detection limits. Based on these results, the growers were advised to allow water to run for 2 min before use, especially if the water was used for rinsing harvested vegetables.

In a third experiment, the effects of the amount of nitrogen fertilizer applied during cultivation and the harvesting procedure on the microbiological quality of harvested lettuce were examined (Luyten and Vergote, 2006). Interestingly, neither the nitrogen fertilizer regime nor the harvesting procedure (whether manual or automatic) affected the levels of indicator microorganisms (data not shown).

21.3 PROCESSING OF FRESH PRODUCE IS A KEY STEP IN QUALITY PRESERVATION

21.3.1 Hand hygiene

Several studies suggest that poor worker hygiene is a major contributor to the occurrence of foodborne illnesses. Specifically, poor hand hygiene of infected food operators contributes significantly to the transfer of pathogenic bacteria and viruses (Bean and Griffin, 1990; Snyder *et al.*, 1998; Olsen *et al.*, 2000; Reij *et al.*, 2004). The same studies also demonstrated that exercising good hand hygiene during all stages of food production is critical for minimizing the risk of foodborne illnesses, especially with ready-to-eat foods and fresh-cut produce that do not require further cooking or heating steps. However, selecting the optimal hand hygiene technique is not an easy task since a clear scientific consensus on this topic is not yet apparent in the literature. At the request of local food companies, iMIK conducted a literature review to clarify the issues regarding hand hygiene in the food industry (Rediers *et al.*, 2008).

In this extensive literature review, iMIK reported on the most important techniques used to minimize the risk of cross-contamination by hands, including washing and disinfecting of hands, and glove use. In addition, the efficacy as well as the inherent advantages and disadvantages of each technique were thoroughly discussed. This review article concluded that neither washing hands, or hand disinfection nor wearing gloves completely eliminates the risk of cross-contamination. Regardless of the hand hygiene technique chosen, it is critical that people handling food strictly adhere to the basic principles of hand hygiene.

A very simple and effective technique to remove microorganisms is washing hands with soap using water of potable quality. The washing procedure described must be performed correctly, complying with the correct time necessary (at least 15 s) to allow for the effective physical removal of pathogens. Several reports indicate that the use of antibacterial soaps is more effective than regular soaps, and therefore these particular studies strongly recommended the use of antibacterial soaps, especially when hands are not sufficiently rubbed (Larson, 2001; Montville *et al.*, 2002). Still, the benefits of antibacterial soaps compared with those of regular soaps are debated; several publications state that plain soap is as effective as antibacterial soap when used properly and frequently (reviewed in Aiello *et al.*, 2007). Irrespective of the kind of soap that is used, hand drying is yet another basic component of effective hand washing. The skin of hands must be dried properly to have an optimal microorganism-reducing effect.

Provided that hands are not soiled, the quickest method of achieving effective hand hygiene is using an alcohol-based sanitizer. The advantage of these sanitizers is that they are applied without water and thus can be used virtually everywhere. Moreover, alcohol-based sanitizers appear to cause less skin damage than soaps due to the addition of skin softeners or emollients (Larson, 2001). Nevertheless, regular hand washing is still highly recommended, even when disinfectants are used. This is particularly true in the food sector, where food production typically results in the formation of an organic film on skin, which reduces the antimicrobial efficacy of the alcohol. Washing hands with soap and potable water is also advised when hands are possibly contaminated with viruses or bacterial endospores, as bacterial endospores and many viruses are insensitive to alcohols.

Wearing sterilized gloves is an alternative technique for reducing the transmission of microorganisms from hands to food or vice versa. The efficacy of wearing gloves is comparable to hand washing or disinfection (Chen *et al.*, 2001). However, wearing gloves can induce a false sense of safety, which may result in food operators being negligent. Even when gloves are used properly, it is highly recommended that hands are washed regularly and gloves are changed frequently (Larson, 1989). Moreover, latex glove use is often linked to skin allergy or eczema.

All of the hand hygiene techniques mentioned may cause skin damage when used frequently. Damaged skin is highly susceptible to the colonization of microorganisms and, in addition, hand hygiene procedures are considerably less effective when skin is damaged. It is therefore essential to maintain healthy hands by applying emollients, hydrating creams or lotions.

In conclusion, a specific hand hygiene technique should be selected based on its inherent advantages and disadvantages, taking into account the specific circumstances and needs of the working environment. Food companies often have to compromise between the desired reduction in the number of microorganisms and the implementation of a hand hygiene technique that is achievable with respect to their specific working environment and operating conditions. Nevertheless, it has been stated that the most effective measure in removing microorganisms is a combination of washing hands with antibacterial soap and water of potable quality, followed by proper hand drying and disinfection using alcohol-based sanitizers (Paulson, 1994).

21.3.2 The use of at-line microbial monitoring in food processing

For fresh produce, the microbial quality of the end product is a crucial factor for determining shelf life. However, monitoring the microbial quality at the end of the production line is often too late, because microbiological quality and safety of fresh produce depend strongly on the controls that can be exercised during production. Furthermore, uncontrolled processes invariably lead to economic loss, which in many cases results from microbiological contamination. In this respect, quality control at the end of the production chain has been proven to be inadequate, and more rapid methods of assessing microbiological quality during the process, i.e. at-line microbial monitoring, are required (Willems *et al.*, 1999).

Traditional culture-based microbiological enumeration and detection methods such as dilution plating are not suitable for at-line monitoring simply because the length of time required to conduct such analyses does not permit swift enough feedback for true process control. Many publications have already focused on fast microbiological methods (Fung and Matthews, 1991; Bird, 1992; Vasavada, 1993; Swaminathan and Feng, 1994; Vanne *et al.*, 1996; Griffiths, 1997). Alternatives for traditional culture-plate enumeration methods are

most probable number (MPN), impedance, turbidity, oxygen consumption, adenosine triphosphate (ATP), immunological, DNA-based and microscopic techniques, each having its own advantages and disadvantages. At-line microbial monitoring techniques should be fast, simple to conduct (preferably automated), inexpensive, sensitive, reliable, and specific enough for routine use for a given process or site. The most sensitive techniques are those that require growth for detection, i.e. MPN, impedance and turbidity. For instance, impedance techniques can achieve very short detection times (less than 2 h for a sample with a high microbial load). Impedance techniques, such as the Rapid Automated Bacterial Impedance Technique (RABIT; Don Whitley Scientific, Shipley, UK), actually detect microbial presence either directly or indirectly by measuring the growth of microorganisms present in a given sample. Furthermore, the impedance technique operates independently from the sample matrix, is easy to perform, is unaffected by clumping of microorganisms and can be selective when appropriate media are used. These properties make impedance technologies, such as the RABIT, highly useful for at-line monitoring.

For the direct method, growth is simply detected and assessed by continuously measuring the cumulative increase in conductivity of the liquid culture media in a sealed conductivity cell. Plotting this increase in conductivity results in a curve that corresponds to the lag, logarithmic and stationary phases of a typical bacterial growth curve. In the indirect method, microorganism levels are determined indirectly by measuring the decrease in conductivity (impedance) in a KOH salt-bridge matrix caused when CO_2 produced during microbial growth is trapped and accumulates in the matrix. This method is considered indirect because conductivity is not measured in the growth medium as with the direct method. Since the indirect method technically measures impedance, the shape of the resulting conductivity curve resembles an inverted bacterial growth curve. With both methods, microbial concentration in a sample is assessed by determining the *time to detection* (TTD) of the microorganisms in the sample. TTD corresponds to the point where the cumulative change in conductivity from the baseline meets or exceeds a set value over a defined time interval. The link between TTD and the initial number of cells can be used for calibration. Silley and Forsythe (1996) present a more elaborate overview of impedance microbiology and summarize a number of its applications. Furthermore, there are many publications on the use of impedance for microbial monitoring in food and food processing (Bolliger *et al.*, 1994; Capell *et al.*, 1995; Glassmoyer and Russell, 2001; Metcalfe and Marshall, 2004; Walker *et al.*, 2005).

In a case study carried out by iMIK, at-line microbial monitoring was applied to evaluate a novel recycled process-water treatment system of a commercial apple and pear processor whose processing activities involved fruit storage, washing, sorting and packing. Process water used for washing fruit prior to sorting and packing is recycled for economic and environmental reasons. Microbial and other contaminants in the recycled water must be removed or inactivated to effectively clean and prevent cross-contamination of the fruit. This water-treatment system was computer-controlled and comprised a cascade that contains the following five components: (i) a conical sedimentation tank; (ii) two alternating stacked-disc filtration units; (iii) a zeolite-based gravimetric filtration unit; (iv) a resin-based ion-exchange unit; and (v) dual low-pressure UV treatment cells.

RABIT was used to assess the total mesophilic bacteria (TMB) level in the process water at the following five sampling points along the process water recovery and treatment system: (i) the fruit-washing basin; (ii) after zeolite filtration; (iii) after ion-exchange treatment; (iv) after UV treatment; and (v) immediately before water returns to the fruit-cleaning basin.

To calibrate RABIT measurements, linear regression analyses were conducted using RABIT TTD values and log-transformed TMB values from dilution plate enumeration on TSB agar of samples collected in technique optimization experiments. Thereafter, this calibration equation was applied to RABIT TTD measurements to calculate TMB values in process water samples. Mean TMB values of process water from each sampling point were analysed and plotted using statistical process control (SPC) routines of Minitab software (Release 15; Minitab, State College, PA, USA). Upper and lower control limits (UCL and LCL, respectively) were calculated as three standard deviations above and below the average lines, respectively, when appropriate. After SPC analysis of the data and comparison of SPC graphs from each of the sampling points, it was clear that only the UV treatment could reduce TMB loads in process water to at least 1 log order of magnitude lower than the process water in the fruit-washing basin (Figure 21.3). SPC analysis also differentiated between apple

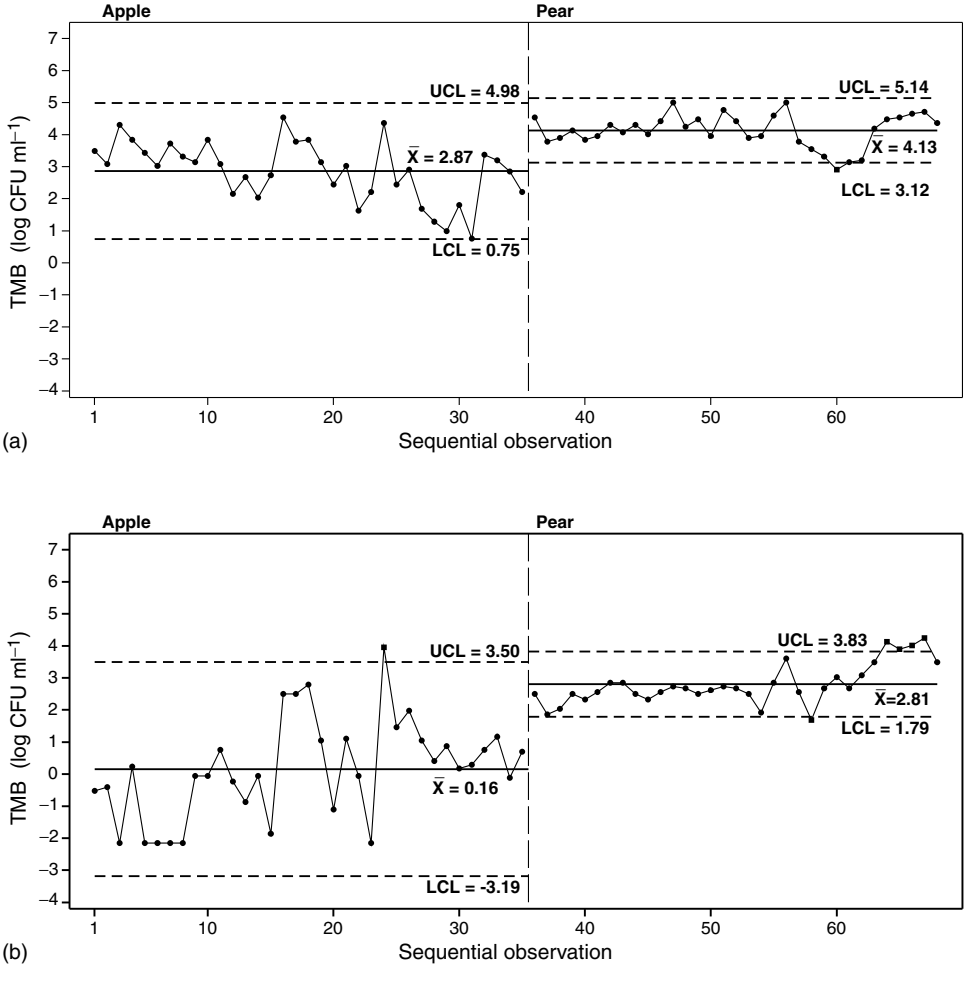

Figure 21.3 SPC charts used to evaluate the impact of a novel water-cleaning system on TMB levels in recycled apple and pear rinse water. RABIT was used to assess TMB levels in water collected from the fruit-rinsing basin (a), and collected after UV light treatment (b). TMB levels are expressed as log CFU/ml. UCL and LCL and the mean value of observations (solid horizontal line) were determined separately for the apple- and pear-processing periods. Square data symbols indicate out-of-control observations. Samples were taken routinely in the period 2004–2006.

processing (mean 2.7 log reduction, observations 1–35) and pear processing (mean 1.3 log reduction, observations 36–68). Coinciding with this distinction, it was also observed that the process water during pear processing was visibly darker than during apple processing. Hence, the reduced effect of the UV treatment during pear processing can be attributed to the fact that the efficacy of UV irradiation is reduced in highly light-scattering or -absorbing solutions (Hua and Thompson, 1999).

This case study shows that fast at-line microbial monitoring techniques, such as RABIT, are highly useful for the implementation of process integrated microbiology (PIM). PIM is an approach in which the responsibility of the microbiological process control is, where possible, handed over to the process operators. It not only includes obtaining and using rapid, simple-to-conduct detection techniques, but also the use of SPC for automated continuous analysis, process evaluation and fault detection. As a result, PIM can facilitate continuous process improvement based on in-process microbial indicators.

21.4 MONITORING THE ENTIRE FOOD SUPPLY CHAIN

21.4.1 Temperature management in the cold chain

Numerous studies have demonstrated that foodborne illnesses are often caused by poor temperature management in the cold chain (Brackett, 1992; McCabe-Sellers and Beattie, 2004; Rosset *et al.*, 2004; Todd *et al.*, 2007). Because cold storage drastically reduces the growth rate of most human pathogens, strict temperature control throughout the fresh-produce supply chain can minimize the risk of foodborne illnesses (Ukuku and Sapers, 2007). However, few pathogens, such as *Listeria monocytogenes*, are still able to grow at refrigeration temperatures on a wide variety of vegetables (Beuchat and Brackett, 1990; Brackett, 1999; Thomas and O'Beirne, 2000).

In addition to minimizing the risk of foodborne illnesses, cold storage is also important for the following reasons. First, low temperatures are required to maintain an optimal product quality. When produce is maintained at low temperatures several physiological activities, such as transpiration and respiration, are reduced. Transpiration causes product weight loss, while respiration causes chemical and enzymatic changes that may result in tissue softening, pigment loss, ripening or discoloration (Brosnan and Sun, 2001; de Castro *et al.*, 2005). Second, low temperature reduces the growth rate of spoilage microorganisms, such as some *Pseudomonas* spp., *Xanthomonas* spp., *Erwinia* spp., *Bacillus* spp., and several yeasts and moulds (Brackett, 1994; Francis *et al.*, 1999). Therefore, maintaining fresh-cut produce at low temperature considerably delays microbial deterioration. For instance, a major causal agent of postharvest bacterial soft rots is *Erwinia coratovora*, which grows optimally at 30°C. However, its growth is drastically reduced during cold storage, and therefore most *Erwinia* spp. are generally not considered to pose a threat at low temperature (Godfrey and Marshall, 2002).

Hence, it is clear that effective cold-chain management is crucial to the preservation of the food safety and quality of fresh-cut produce. In order to reduce the proliferation of spoilage microorganisms and human pathogens, the temperature of fresh produce should be maintained below 5°C. However, this is apparently difficult to achieve since fresh produce is regularly subjected to temperature abuse approximating 8–12°C (Scandella *et al.*, 1990; Giannakourou and Taoukis, 2003).

In assessing fresh produce in Flanders, iMIK investigated the cold chain of fresh-cut endive and its impact on the microbiology of foodstuffs (Luyten and Claes, 2006; Claes *et al.*, 2007e; Rediers *et al.*, 2009). In this survey, endive temperature was monitored throughout the

supply chain from the producer through the processor and distributor to the restaurant. Levels of indicator microorganisms, namely total mesophilic aerobic bacteria, total coliforms and total *Enterobacteriaceae*, were assessed from harvest until the end of the defined shelf-life period in order to observe the effect of temperature abuse on microbial food safety. In addition, the temperature data obtained were used to assess the initial cooling process since the time required for initial cooling is considered to be an important shelf-life determinant (Brosnan and Sun, 2001).

Results obtained in this survey demonstrated that the relative position of the endive in a pallet affects the speed of cooling during the initial cooling process. As expected, endive cools down more rapidly in crates placed at the top of the pile than in the middle. Secondly, it was shown for the specific conditions of the supply chain examined in this study that the outside temperature had negligible effect on the time required for initial cooling of the endive (data not shown). Nevertheless, the endive temperature after 6 h of cooling was below 8°C in all cases. Although it has been stated previously that external temperatures during harvest have a major impact on fresh produce quality and shelf life (Brackett, 1994), our data suggest that the key parameter is the speed of the initial cooling process, rather than the external temperature. This is consistent with Rosset *et al.* (2004), who observed that meteorological conditions and initial product temperature had no effect on initial cooling time.

More importantly, our experiments indicate that this particular cold chain was generally properly maintained and that an endive temperature of approximately 4°C could be maintained throughout the supply chain (Figure 21.4).

Nevertheless, the global temperature profile revealed some critical points in the cold chain. During transport from the farm to the processor and from the processor to the distributor, small increases in temperature were observed. These increases were more pronounced at the

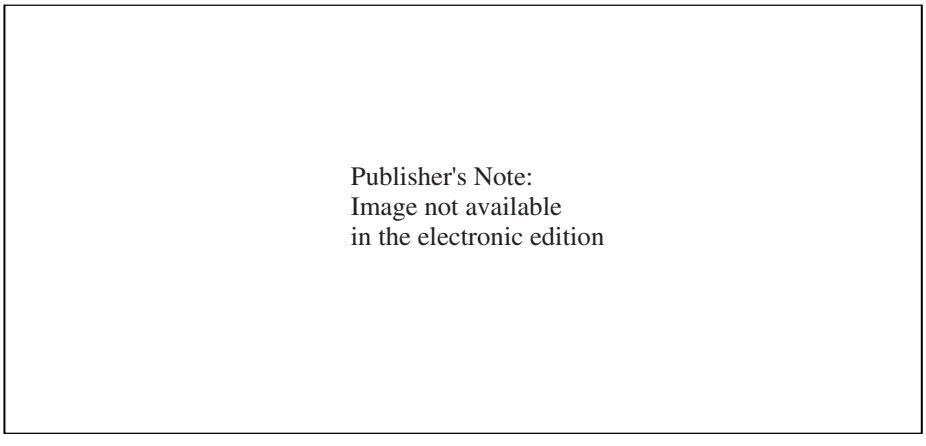

Figure 21.4 Temperature profiles of fresh-cut endive from harvest to distribution. Data loggers were placed in crates located at the top (▪), in the middle (▲) and at the bottom (●) of the pallet. The temperature profile covers the time from harvest (10:00, day 1) until storage at the distributor (05:00, day 3). Shaded boxes represent transport from farm to the processor and from processor to the distributor, respectively. During processing, data loggers were placed next to the processing unit, as indicated by the text box. Temperature was recorded every minute. Data represent the average temperature of four independent experiments. Reprinted with permission from *Postharvest Biology and Technology* 51(2), Rediers, H., Claes, M., Peeters, L., and Willems, K.A., Evaluation of the cold chain of fresh-cut endive from farmer to plate, 2009 with permission from Elsevier.

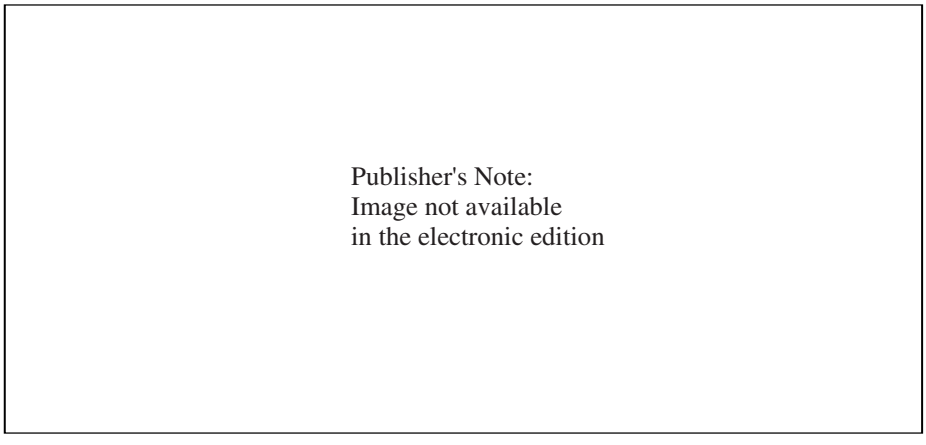

Figure 21.5 Temperature profile during distribution to the restaurants. Endive temperature was recorded every minute during transport (5:00–10:00, day 3) and upon each successive delivery to restaurant 1 (▪), restaurant 2 (▲) and restaurant 3 (●). Data represent mean values from four independent experiments. Arrows indicate time of delivery. Reprinted with permission from *Postharvest Biology and Technology* 51(2), Rediers, H., Claes, M., Peeters, L., and Willems, K.A., Evaluation of the cold chain of fresh-cut endive from farmer to plate, 2009 with permission from Elsevier.

top of the pallet than in the middle or at the bottom. A second critical point appears to be the delivery to the restaurants (Figure 21.5). During delivery, temperature increases of 2–4°C were observed. At both critical points, higher temperature abuse was recorded on hot days.

Storage in the restaurants appeared to be a third critical point since temperature of endive stored in refrigerated rooms of the restaurants showed considerably more fluctuations than endive stored in laboratory refrigerators. Nevertheless, endive temperature never exceeded 8°C (data not shown).

In parallel with temperature monitoring, the total levels of mesophilic bacteria, coliforms, and *Enterobacteriaceae* were assessed at different points throughout the supply chain (Figure 21.6). In general, TMB levels were lower upon arrival in the restaurants compared to the samples taken at harvest. This probably occurred because the fresh-cut endive was washed during processing, which undoubtedly removed a substantial amount of micro-organisms. Levels of all microbiological indicators were significantly higher on endive samples collected and analysed at the end of the defined shelf-life period, more specifically on the 'best-before' date. However, the increase in coliforms (1.6 log CFU/g on average) during storage in the restaurants was more pronounced than the increase in mesophilic bacteria (0.8 log CFU/g) and *Enterobacteriaceae* (0.9 log CFU/g).

To examine whether the temperature fluctuations encountered in the supply chain could affect microbial food safety, microbiological analyses at the best-before date of endive stored in the restaurants were compared to analyses of endive stored under ideal conditions in laboratory refrigerators kept at 2.5°C. TMB levels were slightly but not significantly higher (0.3 log CFU/g; $p = 0.05$) when endive was stored in the restaurant. Conversely, *Enterobacteriacae* and coliform levels were significantly higher (0.4–0.8 log CFU/g and 1.1–1.6 log CFU/g, respectively; $p = 0.05$) when endive was stored in the restaurant. These data also suggest that temperature fluctuations impact coliform growth greater than *Enterobacteriaceae* and mesophilic bacteria growth. This phenomenon was consistent with that observed

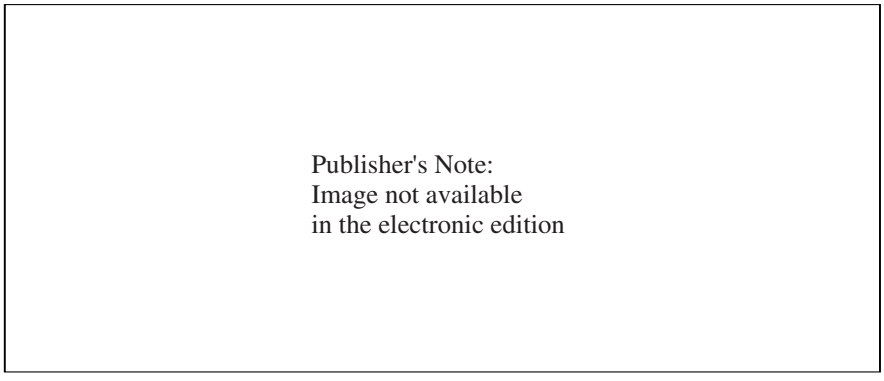

Figure 21.6 Microbiological analysis of endive samples. Levels of total mesophilic aerobic bacteria (black bars), the number of coliforms (grey bars) and *Enterobacteriaceae* (white bars) detected on endive samples are expressed in log CFU/g endive (data from Rediers *et al.*, 2009). Samples were taken after harvest (F, day 1), after arrival in the restaurants (R1, R2, R3, day 3), and at the best-before date (day 7) in the restaurants (BB1, BB2, and BB3) and in the laboratory (BBD). Data are the means from four independent experiments. Enumeration of microorganisms in each experiment was conducted in triplicate. Error bars indicate standard error of the mean. Reprinted with permission from *Postharvest Biology and Technology* 51 (2), Rediers, H., Claes, M., Peeters, L., and Willems, K.A., Evaluation of the cold chain of fresh-cut endive from farmer to plate, 2009 with permission from Elsevier.

when replicated experiments were individually compared with one another. When endive was stored under optimal laboratory conditions (with essentially no temperature abuse), only a small but insignificant increase in the coliform level was observed. The largest increase in coliform levels was observed in restaurant 1 where temperature abuse was greatest (2 log CFU/g compared to arrival in the restaurant). However, increases in coliform levels during storage in restaurants 2 and 3 (where temperature abuse was less) were lower than restaurant 1 (1.3–1.6 log CFU/g). In contrast, the levels of Enterobacteriaceae and mesophilic bacteria vary little between the three restaurants. Our findings agree with the previously stated hypothesis that the effect of temperature abuse on the growth potential of microorganisms varies with the type of produce and the microbial indicators (Thomas and O'Beirne, 2000).

In addition to the indicator microorganisms, the human pathogens *Escherichia coli* and *L. monocytogenes* were also assessed. It was demonstrated that *L. monocytogenes* was absent in all endive samples that were analysed. *E. coli* was present in one-third of the endive samples, but in most cases *E. coli* levels remained below 2 log CFU/g.

In conclusion, endive temperature profiles indicated that, in general, the cold chain was properly maintained, but also revealed some critical points throughout this chain. It was observed that small temperature fluctuations in the supply chain had only a small effect on the level of aerobic mesophilic bacteria. However, at the best before date, total coliforms and *Enterobacteriaceae* levels were significantly higher in endive samples subjected to temperature fluctuations in the supply chain, compared to endive stored under ideal conditions. Nevertheless, the levels of all indicator microorganisms and pathogens were confined within the limits prescribed by European Commission Regulation EC 2073/2005. Similar results were obtained in an analogous experiment carried out previously in Autumn 2005 (Luyten and Claes, 2006), which points to the consistency of our results.

21.4.2 Construction of a microbiological database as a tool for process control

Microbiological analyses are routinely carried out throughout the fresh-produce supply chain. Currently, however, these data are generally used only to evaluate specific processes in the supply chain. One of the objectives of iMIK was to build a centralized database containing microbiological data from all stakeholders in the food supply chain. Individual processes in a particular food company can be evaluated with this database, thus enabling the solid identification of critical control points throughout the company's operating environment. Different processes can be analysed with the use of SPC charts of microbiological parameters, e.g. TMB, and whether or not a specific process is under control can easily be detected.

Furthermore, statistical analysis of this centralized database, which contains data from the entire food supply chain, will also enable conclusions to be drawn for the complete food supply chain, and the identification of critical control points and their control throughout the entire food supply chain. Additionally, such a database enables food companies to benchmark and compare the microbiological quality of their food products with other food companies.

Although this database is still being compiled, the preliminary data presented in Figure 21.7 and Figure 21.8 demonstrate the usefulness of SPC charts as well as trend analysis of microbiological parameters in a food company. Consistent with standard SPC conventions and with the examples in Figure 21.3, UCL and LCL values were calculated as three standard deviations above and below the overall mean value of all observations. In addition, the maximum level of TMB tolerated by Belgian regulations is also indicated for each of the products presented in Figure 21.7 and Figure 21.8.

The SPC chart of TMB levels detected in freshly prepared fruit salads sampled from a fresh fruit processing company presented in Figure 21.7 indicates that all values lie well within the LCL–UCL boundaries, thus signifying that the process is under control. In addition, only one of these values exceeds the maximum tolerable level of 6 log CFU/g, demonstrating that TMB levels of these particular fruit salads prepared during this period ordinarily remain below the legal safety limit.

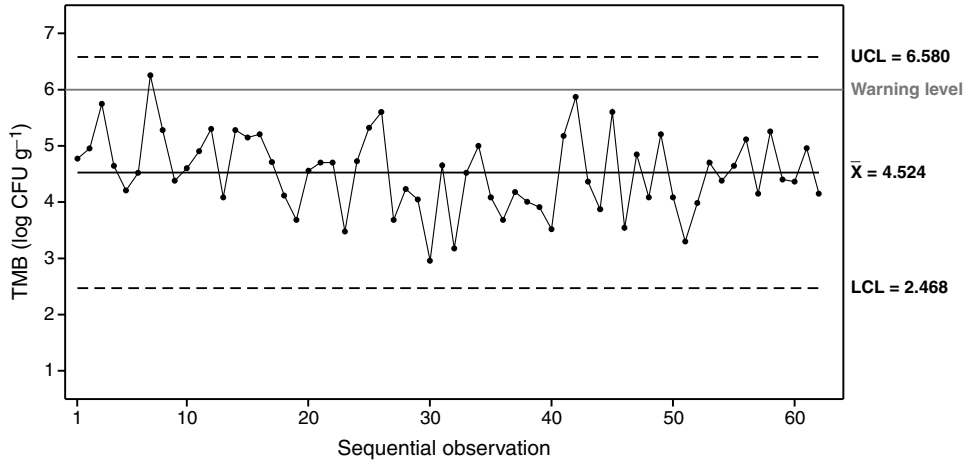

Figure 21.7 SPC chart of TMB detected in miscellaneous freshly prepared fruit salads routinely tested during 2004–2006. TMB levels are expressed as log CFU/g fruit salad. The UCL and LCL (dashed horizontal lines) and mean (solid horizontal line) calculated for all sequential observations are presented. The maximum TMB level in fresh fruit salad products tolerated by Belgian food safety regulations is also plotted (Warning level).

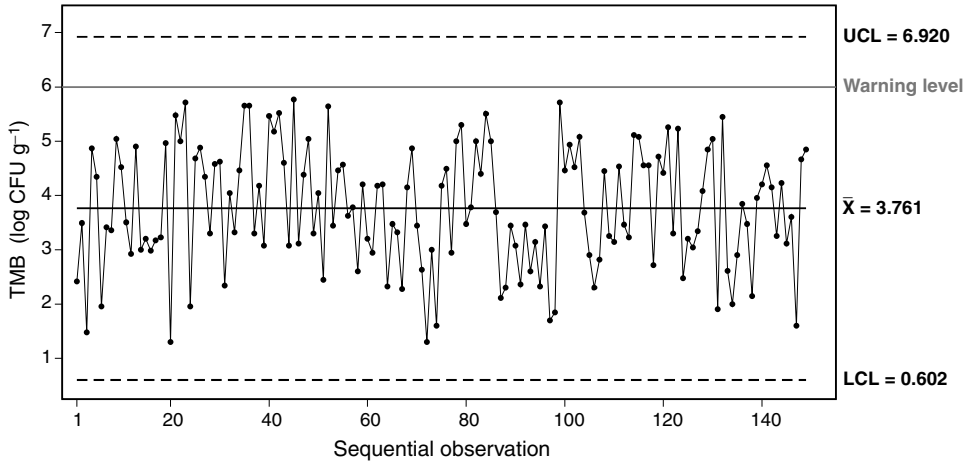

Figure 21.8 SPC chart of TMB levels detected in freshly prepared double hamburger sandwiches topped with lettuce and onion. TMB levels are expressed as log CFU/g. The UCL and LCL (dashed horizontal lines) and mean (solid horizontal line) calculated for all sequential observations are presented. The maximum TMB level tolerated by Belgian food safety regulations (Warning level) is represented by the solid grey line. Samples were taken routinely during 2005–2007.

Likewise, TMB measurements of hamburger sandwich samples obtained from a fast food restaurant are plotted in another SPC chart (Figure 21.8). Clearly, this process is under control, since all TMB values lie within the LCL–UCL boundaries. Furthermore, TMB values never exceeded the maximum tolerable level of 6 log CFU/g.

21.5 THE IMPROVEMENT OF COMPLIANCE BY INCREASING AWARENESS

Several codes of practice have already been implemented at different stages of the food supply chain. For instance, in the primary sector, GAP are a collection of principles that are applied in primary production and post-production processes. Their use is intended to result in safe and healthy food, while accounting for economic, social and environmental sustainability (Food and Agriculture Organization, 2006). Likewise, Good Manufacturing Practices (GMP), Good Transport Practices (GTP), Good Retail Practices (GRP), and Good Catering Practices (GCP) are sets of procedures that should guarantee optimum product quality and safety during processing, transport, retail and catering activities, respectively. In addition, Good Hygienic Practices (GHP) and Good Storage Practices (GSP) contain principles that are important in every stage of the supply chain (Gorris, 2005; Food and Agriculture Organization, 2006; Raspor, 2008). However, strict compliance with these guidelines is required in order to ensure optimal food safety and quality. This applies to all food workers in the food supply chain.

The main barriers for implementation of and compliance with good practices are a lack of money, time, experience and infrastructure, not to mention lack of information and interest (Yapp and Fairman, 2006). For instance, food workers are often not fully aware of the importance of food safety guidelines, and as a consequence they are careless in complying with these rules. Therefore, employees should be better trained in good practices, while

information campaigns should also be conducted to build greater consciousness of food safety practices among employees. Although little scientific support exists in the literature for a direct relationship between awareness and behaviour, it is generally recognized that increased knowledge regarding different aspects of food safety can enhance the food workers' motivation for and adherence to safety guidelines (Bruhn, 1997; Worsfold and Griffith, 1997; Wilcock *et al.*, 2004; Yapp and Fairman, 2006; Raspor, 2008). Therefore, iMIK has published several brochures and other documents, and has organized symposia and workshops for food workers throughout the entire food supply chain. These efforts aimed at informing food workers about food safety concerns (Mariën and Luyten, 2006). Since communication of food safety should be expressed in the simplest way possible to facilitate understanding (Mossel *et al.*, 1998), the information in the iMIK-generated brochures is expressed in an uncomplicated and straightforward way.

The first brochure focuses on the implementation of good hygienic practice in greenhouse horticulture. The main objective of this brochure is to provide horticultural workers with easy-to-understand information regarding good hygienic practices in order to avoid contamination of crops and the spread of plant and human pathogens. The brochure covers the following topics: importance of hygiene measures, procedures for good hygiene and disinfection, visitor procedures, infrastructure points of interest, transportation guidelines, procedures for water disinfection and guidelines for crop rotation (Claes *et al.*, 2007f).

Food operators play a key role in food safety by using safe and hygienic practices during all stages of food production. The objective of the second brochure is to inform food industry workers about good hygiene practices. This brochure was compiled to increase awareness about contamination and cross-contamination of food with human pathogens. As mentioned in section 21.3.1, hand hygiene techniques are only effective if the appropriate procedures are carried out correctly. For this reason, the scientific information provided in the review of Rediers *et al.* (2008) was compiled in a similar brochure and distributed among food operators to improve awareness regarding good hygiene practices. This brochure contains guidelines regarding hand hygiene techniques: specific procedures for hand washing and hand disinfection, and guidelines for maintaining a healthy skin (Claes *et al.*, 2007a).

In addition to these brochures, more scientific information and legal aspects regarding both topics were published in a national technical trade journal (Claes *et al.*, 2007c, 2007d). This journal is specifically published to reach managers in the food industry, such as quality managers, production managers and chief executive officers. In this regard, iMIK also examined and summarized the information in the European General Food Law (Regulations EC852/2004, EC853/2004, EC854/2004 and EC2073/2005) and translated it into a simple and straightforward document (Claes *et al.*, 2007b).

21.6 LAST BUT NOT LEAST: CONSUMERS

Epidemiologic surveillance of foodborne diseases suggests that consumer behaviours, such as ingestion of raw/undercooked foods and inadequate cooling of food, contribute considerably to foodborne diseases (Patil *et al.*, 2004). Clayton and Griffith (2003) estimated that 50–85% of reported foodborne disease outbreaks in various countries around the world are associated with the home environment. The main factors responsible for food poisoning outbreaks are inappropriate storage, inadequate cooking, cross-contamination through

inadequate manipulation, and inadequate cooling of foodstuffs (Djuretic *et al.*, 1996; Evans *et al.*, 1998; Wilcock *et al.*, 2004). This is illustrated by a domestic observation study in which hand washing abuse and poor hygienic practices were frequently observed in the home (Worsfold and Griffith, 1997). In the same study, temperature abuse during transport and in refrigerated storage was reported. In another study, it was also demonstrated that many refrigerators throughout the world are running at higher than recommended temperatures (James *et al.*, 2008).

These observations confirm that every stakeholder in the food supply chain, including the consumer, plays a key role in assuring food safety by using safe and hygienic practices. While there is considerable legislation regarding food safety (e.g. Regulation (Ec) no. 852/2004 of the European Parliament and of The Council of 29 April 2004 on the hygiene of foodstuffs), and general implementation of procedures based on the HACCP principles, these legislative requirements, however, do not apply to the consumers (James *et al.*, 2008). Despite the fact that consumers are currently not associated with the food supply chain, consumers are intrinsically an integral part of this chain because they are the vital link between the retail level and the home. Therefore, Raspor (2008) introduced the term Good Housekeeping Practices (GHKP), which is a selection of principles and techniques for proper food storage and preparation. Most consumers are unaware of the fact that a large proportion of food poisoning originates in the home (Worsfold and Griffith, 1997).

Several authors suggest that a significant proportion of inappropriate handling of foodstuffs could be prevented through an integrated approach in which better knowledge of good food practice in the home is promoted (Bloomfield and Scott, 2003; Kendall *et al.*, 2004; Kennedy *et al.*, 2005; Raspor *et al.*, 2006; Unusan, 2007; Raspor, 2008). Furthermore, Wilcock *et al.* (2004) stated that consumers could benefit from an information campaign regarding food safety in the home. This should include information about temperature control, correct home food preparation practices and cross-contamination as well as basic information about the ubiquity of microorganisms, comprehensive descriptions of foodborne illnesses and foodborne-illness-prevention strategies.

In this regard, iMIK produced a brochure concerning good housekeeping practices, aimed at consumers. This brochure was distributed in schools, at food technology fairs and at the 'Vegetable Auction' (Hanssen *et al.*, 2004). This brochure and other iMIK documents are readily available at www.imik.org. Several of these documents are only available in Dutch at the time of writing.

21.7 CONCLUSION

Food contamination creates a substantial social and economic burden on communities and their health systems worldwide (Raspor, 2008). Ensuring food safety in today's globalized and complex world is a daunting task and is only guaranteed if there is a concerted effort by all stakeholders in the food supply chain (Motarjemi and Mortimore, 2005). It is therefore important to use a whole-chain approach for controlling food safety at all stages of the food supply chain, including consumers. Such an approach will allow identification of food safety hazards throughout the food supply chain as well as evaluate the likelihood of their occurrence and identify viable control measures (Raspor, 2004).

Accordingly, the main objective of the iMIK has been to monitor and evaluate different processes throughout the entire food supply chain to identify critical food safety and quality control points in the supply chain. The main food-category focus of iMIK is on fresh produce,

because fresh-cut produce is usually a ready-to-eat food and is, therefore, not subjected to any heat-inactivation steps, thus increasing the risk of foodborne illness.

First, most key stakeholders in the food supply chain, including consumers, show gaps in their knowledge of food safety (Raspor, 2008). Therefore, food workers and consumers should be provided with sufficient and reader-friendly information, which is currently not always available (Banati and Lakner, 2006). For this reason, iMIK provided the different stakeholders with reader-friendly information about hygiene in the greenhouse (aimed at horticulturists), good hand hygiene (aimed at food processors) and good kitchen practices (aimed at consumers) to increase food safety awareness and knowledge. In addition to these brochures, the available scientific information regarding hand hygiene was collated in a scientific review (Rediers *et al.*, 2008), which was aimed at the quality management level of food businesses.

Next, small studies carried out in the primary production sector, e.g. regarding the harvesting of lettuce and the assessment of water quality, resulted in the identification of critical points that led to several suggestions for improvement of the processes studied. In a study conducted in a food processing company, the introduction of at-line microbial monitoring proved highly useful in the evaluation of a novel water-treatment system that was used in apple and pear processing. Moreover, the data from the at-line monitoring study facilitated further optimization of this water-treatment system and construction and placement of additional systems at other fruit-processing facilities.

Finally, to demonstrate the viability of the whole-chain approach, iMIK followed fresh-cut endive through the entire food supply chain and monitored the temperature from harvest to consumption. The endive temperature profiles indicated that this particular cold chain was properly maintained overall. However, some critical points in the cold chain were identified, and a significant effect of temperature abuse on food microbiology was observed.

The information and study results presented in this chapter clearly demonstrate the usefulness of a whole-chain approach for the effective management of food safety and quality of fresh produce.

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Part IV Use of Natural Preservatives

22 Food Bioprotection: Lactic Acid Bacteria as Natural Preservatives

Graciela Vignolo, Lucila Saavedra, Fernando Sesma, and Raúl Raya

Abstract: Antimicrobial peptides, namely bacteriocins, produced by lactic acid bacteria (LAB) are well known and have been found to be antagonistic toward closely related bacteria and undesirable harmful microorganisms. Several LAB bacteriocins offer great potential in food preservation, and their use in the food industry can help to reduce the addition of chemical preservatives and/or the intensity of processing, satisfying consumer demand for natural-tasting, lightly preserved, and ready-to-eat foods. In the last 30 years a huge number of publications on bacteriocins have been produced and considerable effort has been made recently to develop food applications for many different bacteriocins and bacteriocinogenic LAB strains, either alone or in combination with other hurdles. Depending on the raw materials, processing conditions, distribution, and consumption, the different food ecosystems offer a great variety of scenarios in which pathogenic and spoilage microorganisms may proliferate. Therefore, bacteriocin effectiveness requires careful testing against specific target bacteria in the type of food for which they are intended to be applied. With a view to extending the shelf life of muscle, dairy, and vegetable foods through biopreservation, the application of bacteriocinogenic LAB strains and their bacteriocins is discussed here.

Keywords: bacteriocins; biopreservation; food; lactic acid bacteria

22.1 INTRODUCTION

Consumer perception of food has changed throughout history. Until the 20th century, the food situation around the world was always problematic since food shortages could occur at any time, even in affluent societies. During this early period, the "survival food age," humankind mostly lived for food. Technology, science, communications, and transportation progressed at an accelerated rate during the 20th century and access to food was no longer a problem in rich societies. After World War II, convenience foods (ready-to-eat (RTE) and shelf-stable products) saw their beginnings in the USA as people wanted to save time cooking and obtaining food while, more recently, health-oriented food and nutraceuticals have become major consumer concerns, giving rise to the "functional food era" in the 21st century. However, due to continuing inequality of wealth there is no doubt that improved nutrition standards and greatly increased food security will provide a chance to transform the lives of millions for the better.

Progress in Food Preservation, First Edition. Edited by Rajeev Bhat, Abd Karim Alias and Gopinadhan Paliyath.

The structure of the global food industry is continually changing and evolving as food suppliers, manufacturers, and retailers adjust to meet the needs of consumers, who are increasingly demanding safe foods and a wider variety of higher-quality products. Having first-hand knowledge of consumer preferences and purchase habits, food retailers are positioned to transmit this information upstream to other segments of the supply chain. Trends in technology, trade, and consumption likely to impact microbial food safety in the next few decades include: the pressure on prices and availability of food leading to a dietary shift; the threat of climate change that could impact on food production through taxation or other levy systems; growing populations requiring greater quantities of nutritious and safe food; and the increase in the global food trade due to expected increases in global income levels and improved transportation networks (Quested *et al.*, 2010). Although there is no evidence that imported food, as a whole, poses higher food safety risks than domestically produced food (Zepp *et al.*, 1998), globalization of food supply means that new food safety and risks can be introduced (i.e., emerging bacteria) while previously controlled risks can be re-introduced (i.e., cholera) into countries, and contaminated food can be spread across greater geographical areas, causing illness worldwide. National perception and handling of food safety risks is complicated and partly based on a country's access to and use of science, detection technology, and mitigation methods. In addition, highly publicized food safety incidents and/or scandals also affect consumer perceptions, leading to changes in food purchasing patterns (Buzby, 2001).

Food safety hazards include risks from veterinary drug and pesticide residues, food additives, pathogens such as illness-causing bacteria, viruses, parasites, and fungi, environmental toxins such as heavy metals and persistent organic pollutants such as dioxins, as well as unconventional agents such as prions associated with bovine spongiform encephalopathy in cattle. Undoubtedly the major threat to food safety is the emergence of "new" pathogens. The recent role of *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Vibrio parahaemolyticus* as foodborne microorganisms has been related to the increase in outbreaks when compared to traditional food pathogens (Church, 2004; Elmi, 2004). It is clear that infectious intestinal diseases, whether caused by bacteria, viruses, or parasites, continue to be a major cause of public health concern and social and economic cost worldwide. Data from different surveillance food safety authorities allow estimations of the impact of disease and risks associated with eating different foods. Recently, the preliminary FoodNet (www.foodnetwork.com) data on the incidence of infection with pathogens transmitted through food in the USA showed a decline in infections caused by *Campylobacter*, *Listeria*, *Shigella*, and *Yersinia*; however, those caused by Shiga toxin-producing *E. coli* O57 (STEC O157) and *Salmonella* did not decrease significantly, while *Vibrio* infections increased (Centers for Disease Control and Prevention, 2007). Similarly, summary reports on trends and data collection on zoonotic foodborne outbreaks in the European Union (EU) established in 2008 that a significant decreasing trend in salmonellosis cases continued in the EU for the fifth consecutive year, in agreement with no major changes in *Salmonella* prevalence in fresh broiler chicken, turkey, and pig meats, while *Campylobacter* infections continued to be the most commonly reported for gastrointestinal disorders in humans with fresh poultry and eggs as the main reservoirs (European Food Safety Authority, 2010). In addition, the number of listeriosis cases in humans has decreased by 11% compared to the incidence recorded in 2007, when a high fatality rate of 20% was reported; *L. monocytogenes* was seldom detected in RTE foods above the legal safety limit, with findings over this limit being most often reported from fishery products, cheeses, meat and RTE products (Ammon and Makela, 2010; European Food Safety Authority, 2010).

Awareness by the general public of the consequences of the meat-borne pathogen *E. coli* O157:H7 has increased, making this organism a household name in the 21st century (Ransom *et al.*, 2003; Skovgaard, 2007). The most striking feature of pathogens is their diversity and selection for drug resistance, indicating that infections will continue to emerge and will probably increase, which emphasizes the urgent need for effective surveillance and control (Morse, 2007; Newell *et al.*, 2010). Experience over the last 20 years indicates that the considerable efforts directed at the major bacterial foodborne pathogens appears to have had little impact on the problems in many countries (Newell *et al.*, 2010).

On these bases, the need for solutions regarding the hygienic quality of food is stated. Modern consumers' trends and food legislation have made the successful attainment of food preservation a major challenge. Since consumers demand high-quality, preservative-free, safe, and minimally processed foods with extended shelf life, and legislation has restricted the use as well as the permitted level of some of the currently approved preservatives in different foods, both consumer and legislative needs call for innovative approaches to preserving food. Biological preservation has gained increasing attention as a means of naturally controlling the shelf life and safety of foods; thus the use of bioprotective cultures to ensure the hygienic quality of food has become a promising tool. Lactic acid bacteria (LAB) have been extensively exploited for thousands of years for the production of fermented foods due to their acidifying capacity and hence their ability to inhibit pathogenic and spoilage organisms, and also for their ability to produce desirable taste, flavor, and texture changes. Since LAB naturally dominate the microbiota of raw materials and fermented foods, they are assumed not to pose health risk for humans (they have "generally recognized-as-safe" (GRAS) status). During the last 25 years knowledge about LAB antimicrobial peptides, named bacteriocins, has dramatically increased; nonetheless, their application has not met equal success. Their somewhat limited host-range application, the food composition factors affecting bacteriocin effectiveness and the restrictive legislation concerning food additives are among the reasons that might explain the lack of industrial applications of bacteriocins. From a regulatory perspective, nisin, a bacteriocin produced by *Lactococcus lactis*, is the only bacteriocin approved by the US Food and Drug Administration (FDA) for use in more than 50 countries. An alternative approach to introducing bacteriocins into food is the use of live LAB cultures that produce bacteriocins *in situ* in the food matrix: so-called bioprotective cultures.

22.2 ANTIMICROBIAL POTENTIAL OF LAB

Because LAB have been part of raw materials and fermented foods since ancient times, their association with health has grown among consumers and they have become increasingly popular. The main reasons include the health-friendly perception of natural preservation methods as opposed to chemical or physicochemical treatments; the shelf-life extension of food with no sophisticated technological equipment that is accessible to smaller economies; and the possibility to solving emerging issues such as antibiotic resistance in the food chain, presence of emergent pathogens, and improvement of animal and human health by natural means. Based on the wide spectrum of antimicrobial compounds produced, LAB can be exploited as microbial cell factories for food biocontrol (Gálvez *et al.*, 2010). The antibacterial activity of organic acids produced by LAB and their ability to decrease pH value constitute the main mechanism for biopreservation in fermented foods. However, specific strains of LAB are further known to produce other antimicrobial substances such as low-molecular-weight metabolites (reuterin, reutericyclin, diacetyl, fatty acids), hydrogen peroxide, antifungal

compounds (propionate, phenyl-lactate, hydroxyphenyl-lactate, and 3-hydroxy fatty acids), bacteriocins, and bacteriocin-like molecules which can be exploited to biocontrol pathogens and contaminants through the food chain. The initial breakthrough in the processing of fermented foods was the deliberate addition of LAB as a starter culture; recently the use of a novel generation of starter cultures offering functionalities beyond acidification has begun to be explored (Leroy and De Vuyst, 2004; Leroy *et al.*, 2006; Gillor *et al.*, 2008). LABs are capable of inhibiting different microorganisms in food, displaying crucial antimicrobial effects with a strong impact on preservation and safety. It has also been shown that some LAB strains possess interesting health-promoting properties; the effectiveness of bacteriocins against clinically relevant pathogens highlights their immense potential to improve therapeutic strategies against bacterial infections with a significant impact on human and animal health care. In this regard, the antimicrobial effect was confirmed to be the result of direct antagonism between the probiotic LAB and the pathogen, the anti-infective activity being mediated by the bacteriocin produced *in situ* (Hancock and Sahl, 2006; Corr *et al.*, 2007). The recent understanding that antimicrobial peptides such as bacteriocins are an essential component of microbe–host mutualism underscores their important immunoregulatory role in addition to the well-known direct antimicrobial activity (Sang and Blecha, 2008). On the other hand, as bacteriocins are able to kill bacteria by disruption of membrane integrity they are thought to be less likely to induce resistance; small peptides produced by LAB are largely considered a potential solution to the growing problem of resistance to conventional antibiotics (Rossi *et al.*, 2008; Sang and Blecha, 2008; Sit and Vederas, 2008).

Despite the broad field of application, one of the main limitations of many bacteriocins is their narrow spectrum of inhibition. Although most bacteriocins are only active against Gram-positive bacteria, some LAB bacteriocins recently described are active against Gram-negative organisms of concern in food and in human and animal gastrointestinal tracts (Gotteland *et al.*, 2006; Stern *et al.*, 2006; Line *et al.*, 2008; Svetoch *et al.*, 2008). Antimicrobials produced by probiotic LAB might play a role during *in vivo* interactions occurring in human and animal gastrointestinal tracts, hence contributing to gut health and avoiding pathogen dissemination during processing of food from animal origin. Recently, the availability of bacterial genomes has provided valuable information on the bacteriocinogenic potential of LAB, shedding light on the global response of different ecosystems to bacteriocins as well as the development of adaptation or resistance (Kleerebezem and Vaughan, 2009; Wohlgemuth *et al.*, 2010). In addition, an expanding area of interest is the utilization of bacteriocins in rational drug design. Due to the gene-encoded nature of LAB bacteriocins, bioengineering of existing and novel peptides may lead to the generation of bacteriocins with improved potency and properties for specific applications (Cotter *et al.*, 2005a, 2006; Collins *et al.*, 2010).

22.3 BACTERIOCINS

Bacteriocins are classified on the basis of structural and functional characteristics. Originally, Klaenhammer (1993) proposed four major classes for bacteriocins: Class I, post-translationally modified bacteriocins or lantibiotics, divided into linear (type A) and globular (type B) subtypes; Class II, small (<10 kDa) heat-stable membrane-active bacteriocins with subgroups IIa (anti-listerial peptides having the amino acid motif YGNGV/L in the N-terminal part of the peptide), IIb (two-component peptides), and IIc (thiol-activated peptides requiring reduced cysteine residues for activity); Class III, larger (>30 kDa) heat-labile bacteriocins; and Class IV, complex bacteriocins (lipid or carbohydrate moieties

besides the protein). More recently, Cotter *et al.* (2005b) and Heng *et al.* (2007) suggested some modifications of Klaenhammer's classification scheme, such as the elimination of Classes IIc and IV (thiol-activated peptides and chemically complex bacteriocins). Only two principal categories were proposed, the lanthionine-containing lantibiotics (Class I) and the non-lanthionine-containing lantibiotics (Class II) groups, with four subclasses. On the other hand, Heng *et al.* (2007) retained Class III with a division into IIIa (bacteriolysins) and IIIb (non-lytic proteins) and added Class IV (cyclic bacteriocins, known before as Class IIc). Later on, Nissen-Meyer *et al.* (2009) suggested a division of Class II bacteriocins into four subgroups according to similarities of their C-terminal regions while Zouhir *et al.* (2010) defined new structure-based sequence fingerprints that support a subdivision of the bacteriocins from Gram-positive bacteria into 12 groups.

22.3.1 Biosynthetic pathways

In lantibiotics, the biosynthetic cluster includes: (i) the structural gene(s) encoding the prepeptide; (ii) enzymes responsible for the specific modification reactions; (iii) accessory proteins including processing proteases responsible for removal of the leader peptide; (iv) ABC-superfamily transport proteins responsible for peptide translocation; (v) regulatory proteins; and (vi) proteins involved in producer self-protection (immunity) mechanisms (Chen and Hoover, 2003). On the other hand, Class II bacteriocins exhibit a quite diverse genetic organization; the structural genes might be part of one to four operons which can be even transcribed divergently. In addition, these operons contain the genes encoding for the proteins involved in translocation (ABC family cassettes), immunity, and in some cases those involved in the regulation of their synthesis (two- or three-component system machinery), (Drider *et al.*, 2006). The genes responsible for secretion, maturation, and release of the mature bacteriocin to the extracellular medium have been found in the chromosome, large plasmids, composite and conjugative transposons, and phages, which suggest a potential for horizontal transfer between species (Diep and Nes, 2002). In this regard, the coagulin operon (coaABCD) is identically organized and displays high sequence similarity to the pediocin PA-1/AcH operon; however, this bacteriocin is produced by *Bacillus coagulans* I4, a non-LAB bacterium (Le Marrec *et al.*, 2000).

22.3.1.1 Production and secretion

The structural genes in Class I and II bacteriocins encode ribosomally synthesized precursor prepeptides which are biologically inactive due to the presence of a leader sequence, usually between 15 and 30 amino acids in length, which is attached to the N-terminal part of the molecule. Depending on the type of bacteriocin that it is being produced, they show distinctive features in their structure. For example, type AI lantibiotic leader peptides are generally hydrophilic, possess a high proportion of charged amino acids while typical type AII leader peptides possess highly negative net charges. The leader peptides are processed concomitantly on export and contain a "double-glycine" GG/GA/GS motif immediately preceding the cleavage site, as is also the case for Class II bacteriocin leader peptides (Ennahar *et al.*, 2000; McAuliffe *et al.*, 2001). The role of this leader sequence seems to be the protection of the producer strain from an intracellular high concentration of active peptides and direction of the prepeptide towards the membrane, where bacteriocins are processed. There are also some bacteriocins that have a *sec*-type instead of a double-glycine-type leader sequence (Cintas *et al.*, 1997; Kalmokoff *et al.*, 2001) and some that do not contain leader

peptides at all (Cintas *et al.*, 1998, 2000; Floriano *et al.*, 1998). Once the bacteriocin is synthesized, transport and secretion throughout the membrane is mediated by an exclusive transport system. The first component is a protein belonging to the ABC family of cassettes and which has been described in the majority of Class II bacteriocins. This protein involves three domains: a peptidase domain which is involved in the recognition and subsequent cleavage of the leader peptide, a transmembrane domain, and an ATP-binding domain. The second component is an accessory protein containing a hydrophobic N-terminal domain inserted into the membrane and a hydrophilic C-terminal domain which might facilitate the translocation or help in the processing of the leader peptide. However, the role of this protein in the biosynthetic pathways is not completely understood.

22.3.1.2 Immunity

Most recently published studies have been related to the understanding of the mode of action of immunity protein. All bacteriocin producer strains must also present a mechanism of immunity that protects them from being killed by their own antimicrobial peptide. Class IIa bacteriocins present a gene encoding the immunity protein that generally lies beside and downstream of the structural gene, both forming an operon structure. Exceptions are enterocin CRL35 and mundticin KS in which the immunity gene is located on a different operon just besides the ABC transporter-encoding gene (Kawamoto *et al.*, 2002; Saavedra *et al.*, 2004). Immunity proteins are cytosolic proteins that show high degree of specificity, recognizing and conferring immunity only to their own bacteriocin or in some cases to closely related ones (Fimland *et al.*, 2005). Studies involving hybrid peptides demonstrated that the C-terminal half of this protein recognizes the C-terminal hairpin domain of the cognate bacteriocin (Johnsen *et al.*, 2005). The mechanism of protection is not fully understood and, since no evidence of direct contact between these both peptides is available, it was hypothesized that the immunity protein is involved with the "bacteriocin receptor" (MptC and/or MptD subunits of the mannose-PTS), which in turn blocks the receptor's ability to interact with the bacteriocin (Fimland *et al.*, 2005). For several bacteriocins from subclass IIa and IIc it was demonstrated that a cognate immunity protein binds tightly to the receptor in a bacteriocin-dependent manner and an "on-off type mechanism" was proposed, allowing formation of a complex only in the presence of the bacteriocin (Diep *et al.*, 2007). On the other hand, immunity to lantibiotics involves the combined action of an immunity protein encoded by the gene *lan I* and a dedicated ABC transporter encoded by *lan FEG* genes (Ra *et al.*, 1999; Stein *et al.*, 2003; Draper *et al.*, 2008). More recently, members of Abi protein family had shown to be associated with certain bacteriocin loci and were found to be involved with self-immunity. For instance, the Abi genes *skkI*, *plnI*, and *plnL* were shown to confer immunity to their respective cognate bacteriocins, sakacin K and plantaricins EF and JK, all belonging to subclass IIb two-peptide bacteriocins (Diep *et al.*, 2009; Kjos *et al.*, 2010). Since Abi proteins are proteases, the predicted mode of self-immunity would arise from a proteolytic mechanism. However, preliminary studies did not show any significant bacteriocin degradation when it was exposed to cell extracts from Abi-immune cells or to whole cells (Kjos *et al.*, 2010).

22.4 FOOD APPLICATIONS

The use of antagonistic microorganisms or their metabolic products to inhibit or destroy undesirable (pathogen and contaminant) organisms in food to enhance food safety and extend

shelf life without significantly altering the sensory properties of the product is referred to as bioprotection. Two main approaches are commonly used in the application of bacteriocins for biopreservation of foods: (i) *ex situ* production that involves either the addition of purified or semi-purified bacteriocins as food preservatives (use of which is dependent on legal approval/regulations) or the use of a previously fermented product with a bacteriocin-producing LAB strain as an ingredient in food; and (ii) *in situ* production involving the inoculation of food with bacteriocinogenic LAB, the ability of these strains to grow and produce bacteriocins in the product being crucial for successful application. In the first case, bacteriocins recovered after cultivation of the producer strain in a food-grade substrate can be added as partially purified or purified concentrates which will require specific approval as preservatives from the legislative point of view. As mentioned before, nisin is the only bacteriocin licensed as a food preservative in over 50 countries. International acceptance of nisin was given by the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA, 2009), establishing the daily permitted doses (Hurst and Hoover, 1993); however, maximum limits vary between countries, with dairy products (especially cheeses) and canned foods being the products for which nisin has been regulated. In addition, concentrated supernatants of a bacteriocin LAB producer culture such as ALTA™ 2341 (Quest) and Microgard™ (Danisco) are on the market, while the milk-based preparation lacticin 3147 has also recently been described (Guinane *et al.*, 2005). *Ex situ*-produced bacteriocins have been applied to foods under different forms such as immobilized preparations, in which the concentrated culture broth is bound to different carriers such as silica particles and corn starch powder, encapsulated on liposomes, or incorporated on gel coatings and films of different materials (Gálvez *et al.*, 2007). The second approach of *in situ* bacteriocin production shows legal and cost advantages compared to *ex situ* production, in particular for small-scale economies that benefit from lower-cost biopreservation processes (Holzapfel, 2002).

Although the implementation of bioprotective cultures requires careful selection of bacteriocinogenic strains, the number of publications and patents linked to food preservation has steadily increased since the 1980s (Desriac *et al.*, 2010). Bacteriocin-producing LAB strains can be used either directly as starter cultures, or as as adjuncts or co-cultures in combination with a starter culture, heterologously produced by a starter strain or as protective cultures. When used as starter cultures, bacteriocinogenic strains should be able to optimally drive the fermentation process, besides producing enough bacteriocins to afford protection. In contrast, those used as adjunct cultures are not expected to contribute to fermentation but they must not interfere with the function of the starter culture; therefore the starter culture must be resistant to the bacteriocin. On the other hand, bacteriocinogenic protective cultures are mainly used to inhibit pathogenic and spoilage organisms to extend the shelf life of non-fermented foods, growing and producing bacteriocin during refrigeration storage and/or temperature-abuse conditions. Under refrigeration storage conditions the growth of protective culture should have a neutral impact on the sensory properties of the food, while in temperature-abused foods the protective culture acts as the predominant spoiler, ensuring growth inhibition of pathogenic bacteria and that the food is no longer consumed (Holzapfel *et al.*, 1995).

22.4.1 Bioprotection of meat, poultry, and seafood products

Fresh meat and seafood as well as their fermented and processed products provide an excellent environment for the growth of pathogenic and spoilage organisms. Given that chilled storage is essential for muscle foods, it is usually psychrotrophic microorganisms that

are most problematic in these environments. As meat cannot be pasteurized prior to the addition of a selected LAB starter, a culture for biopreservation of meat must compete with the existing indigenous microbiota. The conditions under which bacteria must grow in muscle raw materials as a substrate determine the different ecosystems which involve particular microbial biodiversity (Vignolo *et al.*, 2010). During aerobic storage, the microbiota of refrigerated meat and fish predominantly comprise a consortium of cold-tolerant Gram-negative and Gram-positive bacteria including LAB, while under modified-atmosphere packaging (MAP) conditions a highly competitive microbiota consisting primarily of LAB develops. Fermented meat products involve ecological determinants that strongly influence the establishment of a specific microbial association, this involving *Lactobacillus sakei*, *Lactobacillus curvatus*, and *Staphylococcus xylosus* strains considered as the best-adapted species to the stringent conditions of meat fermentation (Cocconcelli and Fontana, 2010). Fermented sausages have traditionally been produced by fermentation combined with drying without heat treatment, the various manufacturing processes used to produce different types of sausages involving differences in the ability to reduce pathogens. Although fermented sausages are known as shelf-stable products and the reduction of *L. monocytogenes* and *E. coli* O157:H7 numbers during fermentation and drying has been reported, they have still been involved in a number of poisoning outbreaks (Vignolo *et al.*, 2008). The use of bacteriocinogenic LAB strains as functional starter culture or co-culture for sausage fermentation must be able to produce acid beyond the ability to produce bacteriocins. On the other hand, vacuum-packaged (VP) and MAP meat, poultry, and seafood processed products constitute a wide spectrum of different RTE meat-based products whose microbiological status is highly dependent on raw materials, hygiene, and handling procedures. In particular, frequent *L. monocytogenes* contamination occurs as a consequence of mishandling practices after post-packaging thermal treatments. An additional hurdle to reduce the risk of this ubiquitous emergent pathogen is the use of competitive-bacteriocin-producing LAB and/or their bacteriocins.

Examples on the application of bacteriocins and bioprotective cultures on muscle foods are shown in Table 22.1. Since nisin is the only approved bacteriocin for food preservation, its use has been widely documented in the literature. Different concentrations of nisin (sometimes combined with other hurdles) have been applied as spray or in scalding water to sanitize the surface of red meat (Cutter and Siragusa, 1994; Pawar *et al.*, 2000; Barboza de Martínez *et al.*, 2002) and poultry carcasses (Shefet *et al.*, 1995) and for RTE aquatic food preservation (Al-Holy *et al.*, 2004) resulting in a significant reduction (approximately 2.0–3.0 log CFU/cm^2) of *Listeria*, *Salmonella typhimurium*, *Brochothrix thermosphacta*, and spoilage LAB species during cold storage. The evaluation of anti-listerial activity of nisin A and pediocin AcH in artificially contaminated raw pork and chicken meat showed nisin to be more efficient than pediocin initially, a loss of effectiveness attributed to degradation by meat proteases being produced after 2 days of storage (Murray and Richard, 1997). When pediocin AcH (prepared by binding to heat-killed *Pediococcus acidilactici* H producer cells) was added to raw chicken breast meat, a strong anti-listerial activity was found, offering protection from post-processing recontamination (Goff *et al.*, 1996). In brined shrimps, three different LAB bacteriocins (nisin Z, carnosin UI49, and bavaricin A) were demonstrated to be highly effective as natural preservatives (Einarsson and Lauzon, 1995). LAB bacteriocins other than nisin were also assayed, among which enterocins, lacticin 3147, sakacin P, pediocin AcH, and lactocin 705 were found to control the growth of *Listeria*, *Clostridium perfringens*, and *Salmonella* in raw beef and poultry, smoked salmon, fresh pork sausages, and different fermented sausages (Vignolo *et al.*, 1996; Lauková *et al.*, 1999; Aymerich *et al.*,

Table 22.1 Bacteriocinogenic LAB and/or their bacteriocins applied to extend the shelf life of muscle foods.

Product	Bacteriocin/protective culture	Target organisms	References
Fresh meats			
Beef carcasses	Nisin	*Listeria innocua/Brochothrix thermosphacta/ Carnobacterium divergens*	Cutter and Siragusa (1994)
Broiler carcasses	Nisin	*S. typhimurium*	Shefet *et al.* (1995)
Brined shrimps	Nisin Z/carnocin UI49/bavaricin A	*Moraxella/Coryneforms/ B. thermosphacta*	Einarsson and Lauzon (1995)
Raw chicken breast	Pediocin AcH	*L. monocytogenes*	Goff *et al.* (1996)
Raw minced beef	Lactocin 705	*L. monocytogenes*	Vignolo *et al.* (1996)
Ground pork	Nisin A/pediocin AcH	*L. innocua/Listeria ivanovii*	Murray and Richard (1997)
Raw buffalo mince	Nisin	*L. monocytogenes*	Pawar *et al.* (2000)
Minced pork/deboned chicken	Enterocins A and B	*L. innocua*	Aymerich *et al.* (2000)
Fresh pork sausages	Lacticin 3147	*C. perfringes/Salmonella* Kentucky/*L. innocua*	Scannell *et al.* (2000)
Red meat carcasses	Nisin	*E. coli*/coliforms	Barboza de Martínez *et al.* (2002)
Cold smoked salmon	*Carnobacterium piscicola* CS526	*L. monocytogenes*	Yamazaki *et al.* (2003)
Salmon/sturgeon caviar	Nisin	*L. innocua*	Al-Holy *et al.* (2004)
Raw chicken meat	*Enterococcus faecium* PCD71/ *Lactobacillus fermentum* 179	*L. monocytogenes/S. enteritidis*	Maragkoudakis *et al.* (2009)
Fermented products			
Hornád salami	Enterocin CCM4231	*L. monocytogenes*	Lauková *et al.* (1999)
Sausages	Pediocin AcH	*L. monocytogenes*	Mattila *et al.* (2003)
Ostrich meat salami	*Lactobacillus plantarum* 423/ *L. curvatus* F126	*L. monocytogenes*	Dicks *et al.* (2004)
Sausage	Enterocin AS-48	*L. monocytogenes*	Ananou *et al.* (2005)
Sausage	*Lactobacillus pentosus* 31-1	*L. innocua/Staph. aureus*	Liu *et al.* (2010)
VP/MAP products			
Raw beef	*Leuconostoc gelidum* UAL187	LAB	Leisner *et al.* (1996)
Poultry breast/cooked pork	*L. sakei* CTC494/Sakacin K	*L. innocua*	Hugas *et al.* (1998)
Bologna-type sausage	Nisin	LAB	Davies *et al.* (1999)
Cooked ham/servelat sausage	*L. sakei* TH1	*L. monocytogenes*	Bredholt *et al.* (2001)

(*Continued*)

Table 22.1 (*Continued*)

Product	Bacteriocin/protective culture	Target organisms	References
Brazilian sausage	*L. sakei* 2a	*L. monocytogenes*	Liserre *et al.* (2002)
Chicken cold cuts/smoked salmon	Sakacin P/*L. sakei* Lb190	*L. monocytogenes*	Katla *et al.* (2001, 2002)
Cooked meat products	*Leuc. carnosum* 4010/leucocin	*L. monocytogenes*	Jacobsen *et al.* (2003)
Raw beef	*L. curvatus* CRL705	*L. innocua/B. thermosphacta*	Castellano and Vignolo (2006)
Cooked meat products	*L. sakei* 10A	LAB/*L. monocytogenes*/ *B. thermosphacta*	Vermeiren *et al.* (2006)
Sliced cooked ham	*L. sakei* 1	*L. monocytogenes*	Alves *et al.* (2006)
Cold smoked salmon	*E. faecium* ET05/*L. curvatus* ET30	*L. innocua*	Tomé *et al.* (2008)

2000; Scannell *et al.*, 2000; Katla *et al.*, 2001, 2002; Mattila *et al.*, 2003; Ananou *et al.*, 2005). Given that among the main LAB isolated from meat and fish ecosystems different species of *Lactobacillus*, *Leuconostoc*, *Enterococcus*, and *Carnobacterium* were often identified (Najjari *et al.*, 2008; Belfiore *et al.*, 2010; Vignolo *et al.*, 2010), it is expected that strains from these genera are likely to be applied as protective culture. As shown in Table 22.1, different bacteriocinogenic LAB strains were assayed as protective cultures in fresh meats, fermented sausages, and VP and MAP meat and poultry products, in which nisin and the bacteriocins produced by *L. sakei* and *L. curvatus* were the most effective applied. Although fresh fish and seafood deterioration is generally caused by Gram-negative microorganisms, spoilage organisms such as *Clostridium botulinum* and *L. monocytogenes* have also been the cause of contamination. In addition to nisin, bacteriocin-producing LAB were applied to inhibit *L. monocytogenes* on refrigerated cold smoked salmon stored aerobically and under vacuum. *Carnobacterium piscicola*, *L. sakei*, *L. curvatus*, and *Lactobacillus delbrueckii* as bacteriocin-producing strains were used as an extra hurdle to minimize the risk of listeriosis for shelf-life extension of different muscle foods (Katla *et al.*, 2001; Yamazaki *et al.*, 2003; Tomé *et al.*, 2008).

22.4.2 Bioprotection of dairy products

LAB have a long history of use as preservatives in dairy fermentations where they are commonly employed as starter cultures, in particular for cheese manufacture; the early use of bacteriocin-producing cultures was related to the retardation of late gas blowing in Swiss-type cheeses by clostridia. Nisin (Nisaplin™) has been used as an effective preservative in fresh cheeses with high water activity (a_w) such as ricotta-type cheese to control *L. monocytogenes*, resulting in an extended shelf life (Davies *et al.*, 1997). Although nisin, the only approved and commercially available bacteriocin, is the more frequently used to control spoilage and pathogenic bacteria in the dairy industry, other lantibiotics such lacticin 3147 and lacticin 481 have displayed great potential when used as preservatives (Collins *et al.*, 2010). Another alternative to the use of lantibiotics in foods is the use of lantibiotic-producing starter cultures. Nisin-producing lactococci were effectively applied against clostridial (Rilla *et al.*, 2003) and listerial (Rodriguez *et al.*, 1998) spoilage; however, interferences with starter performance and cheese ripening as well as a high sensitivity to bacteriophages were demonstrated (O'Sullivan *et al.*, 2002a). To overcome these disadvantages, different strategies have been designed, such as the application of lacticin 3147-producing *Lc. lactis* on the surface of cheeses to control *L. monocytogenes* to improve safety (O'Sullivan *et al.*, 2006); the use of a lacticin 481-producing *Lc. lactis* strain as an adjunct for Cheddar cheese manufacture to control non-starter LAB; and to increase starter cell lysis during cheese making to speed up enzyme release to degrade peptides into small peptides and amino acids, thus contributing to quality, texture, and flavor (O'Sullivan *et al.*, 2002b, 2003a). As stated above, another strategy to limit *L. monocytogenes* levels in cheese was the heterologous production of enterocin A and pediocin PA-1 by lactococcal starter strains (Reviriego *et al.*, 2007; Liu *et al.*, 2008). In addition, the construction of food-grade lactococcal strains which co-produce the lantibiotics lacticin 3147 and lacticin 481 was achieved either by conjugating the lacticin 3147 genetic determinants into a 481-producing recipient or vice versa, this proving more efficacious for food applications (O'Sullivan *et al.*, 2003b). Moreover, enterotoxigenic *Staphylococcus aureus*, a common cause of bovine mastitis and methicillin-resistant *Staph. aureus* associated with intra-hospital infections, has also been reported as a causative outbreak agent, with animals and food handlers being

important vehicles for contamination of dairy products. Enterocin AS-48, a broad-spectrum cyclic bacteriocin *in situ* and *ex situ* produced by *Enterococcus faecalis* A-48-32, and nizin Z, produced by *Lc. lactis* IPLA729, proved to be effective for the inhibition of *Staph. aureus* as well as enterotoxigenic *Bacillus cereus* in different cheeses (Muñoz *et al.*, 2004, 2007; Rilla *et al.*, 2004). It is known that enterocins are among the most active anti-listerial bacteriocins identified to date; however, because of concerns associated with *Enterococcus* the use of these antimicrobials in food has been curtailed. Although *ex situ*-produced enterocins have proved to be highly effective, in particular for *L. monocytogenes* inhibition, their use in foods are legally restricted so far; with a view to harness enterocin activity, heterologous production by food-grade LAB remains a promising strategy.

In addition to cheese, *in situ* bacteriocin production by bacteriocinogenic *Streptococcus salivarius* ssp. *thermophilus* B during yogurt storage refrigeration or temperature abuse was effective in controlling *L. monocytogenes* and *Staph. aureus*, resulting in a 5-day extension of shelf life (Benkerroum *et al.*, 2002). Moreover, an exceptionally high level of physiological activity (concentration of bacteriocin and probiotic viable cells) of the probiotic yogurt culture *L. delbrueckii* ssp. *bulgaricus* BB18 was obtained by a continuous prefermentation process of cultivation (Simova *et al.*, 2008). Most of the bacteriocinogenic LAB used as bioprotective culture in dairy products belong to the genera *Lactococcus* and *Enterococcus*, as their species are mainly isolated from cheeses; some examples being presented in Table 22.2.

22.4.3 Bioprotection of vegetable products

The application of bacteriocinogenic LAB and/or their bacteriocins as biopreservatives in vegetable food matrices has focused on the inhibition of spoilage and/or human pathogens vehiculized in vegetable foods and beverages; utilization of bacteriocins appears as a natural alternative to chemical additives and antibiotics (Table 22.2). In traditional vegetable fermentations such as table olives, sauerkraut, sourdough, kimchi, and miso, LAB starter cultures are selected for their ability to improve nutritional quality and to reduce anti-nutritional and/or toxic compounds of raw materials as well as their probiotic aptitude, in addition to their technological potential (i.e., acid, exopolysaccharide, and aroma-compound formation). Production of antimicrobial compounds constitutes a secondary contribution of LAB, which, applied as bioprotective culture or co-cultures, are considered as an additional safety hurdle warranting microbiological food stability, reducing risks of growth and survival of pathogens and spoilage organisms (Settani and Corsetti, 2008). Table olives undergo a complex fermentation process in which LAB that are resistant to the high sodium chloride concentration in brine will remain as the dominant organisms. Starter cultures have been selected with bacteriocin production as a desired feature; *Lactobacillus plantarum* LPCO10 and *Enterococcus faecium* BFE900 were screened by Jiménez-Díaz *et al.* (1993) and Franz *et al.* (1996), respectively. Plantaricins S and T, produced by *L. plantarum* LPCO10, were responsible for the control of epiphytic microbiota during fermentation of Spanish-style green olives, allowing high lactic acid production in brines and stabilization of the final product (Ruiz-Barba *et al.*, 1994). Sauerkraut (cabbage), kimchi (spiced cabbage or radish), as well as miso (soybean and rice paste) fermentations involve a complex succession of different LAB species, *Bacillus*, and yeasts, with the correct sequence of organisms being essential in achieving a stable product with typical flavor and aroma. Because spoilage lactobacilli, particularly homofermentative *L. plantarum*, are responsible for product over-acidification, nisin and nisin-producing lactococcal strains were assayed to control these acidifying organisms as well as *Bacillus* and *L. monocytogenes* (Harris *et al.*, 1992; Choi and

Table 22.2 Bacteriocinogenic LAB and/or their bacteriocins applied to extend the shelf life of dairy and vegetable foods.

Product	Bacteriocin/protective culture	Target organisms	References
Dairy products			
Ricotta-type cheese	Nisin	*L. monocytogenes*	Davies *et al.* (1997)
Raw ewes' Manchego cheese	Nisin/*Lc. lactis* ssp. *lactis* ESI515	*L. innocua*	Rodriguez *et al.* (1998)
Yogurt	*Streptococcus salivarius* ssp. *thermophilus*	*L. monocytogenes/Staph. aureus*	Benkerroum *et al.* (2002)
Vidiago cheese	Nisin Z/*Lc. lactis* ssp. *lactis* IPLA729	*Clostridium tyrobutyricum*	Rilla *et al.* (2003)
Cheddar cheese	Lacticin 481/*Lc. lactis* CNRZ481	Non-starter LAB	O'Sullivan *et al.* (2003a)
Asfuega'l Pitu cheese	Nisin Z/*Lc. lactis* ssp. *lactis* IPLA729	Methicillin-resistant *Staph. aureus*	Rilla *et al.* (2004)
Non-fat hard cheese	Enterocin AS-48/*E. faecalis* A-48-32	*B. cereus*	Muñoz *et al.* (2004)
Smear-ripened cheese	Lacticin 3247/*Lc. lactis* DPC3147	*L. monocytogenes*	O'Sullivan *et al.* (2006)
Model cheese	Pediocin PA-1/nisin/*Lc. lactis* ESO515	*L. monocytogenes*	Reviriego *et al.* (2007)
Skim milk, fresh cheese	Enterocin AS-48/*E. faecalis* A-48-32	*Staph. aureus*	Muñoz *et al.* (2007)
Cottage cheese	Enterocin A/*Lc. lactis* IL1403	*L. monocytogenes*	Liu *et al.* (2008)
Yogurt	*L. delbrueckii* ssp. *bulgaricus* BB18	Biological activity as probiotic	Simova *et al.* (2008)
Vegetable products			
Spanish-style green olives	Plantaricins S and T/*L. plantarum* LPCO10	Epiphytic microbiota	Ruiz-Barba *et al.* (1994)
Sauerkraut	Nisin/*Lc. lactis*	*L. plantarum*	Harris *et al.* (1992), Breidt *et al.* (1995)
Kimchi	Sakacin A/*L. sake*; pediocin/*P. acidilactici*	*L. monocytogenes*	Choi and Beauchat (1994)
Fruit juices	Nisin	*Alicyclobacillus acidoterrestris*	Komitopoulou *et al.* (1999)
Kimchi	Nisin	*L. plantarum*	Choi and Park (2000)
Miso	Nisin/*Lc. lactis*	*B. subtilis*	Kato *et al.* (1999, 2001)
Mashed potato/zucchini	Nisin/enterocin EJ97	*Bacillus/Clostridium*	Thomas *et al.* (2002); García *et al.* (2004)
Wheat sourdough	*L. pentosus* 2MF8/*Lc. lactis* M30	LAB/*Bacillus*	Corsetti et al (2004); Settanni *et al.* (2005)
Rye sourdough	Amylovorin L471/*L. amylovorus* DCE471	Biological activity as probiotic	De Vuyst et al (2004)
Melon and cantaloupe	Nisin/EDTA/other additives	*Salmonella stanley/L. monocytogenes*	Ukuku and Fett (2004); Ukuku *et al.* (2005)
Fresh-cut produce	Nisin/pediocin/phytic acid	*L. monocytogenes*	Bari *et al.* (2005)
Fresh fruit; vegetable juices	Enterocin AS-48	*L. monocytogenes/Bacillus/Staphylococcus*	Grande *et al.* (2005b, 2006)

(*Continued*)

Table 22.2 (*Continued*)

Product	Bacteriocin/protective culture	Target organisms	References
Soybean/alfalfa sprouts	Enterocin AS-48	*L. monocytogenes/Bacillus*	Cobo Molinos *et al.* (2005, 2008)
Canned vegetables	Enterocin AS-48/other additives	*B. coagulans*	Lucas *et al.* (2006)
Sourdough	*L. plantarum* LMO25/*L. alimentarius* LMO7	*B. subtilis/B. licheniformis*	Menteş *et al.* (2007)
Apple and lettuce	*Leuconostoc*	*L. monocytogenes/Salmonella*	Trias *et al.* (2008)
Apple cider	Enterocin AS-48	LAB/*B. licheniformis*	Martínez Viedma *et al.* (2008, 2010)
Beer	Enterocins L50, P and Q	LAB	Basanta *et al.* (2008)

EDTA, ethylenediaminetetra-acetic acid.

Beauchat, 1994; Breidt *et al.*, 1995; Kato *et al.*, 1999, 2001; Choi and Park, 2000). Similarly, sourdough fermentation is characterized by a complex microbial ecosystem, mainly represented by LAB and yeasts, whose fermentation confers to the resulting dough its particular features such as palatability and high sensory quality (Corsetti and Settanni, 2007). Wheat and rye sourdough fermentation stability was increased with the contribution of bacteriocinogenic LAB, among which the *in situ* activity of bacteriocins produced by *Lactobacillus pentosus* 2MF8, *Lactobacillus amylovorus* DCE471, and *Lc. lactis* ssp. *lactis* M30 was reported (Corsetti *et al.*, 2004; De Vuyst *et al.*, 2004; Settanni *et al.*, 2005). More recently, Menteş *et al.* (2007) showed the ability of bacteriocin producers *L. plantarum* LMO25 and *Lactobacillus alimentarius* LMO7 during sourdough propagation to inhibit rope-forming *Bacillus subtilis* and *Bacillus licheniformis* in white bread.

The increasing popularity of non-fermented vegetables packaged and RTE as convenience products has introduced new environments which support the growth of foodborne pathogens. During growth in field, vegetables are frequently in contact with soil and their surfaces may be contaminated with both Gram-positive (*Bacillus*, *L. monocytogenes*) and Gram-negative (*Salmonella*, *E. coli*) pathogens; subsequent handling and packaging of these foods and the occasional difficulty with maintaining cold temperatures, provide an opportunity for pathogens to grow. Bacteriocin-producing cultures in combination with other food additives are commonly employed as protective agents in this kind of product. The effectiveness of treatments combining nisin or pediocin with chlorine, sodium lactate, potassium sorbate, ethylenediaminetetra-acetic acid (EDTA), and organic acids (citric and phytic) on whole and fresh-cut pieces of melons, cantaloupe, as well as fresh-cut cabbage, broccoli, and mungbean sprouts has been reported (Ukuku and Fett, 2004; Bari *et al.*, 2005; Ukuku *et al.*, 2005). Immersion solutions of the cyclic bacteriocin enterocin AS-48, produced by *E. faecalis* A-48-32, alone or combined with chemical preservatives, were also assayed on fresh green asparagus and alfalfa and soybean sprouts, demonstrating to be effective inhibiting *L. monocytogenes*, *B. cereus*, and *Bacillus weihenstephanensis* (Cobo Molinos *et al.*, 2005, 2008). Moreover, bacteriocin-producing *Leuconostoc* strains also provided positive and encouraged results showing potential as bioprotective agents against *Salmonella* and *L. monocytogenes* in Golden Delicious apples and iceberg lettuce (Trias *et al.*, 2008). Stability of fruit and vegetable juices, in particular ropy appearance and off-flavor formation during storage, was greatly improved by applying bacteriocins. Enterocin AS-48 showed the ability to reduce viable counts of *L. monoctyogenes*, *B. cereus*, and *Staph. aureus* in fresh fruit and vegetable juices; however, different activity responses from complete to negligible losses were observed (Grande *et al.*, 2005a). Under refrigeration storage, bacteriocin activity was exhibited during the first 48 h in vegetable juices (avocado, cabbage, cauliflower, celery, green beans, and lettuce), while enterocin AS-48 stability was longer (15 days) in fresh fruit juices (orange, grapefruit, kiwi fruit, pineapple, apple, and pear). Moreover, the antimicrobial activity of nisin and enterocin AS-48 against vegetative cells and endospores of *Alicyclobacillus acidoterrestris*, a thermoacidophilic Gram-positive spoilage organism, was assayed in fruit juices under different storage conditions, obtaining extra protection with reduced heat treatment (Komitopoulou *et al.*, 1999; Grande *et al.*, 2005b).

On the other hand, since canned seasonal vegetables are usually preserved by acidification and thermal treatments, bacteria that tolerate these conditions (e.g., *Bacillus*) can proliferate during storage and may cause spoilage. In canned vegetables with pH values between 4 and 4.5, the presence of *Bacillus coagulans* represents a big concern due to its ability to increase food pH to values that can allow germination of surviving *C. botulinum* spores. The efficacy of enterocin AS-48 together with heat treatment and other additives (lactic acid, glucose, and

sucrose) was demonstrated and proposed as an additional hurdle to protect canned vegetables from *B. coagulans* damage (Lucas *et al.*, 2006). "Refrigerated processed foods of extended durability" are cooked chilled foods, mildly heated in-package, which are non-sterile by design and rely on refrigeration for preservation. Endospore-forming bacteria, especially *Bacillus* and *Clostridium*, represent the main bacterial population of mashed potatoes, zucchini purée, and vegetable soups. Nisin and enterocin AS-48 have been tested for their useful contribution to extending the shelf life of this type of food by controlling spore-forming organisms, with the bacteriocin remaining active after pasteurization, protecting consumers against the possibility of food poisoning caused by heat-resistant bacterial pathogens (Thomas *et al.*, 2002; Grande *et al.*, 2007). Moreover, the efficacy of enterocin EJ97 in the control of *Bacillus macroides*/*Bacillus maroccanus* when used in concentrated pure form or combined with chemical preservatives was also demonstrated in zucchini purée (García *et al.*, 2004). LAB bacteriocins have also been applied in the control of spoilage organisms in alcoholic beverages such as cider, beer, and wine. Enterocin AS-48 was used to inactivate rope-forming LAB and *B. licheniformis* in cider (Grande *et al.*, 2006; Martínez Viedma *et al.*, 2008) while enterocins L50, P, and Q produced by *E. faecium* L50 were investigated against beer-spoilage LAB with promising results (Basanta *et al.*, 2008). In addition, appropriate combinations of nisin and metabisulfite may control the growth of spoilage bacteria in wine, allowing malolactic fermentation to occur and a decrease in sulfur dioxide to the level currently used by the wine industry (Rojo-Bezares *et al.*, 2007).

22.5 HURDLE TECHNOLOGY TO ENHANCE FOOD SAFETY

The microbial safety and stability as well as the nutritional and sensory quality of food are based on the application of combined preservative factors or "hurdles." Since the major functional limitations of bacteriocins in foods are their relatively narrow activity spectra and moderate antibacterial effect, they should be considered only as an additional measure to good manufacturing, processing, storage, and distribution practices as highlighted by Holzapfel *et al.* (1995). Hurdle technology, derived from the understanding of the hurdle effect (Leistner, 1997, 2000), refers to the deliberate combination of existing (temperature, a_w, Eh, pH, preservatives, competitive microbiota) and novel (antimicrobial compounds and physicochemical treatments) preservation techniques to establish a series of more selective preservative factors (hurdles) that spoilage and pathogenic microorganisms should not be able to overcome. Examples of the application of hurdle technology to improve shelf life and enhance food safety are shown in Table 22.3.

Selected LAB protective cultures and/or their bacteriocins were assayed against food-borne pathogenic and contaminant organisms, providing a wide array of treatment options based on different hurdle combinations. It is well documented that nisin enhances thermal inactivation of bacteria, reducing treatment time and resulting in better food quality and cost savings. This was found in eggs and lobster meat by Boziaris *et al.* (1998) and Budu-Amoako *et al.* (1999), respectively. It was also reported that chelating agents (EDTA, lactate, citrate) when combined with bacteriocins can bind magnesium ions from the lipopolysaccharide layer, disrupting the outer membrane of Gram-negative bacteria. This allows nisin and lactocin 705 to penetrate the cytoplasmic membrane of *E. coli* O157:H7, *Salmonella enteritidis*, *S. typhimurium*, and *Pseudomonas aeruginosa*, causing cell death.

The synergistic effect between bacteriocins and other processing technologies on the inactivation of microorganisms has also been frequently reported in the literature. Different

Table 22.3 Bacteriocins combined with other hurdles in food preservation.

Bacteriocin	Other hurdles	Target organisms	References
Nisin	Heat	*S. enteritidis*	Boziaris *et al.* (1998)
		L. monocytogenes	Budu-Amoako *et al.* (1999)
Enterocin AS-48		*B. coagulans*	Lucas *et al.* (2006)
Nisin	Chelating agents (EDTA/others)	*S. typhimurium/E. coli* O157:H7	Cutter and Siragusa (1995)
		E. coli/Pseudomonas aeruginosa/ S. enteritidis	Boziaris and Adams (1999)
		L. monocytogenes	Cleveland McEntire *et al.* (2003)
		Salmonella	Ukuku and Fett (2004)
Lactocin 705, nisin		*E. coli* O157:H7	Belfiore *et al.* (2007)
Nisin	MAP	*L. monocytogenes*	Szabo and Cahill (1998)
		L. monocytogenes	Nilsson *et al.* (2000)
Lactocin 705		*L. innocua/Br. thermosphacta*	Castellano and Vignolo (2006)
Nisin	Fatty acid esters	Gram-positive	Thomas *et al.* (1998)
	Carvacrol	*B. cereus*	Periago and Moezelaar (2001)
	Monolaurin	*Bacillus sp.*	Mansour and Millière (2001)
Enterocin AS-48	Essential oils	*L. monocytogenes*	Antonio *et al.* (2009)
Nisin	Essential oils	*S. enteritidis*	Govaris *et al.* (2010)
	Lactoperoxidase system	*L. monocytogenes*	Zapico *et al.* (1998); Boussouel *et al.* (2000)
	Lysozyme	*Br. thermosphacta*/LAB	Nattress *et al.* (2001)
	Leucocin F10	*L. monocytogenes*	Parente *et al.* (1998)
	Curvaticin 13	*L. monocytogenes*	Bouttefroy and Millière (2000)
Lactocin 705	Nisin; enterocin 35	*Listeria*	Vignolo *et al.* (2000)
Pedioin AcH	HHP	*Staph. aureus/L. monocytogenes*/LAB/Gram-negative	Kalchayanand *et al.* (1994, 1998)
Nisin	HHP/enzymes	*E. coli/S. enteritidis/Staph. aureus*	Masschalck *et al.* (2000, 2001)
	HHP	*B. subtilis/Clostridium sporogenes*	Stewart *et al.* (2000)

(*Continued*)

Table 22.3 (*Continued*)

Bacteriocin	Other hurdles	Target organisms	References
Enterocins A and B, sakacin K		*L. monocytogenes/E. coli/ Salmonella*/LAB	Garriga *et al.* (2002)
Lacticin 3147		*Staph. aureus/L. innocua*	Morgan *et al.* (2000)
Nisin	PEF	*B. cereus/E. coli*	Pol *et al.* (2000); Terebiznik et al (2000)
Enterocin AS-48		*Salmonella enterica/Pediococcus parvulus*	Martínez Viedma *et al.* (2010); Ananou *et al.* (2010)

EDTA, ethylenediaminetetra-acetic acid; MAP, modified-atmosphere packaging; HHP, high hydrostatic pressure; PEF, pulsed electric field.

antimicrobials (fatty acid esters, monolaurin, and essential oils) were applied in combination with nisin to reduce and/or inhibit *Bacillus* and *L. monocytogenes* growth in media and food (milk and RTE salads); the combined action of essential oils and enterocin AS-48 also exhibited stronger antimicrobial activity against *S. enteritidis* than bacteriocin alone (Govaris *et al.*, 2010). In addition, a synergistic and bactericidal effect of nisin and the lactoperoxidase system (LPS) on *L. monocytogenes* inactivation in non-fat milk was reported (Zapico *et al.*, 1998; Boussouel *et al.*, 2000), while mixtures of lyzozyme and nisin proved to be highly effective to control meat-spoilage bacteria (Nattress *et al.*, 2001). Combinations of various bacteriocins were also assayed as a strategy for shelf-life extension of food, enhancing antibacterial activity. When used in combination with nisin it was found that leucocin F10, curvaticin 13, lactocin 705, and enterocin CRL35 showed higher activity against *L. monocytogenes*, avoiding re-growth of resistant cells (Parente *et al.*, 1998; Bouttefroy and Millière, 2000; Vignolo *et al.*, 2000).

In view of consumer demands for more natural and nutritious food, different non-thermal processing technologies, such as high hydrostatic pressure (HHP) and pulsed electric fields (PEFs), have been applied in the food industry. Bacteriocins in combination with these processing techniques enhanced bacterial inactivation (Table 22.3). Sublethal injury to Gram-negative bacteria (*S. typhimurium* and *E. coli* O157:H7), *L. monocytogenes*, and LAB (*L. sakei* and *Leuconostoc mesenteroides*) induced by HHP showed an increased sensitivity to nisin and pediocin AcH (Kalchayanand *et al.*, 1994, 1998). Similarly, HHP showed an increase of the bactericidal activity of *Staph. aureus* as well as food Gram-negative pathogens and contaminants when applied in combination with nisin and lactoferrin (Masschalck *et al.*, 2001; Lee and Kaletunç, 2010), while the combined effects of HHP and nisin was reported to enhance sensitivity of *B. cereus*, *B. subtilis*, and *Clostridium sporogenes* spores (Stewart *et al.*, 2000; López-Pedemonte *et al.*, 2003). Moreover, bactericidal synergism through nisin and other bacteriocins such as enterocins A and B, sakacin K, pediocin AcH, lacticin 3147, and HHP was reported to increase death rate of *Staph. aureus*, *E. coli*, *Salmonella*, *Listeria*, and slime-producing LAB in meat systems and dairy products (Morgan *et al.*, 2000; Garriga *et al.*, 2002; Ananou *et al.*, 2010). PEF was also applied as a non-thermal preservation method in an application of the hurdle concept. Mild PEF and nisin were able to enhance bactericidal effect of nisin on vegetative cells of *B. cereus* (Pol *et al.*, 2000) while the combined action of nisin and high-intensity PEF was able to inactivate *E. coli* and *Salmonella enterica* in simulated milk ultrafiltrate medium (Terebiznik *et al.*, 2000). The enhanced bactericidal effect of the broad-spectrum cyclic peptide enterocin AS-48 was also demonstrated when tested in combination with high-intensity PEF on *S. enterica* and exopolysaccharide-producing *Pediococcus parvulus* on apple juice (Martínez Viedma *et al.*, 2008, 2010).

22.6 BACTERIOCINS IN PACKAGING FILMS

Active packaging is an innovative and challenging technology that has been introduced as a response to the continuous changes in current consumer demands and market trends. Active packaging performs important roles other than providing an inert barrier between the product and external environment (Church and Parsons, 1995). A variety of active-packaging technologies have been developed to assure better quality, wholesome and safe foods, and also to limit package-related environmental pollution and disposal problems. In general, active food packaging can provide several functions that do not exist in conventional packaging systems, including scavenging of oxygen, moisture, or ethylene, emission of

ethanol and flavors, and antimicrobial activity. The incorporation of antimicrobial agents into polymeric materials allows the industry to combine the preservative function of antimicrobials with the protective function of packaging.

In recent years, attention has focused preventing the initial adhesion of microbial contaminants by the application of antimicrobial substances to the surface of food, rather than trying to remove undesirable bacteria once they have adhered. Unfortunately, some of the traditional methods used for food preservation (thermal processing, drying, freezing, refrigeration, irradiation, MAP, and adding antimicrobial agents or salts) cannot be applied to foods such as fresh meats and RTE products. Moreover, addition of antibacterial substances into foods (in the food formulation) and/or onto foods (as sprays or dip solutions) may have limited benefits since the active substances can become inactivated by food constituents or neutralized on contact, or diffuse rapidly from the surface into the bulk of the food (Quintavalla and Vicini, 2002; Vermeiren *et al.*, 2002; Cooksey, 2005; Joerger, 2007). Therefore, the use of packaging films as a vehicle for antimicrobial compounds could be more efficient, by slow migration of the agents from the material to the surface of the product, thus helping to maintain high concentrations where they are needed. A range of antimicrobials have been incorporated into or coated onto different film types, such as bacteriocins, acids and their salts and anhydrides, plant extracts, enzymes, triclosan, and antifungal agents. Even though it is difficult to draw a strict line where the technical function of an additive is solely its antimicrobial effect on the packaging without having any impact on the food itself, the regulatory status of packaging in the EU permits the use of antimicrobial additives by Directive 2002/17/EC and its amendments (EU, 2002), whereas in the USA no specific regulation exists for active packaging (Code of Federal Regulations, 2001). The composition of antimicrobial films is dictated by its intended use; primarily if it is to be ingested along with the food or if it is removed prior to consumption. The range of potential inedible packaging materials has not only included synthetic polymers already approved for food packaging, such as polyethylene (PE), low-density polyethylene (LDPE), ethylene vinyl alcohol/linear low-density polyethylene (EVA/LLDPE), polyvinyl chloride (PVC), polyvinyl alcohol (PVOH), and nylon, but also, cellophane, paper, and mixtures of natural compounds such as chitosan and synthetic cross-linkers. On the other hand, for edible films, the range of matrices is restricted and is dominated by the polysaccharides chitosan, alginate, κ-carrageenan, cellulose ethers, high-amylose products, and starch derivatives as well as protein-based films made with wheat gluten, soy, zein, gelatin, whey proteins, and casein (Joerger, 2007). Inedible bioactive packaging films may be achieved by direct incorporation of bacteriocins into polymers or by binding of antimicrobials to polymeric surfaces of materials using soaking and contact (spray- and pouch-contact) as activation procedures. Due to the thermal stability of bacteriocins they are good candidates to be directly incorporated into polymers during melting or to be intergrated with a solvent during processing methods such as extrusion and injection molding (Appendini and Hotchkiss, 2002). For biodegradable films obtained from renewable sources, casting methods are usually applied for activation and often film plasticizers such as glycerol, propylene, or polyethylene glycol are necessary to overcome disadvantages in mechanical properties and brittleness (Hong *et al.*, 2005).

Among the bacteriocins used as antimicrobials, nisin produced by *Lc. lactis* has been the most frequently incorporated into films, either singly or combined with other antimicrobials. The reason for the predominance of nisin as an antimicrobial in films is perhaps its relatively settled regulatory status as a food additive, but the important goal of inhibiting *L. monocytogenes* in foods is also a contributing factor. Nisin's small molecular size allows the production of films that release the peptide after contact with the food or liquid; this

involves nisin being incorporated into coatings together with acids and the chelator EDTA, in particular when antimicrobial action is targeted against Gram-negative bacteria (Natrajan and Sheldon, 2000; Hoffman *et al.*, 2001). Reduction in bacterial counts determined for nisin-containing films have been around 3 $\log_{10}$ or lower; however, other studies reported $\log_{10}$ reductions between 6 and 9 (Joerger, 2007). Studies done with paperboard coated with nisin in binder materials and exposure of microbes in liquid media (nutrient broth, milk, and orange juice) showed high reductions in microbial counts, possibly due to a more universal contact with the bacteria than that obtained on a solid food surface (Kim *et al.*, 2002; Lee *et al.*, 2004). Nevertheless, reductions of approximately 6 $\log_{10}$ were reported for nisin-coated PE on *L. monocytogenes* on tofu and hot dogs (Cha *et al.*, 2003; Franklin *et al.*, 2004) and on beef in which polymer film performance was improved with the addition of a food-grade chelator (Cutter *et al.*, 2001), suggesting that nisin-coated films potentially have considerable efficacy in environments other than liquid. Also, coatings made with proteins such as zein, soy, wheat gluten, and gelatin containing nisin alone or in combination with other antimicrobials were found to produce considerable $\log_{10}$ reductions for *L. monocytogenes*, *B. thermosphacta*, contaminant LAB, and Gram-negative bacteria in different meat and poultry products (Gill and Holley, 2000; Hoffman *et al.*, 2001; Dawson *et al.*, 2002; Janes *et al.*, 2002; Lungu and Johnson, 2005; McCormick *et al.*, 2005). Compared to nisin, application of other bacteriocins as antimicrobials for film activation has not been widely reported, presumably due to poor availability and regulatory concerns. Even so, reductions of between 2 and 3 $\log_{10}$ in counts of *L. monocytogenes* were observed in different muscle-food systems when films of cellulose incorporated with pediocin powder and polythene and PE activated with a bacteriocin from *L. curvatus* were used as packaging materials (Ming *et al.*, 1997; Mauriello *et al.*, 2004; Ghalfi *et al.*, 2006). Higher reductions (approximately 6.5 $\log_{10}$) were observed in a challenge test of storage of frankfurters superficially contaminated with *L. monocytogenes* packed in polythene film activated with a bacteriocinogenic *L. curvatus* strain (Ercolini *et al.*, 2006).

22.7 CONCLUSIONS

Nowadays consumers are greatly concerned about the relationship between food and health, demanding more natural food that has undergone less severe processing and containing fewer additives. Calls for reduced use of additives and processing seem contradictory for a market asking for safer and tastier foods; these demands put the food industry under pressure to search for innovative solutions. Within the available preservation methods, the food industry is increasingly investigating the replacement of traditional food preservation techniques with new technologies. Although intensive research efforts and investment have been made, very few of these new preservation methods have been implemented by the food industry to date. Indeed, a huge increase in the number of publications on bacteriocins, particularly those produced by LAB (Desriac *et al.*, 2010), and a considerable body of experimental applications in food systems have been reached in the last 30 years. While the great potential of bacteriocins has been highlighted previously, recent developments have made the realization of this potential a more likely prospect than ever before. Although most efforts have been devoted to preservation of meat and dairy products, bacteriocins also offer great potential for fish and uncooked RTE vegetable foods. In addition, recent expanding areas of interest include bacteriocin production for target-specific anti-infective therapy and for rational drug design as clinical antimicrobials. It would seem that the knowledge that has been accrued about these peptides will finally be applied to reaching their full potential. New starter LAB

cultures with an industrially important functionality are being developed, mainly contributing to microbial safety or offering one or more sensorial technological, nutritional, or health advantage. The use of competitive bacteriocinogenic LAB as bioprotective cultures in foods may well provide at least part of the solution. However, the application of biopreservation technology to different food systems should be considered only as an additional hurdle to good manufacturing practices. As shown in this review, there are many bacteriocins and bacteriocinogenic strains that perform satisfactorily in different food ecosystems. The proper selection of bacteriocinogenic strains for each particular type of food as well as the adequate choice of bacteriocin treatment (either alone or in combination with other hurdles) may greatly reinforce the competitiveness on pathogen biocontrol in the food industry.

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23 Bacteriocins: Recent Advances and Opportunities

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Abstract: Lactic acid bacteria (LAB) produce bacteriocins which are ribosomally synthesized antimicrobial proteins or peptides directed against certain microorganism species. The application of bacteriocins as food preservatives is a promising biotechnological approach in food safety. In this chapter we have reviewed some of the research and published literature on bacteriocins produced by LAB. We have described the importance of LAB bacteriocins, with a focus on topics such as detection, classification, mechanisms of action, genetic organization, regulation and immunity. Further, we have reviewed the potential applications of LAB bacteriocins and discussed the monitoring and synergetic approaches used to improve food safety. Due to the abundance of literature on LAB bacteriocins and the availability of relevant reviews, we have lessened the scope of this review to studies published mainly during the past 10 years.

Keywords: bacteriocin; biopreservation; *Clostridium*; lactic acid bacteria; *Listeria*; nisin; pediocin

23.1 INTRODUCTION

In spite of modern food processing and safety concepts currently used such as Hazard Analysis and Critical Control Points (HACCP; Jin *et al.*, 2008), Good Manufacturing Practice (GMP; Mucchetti *et al.*, 2008) and Good Agricultural Practice (GAP; Kay *et al.*, 2008), the risk of serious illness to consumers is still significant (O'Sullivan *et al.*, 2002; Centers for Disease Control and Prevention (CDC), 2010; Quested *et al.*, 2010). In addition, increasing consumer demands for safe, healthy, fresh-tasting, minimally processed foods with a longer shelf life encourage food companies to apply novel hurdle strategies based on 'biopreservation'.

Many bacteria produce various antimicrobial proteins or peptides, referred to as bacteriocins, directed against closely taxonomically related bacteria. Those produced by lactic acid bacteria (LAB) have received increasing attention in the past 20 years as a means of using naturally preservative agents to control the growth of pathogenic and spoilage bacteria in the food industry (Holzapfel, 2002). Consequently, research on LAB bacteriocins has increased and many avenues are currently being investigated for food-manufacturing processes in attempts to improve the safety and shelf life of food products (Deegan *et al.*, 2006). In addition, bacteriocin-producing strains that grow in fermented foods could be selected as protective starter cultures or co-cultures to inhibit pathogens and prolong shelf life, if the sensory properties of the final food product are not affected (Bredholt *et al.*, 2001).

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In general, two essential methods can be used in the application of bacteriocins in food: either *in situ* inoculation of the bacteriocinic strain as a bioprotective culture, or addition of purified or partially purified bacteriocin solutions previously produced with food-grade techniques as food preservatives. Furthermore, semi-purified bacteriocins can also be used in combination with novel food-processing technologies (such as high hydrostatic pressure (HHP), modified-atmosphere packaging (MAP), bioactive composite coatings, high-intensity pulsed electric fields (HIPEFs) or food irradiation) to improve food safety and quality, especially for ready-to-eat (RTE) products (Leistner, 2000). In addition, single bacterial strains producing many bacteriocins or genetically engineered bacteriocin variants with a broader activity spectrum might be used to complement or replace the bacteriocins or the LAB currently used in the food industry (Reviriego *et al.*, 2007; Field *et al.*, 2008; Bravo *et al.*, 2009).

On the other hand, the resurgence of antibiotic-resistant pathogenic bacteria has highlighted the need for new strategies of antibiotherapy. The possibility of the use of bacteriocins, particularly lantibiotics, as a source of alternative antibiotics is being considered (Brumfitt *et al.*, 2002). Further studies are needed to develop such a strategy.

23.2 BACTERIOCINS PRODUCED BY LAB

23.2.1 Detection

In general, agar-well diffusion assays (Ghrairi *et al.*, 2004) are the standard bacterial method for screening bacteriocin producer strains. In addition, several analytical methods based on the ATP-bioluminometric method (Valat *et al.*, 2003), turbidimetric assay (Turcotte *et al.*, 2004), microplate-based assay (Chen *et al.*, 2007) and the fluorogenic method based on berberine (Fuente-Salcido *et al.*, 2007) have been proposed. Nevertheless, these methods include only a fraction of the bacterial population, and require extended calibration and/or are laborious.

The use of molecular tools such as polymerase chain reaction (PCR)-based methods to detect LAB and the development of genomics by sequencing LAB genomes allows an invaluable knowledge-based approach to the identification of new bacteriocins from the environment, e.g. penocin A (Diep *et al.*, 2006), and the exploitation of bacteria for food preservation (Hoskins *et al.*, 2001; Magnusson *et al.*, 2003; Nes and Johnsborg, 2004). Recently, a colony PCR-based method has been developed for screening bacterial isolates for the production of class IIa bacteriocins (Yi *et al.*, 2010). This proposed method was significantly more rapid than previous techniques and sufficient for detecting only class IIa bacteriocinogenic strains. Although, several studies have begun to apply these molecular tools to study bacteriocin-producing strains in fermented food, a substantial number of studies in this area are still performed with conventional culture-based techniques.

23.2.2 Classification

LAB bacteriocins have a large degree of structural and chemical diversity and recent advances in bacterial molecular genetics have further contributed to the characterization of these compounds. In general, bacteriocins produced by LAB are commonly divided into three classes (Klaenhammer, 1993; Nes *et al.*, 1996), as follows.

23.2.2.1 Class I bacteriocins

Class I bacteriocins are known as lantibiotics (see Table 23.1) and are characterized by their unusual amino acids, such as thioeter cross-linked amino acids in lanthionine and 3-methyl-lanthionine and dehydrated amino acids in 2,3-didehydrobutyrine and 2,3-didehydroalanine as a result of extensive post-translational modifications. These unusual amino residues have a role in the stability and activity of lantibiotics (McAuliffe *et al.*, 2001). The first and the most well-known lantibiotic is nisin, which has found application as a shelf-life extender in a broad range of products for over 40 years in more than 50 countries worldwide (E234), without the development of natural resistance (Mangalassary *et al.*, 2008).

Based on their topological structures, lantibiotics have been further subdivided into three subclasses: type A(I), flexible and elongated molecules; type A(II), tail- and ring-region-containing molecules; and type B, globular molecules with no net positive or negative charge (Papagianni, 2003; Asaduzzman *et al.*, 2009). In addition, a separate group is formed by the two component lantibiotics consisting of two post-translationally modified peptides. Antibacterial activity requires the complementary action of both peptides in approximately equal amounts, examples of which are lactacin 3147 (Ryan *et al.*, 1999), staphylococcin C55 (Navaratna *et al.*, 1998) and plantaracin W (Holo *et al.*, 2001).

23.2.2.2 Class II bacteriocins

Class II bacteriocins consist of small, heat-stable, non-modified cationic peptides usually synthesized as inactive prepeptides in the cell; the mature bacteriocins are generated by cleavage of the N-terminal leader peptide during export. They have been further subdivided into three subclasses: IIa, IIb and IIc. Table 23.2 provides a list of class II bacteriocins. Class IIa, also called the pediocin group, include anti-*Listeria* active peptides, which contain a conserved N-terminal amino acid motif, YGNGVXC, and cysteine residues forming a disulphide bridge in the N-terminal half of the peptide. Approximately 40 classes IIa bacteriocins have been identified and only pediocin PA-I/AcH has been exploited commercially, as a biopreservative in meat products. Class IIb bacteriocins require two peptides for full antibacterial activity; examples include lactacin F, lactococcin M and lactococcin MMT24. Class IIc bacteriocins are secreted by the general sec system (Nes *et al.*, 1996). However, it has been shown that class IIa bacteriocins can also be secreted by this system and therefore the subclass may not be necessary (Cintas *et al.*, 1997).

23.2.2.3 Class III bacteriocins

Non-lantibiotics and large and heat-labile bacteriocins make up the Class III bacteriocins. This group is not well documented. Only a few large bacteriocins produced by LAB are described at the molecular level, such as helveticin J produced by *Lactobacillus helveticus* 481 (Joerger and Klaenhammer 1990) and enterolysin A from *Enterococcus faecalis* LMG 2333 (Nilsen *et al.*, 2003). However, a number of the as-yet incompletely described antibacterial compounds are suspected to be class III bacteriocins, although not yet classified, such as helveticin V1829 from *L. helveticus* 1829 (Vaughan *et al.*, 1992), the enterocin R69 from *E. faecalis* R69 (Elotmani *et al.*, 2002) or the recently reported enterocin MMT05 from *E. faecalis* MMT05 (Ghrairi *et al.*, 2004).

A fourth class of complex bacteriocins containing protein and lipid or carbohydrate moieties has been proposed (Klaenhammer, 1993) but bacteriocins belonging to this class are still not purified.

Table 23.1 Some examples of class I bacteriocins, or lantibiotics.

Bacteriocin	LAB producer	Origin	Molecular mass (Da)	Activity spectrum	References
Class I type A(I)					
Gallidermin	*Staphylococcus gallinarum*	Chickens	2164	*Listeria*, *Clostridium*, *Staphylococcus*, *Bacillus*, other LAB	Kellner *et al.* (1988)
Mutacin 1140	*Streptococcus mutans*	Human oral cavity	2263	*Listeria*, *Clostridium*, *Staphylococcus*, *Bacillus*, other LAB	Smith *et al.* (2003)
Epidermin Tu3298	*Staph. epidermidis*		2163	*Listeria*, *Clostridium*, *Staphylococcus*, *Bacillus*, other LAB	Allgaier *et al.* (1986)
Mutacin II	*Strep. mutans*	Human oral cavity	3245	*Listeria*, *Clostridium*, *Staphylococcus*, *Bacillus*, other LAB	Novàk *et al.* (1994)
Mutacin B-Ny266	*Strep. mutans*	Human oral cavity	2270	*Listeria*, *Clostridium*, *Staphylococcus*, *Bacillus*, other LAB	Mota-Meira *et al.* (1997)
Nisin A	*Lactococcus lactis*	Diary product	3353	*Listeria*, *Clostridium*, *Staphylococcus*, *Bacillus*, other LAB	Hurst (1981)
Nisin Z	*Lc. lactis*	Diary product	3353	*Listeria*, *Clostridium*, *Staphylococcus*, *Bacillus*, other LAB	Ghrairi *et al.* (2004)
Class I type A(II)					
Lacticin 481	*Lc. lactis*	Diary product	2901	*Clostridium*, other LAB	Piard *et al.* (1992)
Lactocin S	*Lactobacillus sake*	Fermented meat	3778	*Clostridium*, other LAB	Mortvedt *et al.* (1991)
Carnocin U149	*Carnobacterium piscicola*	Fish	4635	*Listeria*, *Clostridium*, other LAB	Stoffels *et al.* (1992)
Class I type B					
Duramycin C	*Streptococcus griseoluteus*		1951	*Listeria*, *Micrococcus*	Zimmermann *et al.* (1993)
Mersacidin	*Bacillus subtilis*		2315	*Bacillus*, *Listeria*, *Staphylococcus*	Altena *et al.* (2000)

Table 23.2 Some examples of class II bacteriocins.

Bacteriocin	Producer	Origin	Molecular mass (Da)	Inhibitory spectra	References
Class IIa					
Bavaracin MN	*L. sake*	Retailed beef	4769	*Listeria*, *Clostridium*, other LAB	Kaiser and Montville (1996)
Divercin V41	*Carnobacterium divergens*	Fish viscera	4509	*Listeria*, *Clostridium*, other LAB	Metivier *et al.* (1998)
Enterocin A	*Enterococcus faecium*	Spanish dry-fermented sausage	4828	*Listeria*, *Clostridium*, *Staphylococcus*, *Propionibacterium*, other LAB	Aymerich *et al.* (1996)
Enterocin P	*E. faecium*	Spanish dry-fermented sausage	4493	*Listeria*, other LAB	Cintas *et al.* (1997)
Enterocin SE-K4	*Enterococcus faecalis*	Grass silage	5356	*Listeria*, other LAB	Eguchi *et al.* (2001)
Lactococcin MMFII	*Lc. lactis*	Tunisian dairy product	4144	*Listeria*, other LAB	Ferchichi *et al.* (2001)
Mesentericin Y105	*Leuconostoc mesenteroides*	French goat's milk	3868	*Listeria*, other LAB	Hechard *et al.* (1992)
Mundticine	*Enterococcus mundtii*	Fresh chicory endive	4287	*Listeria*, *Clostridium*, other LAB	Bennik *et al.* (1998)
Pediocin PA-1	*Pediococcus acidilactici*	Meat	4628	*Listeria*, *Clostridium*, *Staphylococcus*, *Bacillus*, other LAB	Henderson *et al.* (1992)
Plantaracin C19	*Listeria plantarum*	Fermented cucumbers	3845	*Listeria*, other LAB	Atrih *et al.* (2001)
Plantaracin 423	*Lactobacillus plantarum*	Sorghum beer	3930	*Listeria*, *Staphylococcus*, other LAB	van Reenen *et al.* (2003)
Sakacin A	*L. sake*	Meat	4306	*Listeria*, other LAB	Holck *et al.* (1992)
Sakacin G	*L. sake*	Rhodia-food collection	3834	*Listeria*, other LAB	Simon *et al.* (2002)
Ubericin A	*Streptococcus uberis*	Bovine mastitis	5270	*Listeria*, other LAB	Heng *et al.* (2007)

(Continued)

Table 23.2 (*Continued*)

Bacteriocin	Producer	Origin	Molecular mass (Da)	Inhibitory spectra	References
Class IIb					
Lactococcin Q	*Lc. lactis*	Diary product	α = 4260 β = 4018	Other LAB	Zendo *et al.* (2006)
Lactococcin MMT24	*Lc. lactis*	Tunisian dairy product	α = 3756 β = 3255	Other LAB	Ghrairi *et al.* (2005)
Lactocin 705	*Lactobacillus curvatus*	Meat starter culture	α = nd* β = nd	*Lactobacillus*, *Streptococcus pyogenes*, *Staphylococcus aureus*, other LAB	Cuozzo *et al.* (2000)
Plantaricin NC8	*L. plantarum*	Grass silage	α = 3587 β = 4000	Other LAB	Maldonado *et al.* (2003)
Enterocin C	*E. faecalis*	Human colostrum	α = 4284 β = 3867	*Actynomyces neuii*, *Streptococcus anginosus*, other LAB	Maldonado *et al.* (2009)
Enterocin 1071	*E. faecalis*	Feces of minipigs	α = 4284 β = 3898	Other LAB	Balla *et al.* (2000)
Mutacin IV	*Strep. mutans*	Dental caries	α = 4169 β = 4826	Other LAB	Qi *et al.* (2001)
Class IIc					
Enterocin B	*E. faecium*	Spanish dry-fermented sausage	5463	*Listeria*, *Clostridium*, *Staphylococcus*, other LAB	Casaus *et al.* (1997)
Carnobacteriocin A	*C. piscicola*	Fish	5049	*Listeria*, other LAB	Worobo *et al.* (1994)

*nd, not determined.

23.2.3 Mechanisms of action

The antimicrobial spectrum of LAB bacteriocins varies significantly for the different classes. This diversity of antimicrobial action suggests that bacteriocins have diverse activities which may explain the range of sensitivities of various bacterial species. In general, permeabilization of the cell membrane by pore formation is the primary mechanism by which most LAB bacteriocins exert their biological action. We shall focus here on the case of class I and IIa bacteriocins.

23.2.3.1 Class I bacteriocins

Nisin and related bacteriocins use the cell-wall precursor lipid II bound to membrane as a receptor-like molecule for pore formation and inhibition of peptidoglycan synthesis in sensitive cells (Hasper *et al.*, 2006). Electrostatic interactions between the positive charge of the bacteriocin and the negative charge of the bacterial membrane are essential for initial binding. Various mutational analyses have revealed that the rings situated in the N-terminal region of nisin are involved in lipid II binding, whereas mutations in the more flexible middle part of the peptide affect the ability of nisin to form pores in the membrane of the target cell (Breukink and de Kruijff, 1999; Wiedemann *et al.*, 2001). It has been shown that the pyrophosphate moiety of lipid II interacts with the N-terminal backbone amides of nisin rings A and B via intermolecular hydrogen links (Hsu *et al.*, 2004). Lipid II switches the orientation of the bacteriocin from parallel to perpendicular without altering the target cell membrane surface and this is essential for efficient insertion of nisin and pore formation (Breukink *et al.*, 2003). It has been suggested that the pore is formed by five to eight nisin molecules and an identical number of lipid II molecules (Breukink *et al.*, 2003). The formation of pores in the cytoplasmic membrane leads to the release of monovalent cations and ATP and the dissipation of both transmembrane potential ($\Delta\psi$) and pH gradient (ΔpH) of the protonmotive force (PFM), especially in energized cells (Bruno *et al.*, 1992). These data suggest that the loss of permeability of the cytoplasmic membrane occurs in a voltage-dependent manner. The collapse of the PFM leads to cell death through cessation of all biosynthetic processes and depletion of ATP due to a futile cycle of ATP-driven potassium uptake.

23.2.3.2 Class IIa bacteriocins

Class IIa bacteriocins also destabilize the cytoplasmic membrane in target cells through the formation of pores in the membrane. However, the mechanism by which they achieve this appears to differ somewhat from that described for the lantibiotics. Upon permeablizing the membrane, the rapid escape of UV-absorbing materials (e.g. amino acids, *o*-nitrophenol) and phosphate ions is observed, which results in total dissipation of ΔpH, with a partial dissipation of the $\Delta\psi$ (Bhunia *et al.*, 1991; Minahk *et al.*, 2002; Nielsen *et al.*, 2010). However, mundticin, a class IIa bacteriocin, causes the complete dissipation of $\Delta\psi$ (Bennik *et al.*, 1998). In addition, different studies have shown that the interaction of the class IIa subgroup of bacteriocins with the cytoplasmic membrane of sensitive cells is generally different from that of lantibiotics. Indeed, they are introduced into the membrane in voltage-independent manner (Bruno and Montville, 1993; Chikindas *et al.*, 1993; Sahl and Bierbaum, 1998).

Similar to nisin, interaction of class IIa bacteriocins with the membrane and their insertion are probably mediated by a membrane protein receptor (Chen *et al.*, 1997; Yan *et al.*, 2000; Hechard *et al.*, 2001). The subgroup IIa bacteriocins exhibit a narrow and strain-specific spectrum of activity, and the lipid composition of the membrane plays an important role in this

specificity (Chen *et al.*, 1998). Moreover, it has been shown that the antimicrobial activity of leucocin A is dependent on a stereospecific interaction at the bacterial surface, suggesting that the subgroup IIa bacteriocins interact with a specific receptor molecule in the target cells (Yan *et al.*, 2000). Further studies provided evidence for the role of the subunit IIC of the EIItMan mannose phosphotransferase system in sensitivity of *Listeria monocytogenes* and *E. faecalis* towards pediocin-like bacteriocins (Hechard *et al.*, 2001; Ramnath *et al.*, 2004). In light of these findings, EIItMan could be considered as the putative receptor-type molecule for class IIa bacteriocins (Ramnath *et al.*, 2004).

23.2.3.3 Bacteriocin–membrane interaction and pore formation

To understand details of the mechanism of pore formation, a barrel-stave mechanism has been proposed as a model for pore formation for both subgroup class I and IIa peptides (Abee, 1995). In this model, peptides first adopt a transmembrane orientation before they aggregate in a cooperative process to form water-filled pores, as shown by Breukink and de Kruijff (1999). According to these authors, the bacteriocin insertion into the lipid phase of the membrane is influenced by its concentration and, more precisely, by the cell wall composition and the chemical composition of the environment. The majority of bacteria are negatively charged but still contain hydrophobic surface components. Published results indicate that initial adsorption occurs through electrostatic attraction between the bacteriocin molecule and the cell surface. Similarly, initial electrostatic interaction between the bacteriocin and the cytoplasmic membrane can be adversely affected by the presence of charged ions or at pH values that change the net charge of bacteriocin molecules, thereby affecting the antimicrobial activity (Ennahar *et al.*, 2000).

In addition, the outer lipopolysaccharide membrane of Gram-negative cells, impenetrable to these bacteriocins, provides barrier properties that prevent antimicrobial activity. However, the addition of chelating agents, such as ethylenediaminetetra-acetic acid (EDTA), or diverse stress, can result in the disruption of the outer membrane, thus enhancing the effectiveness of LAB bacteriocin (Boziaris and Adams, 1999). Cutter and Siragusa (1994) showed that when used with EDTA, citrate or lactate, nisin (2000 IU/ml) was effective against Gram-negative bacteria (*Escherichia coli* O157:H7 and *Salmonella enterica* serovar *typhimurium*). Gänzle *et al.* (1999) reported that nisin and curvacin A in combination with low pH, high sodium chloride concentration or propylparabene also increased the susceptibility of *S. typhimurium* and *E. coli* towards those bacteriocins. Pre-adaptation of these organisms to these adverse environmental conditions did not result in decreased bacteriocin sensitivity.

23.2.4 Genetic organization and regulation

Bacteriocins are synthesized as prebacteriocins that contain an N-terminal extension that is cleaved to form the active peptide. There are typically eight to 12 clustered genes involved in bacteriocin biosynthesis. In addition to the bacteriocin and immunity genes, LAB bacteriocin gene clusters contain genes encoding post-translational modifications and regulatory, activation and export functions of these compounds. The gene cluster may be found on plasmids, on the chromosome or on conjugative transposable elements (for reviews see Drider *et al.*, 2006; Asaduzzaman *et al.*, 2009).

In general, bacteriocin biosynthesis appears to be dependent on the growth phase, the composition of the culture medium and the physicochemical properties of the environment (Diep *et al.*, 2000; Guerra *et al.*, 2001). It also appears to be under the control of a signal

transduction system (quorum-sensing system) with three components: a histidine protein kinase (HPK), which allows bacteria to sense the environment around them; a peptide pheromone (induction peptide, IP); and a cytoplasmic protein (response regulator, RR) regulating the transcriptional cellular response through its DNA-binding domain (Nes and Eijsink, 1999). In addition, the genes encoding the proteins responsible for generating and sensing the pheromones are co-transcribed and are under auto-feedback regulation.

23.2.5 Immunity

An antimicrobial producer strain protects itself against its own bacteriocin by means of a system referred to as immunity, which is expressed concomitantly with the antimicrobial peptides (Nes *et al.*, 1996). The immunity open reading frame (ORF) is generally located next to, and downstream from, the bacteriocin structural ORF.

For lantibiotics, two systems of immunity working synergistically have been identified in the producing cell: the protein LanI, and the dedicated ABC transport proteins (proteins LanF, E and G) (Klein and Entian, 1994; Peschel and Gotz, 1996; McAuliffe *et al.*, 2001). LanI, which is most likely attached to the outside of the cytoplasmic membrane, probably confers immunity to the producer cells by preventing pore formation by the bacteriocin. The second transporter system LanFEG acts by transporting bacteriocin molecules that have inserted into the membrane back to the surrounding medium and thus keeping the concentration of the bacteriocin in the membrane below a lethal level.

In general, the immunity gene of class II bacteriocins codes for a dedicated cationic protein with a high pI value containing hydrophobic domains and varying in size from 51 to 154 amino acids. For two-peptide systems, only one immunity protein is required, for which the gene is located on the same operon as the genes encoding the two peptides (Nes and Holo 2000). There is no or only weak homology between class II immunity proteins, except for the type IIc bacteriocins carnobacterioin A and entrocin B (Franz *et al.*, 2000). In addition, the immunity protein provides total immunity against the bacteriocin but cross-immunity has not been observed except for the two bacteriocins mentioned above (Franz *et al.*, 2000; van Belkum and Stiles, 2000; Fimland *et al.*, 2002). Several studies have shown that the major part of the immunity protein for class IIa is found in the cytoplasm and that a smaller proportion is associated with the membrane (Quadri *et al.*, 1995; Abdel-Dayem *et al.*, 1996). Further studies suggested that immunity proteins act either by disturbing the process of bacteriocin aggregation and pore formation or by disturbing the interaction between the bacteriocin and the membrane-located receptor molecule (Quadri *et al.*, 1995).

23.3 BIOPROTECTION AGAINST PATHOGENIC BACTERIA

23.3.1 Biocontrol of *Listeria monocytogenes*

Listeriosis is a frequent and dreaded food infection and it is not surprising that most investigations on the inhibition of food pathogen bacteria by bacteriocins or by bacteriocin-producing strains are devoted to *L. monocytogenes* and *Listeria* spp. This bacterium is ubiquitously distributed in nature and contaminates a wide range of foodstuffs such as dairy products, egg products, meat and vegetables. It can grow in a broad range of pH values (pH 4.6–9.6), in the presence of high salt concentrations and at 4°C, the storage temperature for many foodstuffs. In addition, *L. monocytogenes* can persist in biofilms in processing

equipment despite repeated treatment with disinfectants. This could be a potential contamination source (Autio *et al.*, 1999).

Several anti-*Listeria* bacteriocins (Table 23.2) have been isolated from various food products (Lee *et al.*, 1999; Ayad *et al.*, 2002; Mataragas *et al.*, 2002; Ghrairi *et al.*, 2004; Albano *et al.*, 2007; Cocolina *et al.*, 2007; Chaftar *et al.*, 2009). In most cases, a rigorous characterization of these peptides is still incomplete. However, the majority of them belong to the class IIa bacateriocins and exhibit significant biological activities and important qualities in technology. Bioprotection against *L. monocytogenes* in meat, dairy or seafood products shows that bacteriocins or bacteriocin-producing strains more or less inhibited the growth of *L. monocytogenes* (Motlagh *et al.*, 1992; Stecchini *et al.*, 1995; Goff *et al.*, 1996; Campos *et al.*, 1997; Davies *et al.*, 1997; Nilsson *et al.*, 1997; Ross *et al.*, 1999; Pawar *et al.*, 2000; Katla *et al.*, 2001; Schillinger *et al.*, 2001; O'Sullivan *et al.*, 2002; Reunanen and Saris, 2003; Castellano *et al.*, 2004; Tahiri *et al.*, 2009; Nieto-Lozano *et al.*, 2010; Ruiz *et al.*, 2010). The anti-listerial effect is more apparent in the culture media than model food systems, when the initial concentration of *Listeria* cells is low and there are variations in storage temperature. Among others, the emergence of bacteriocin-resistant *Listeria* cells in food systems supplemented with bacteriocins has been reported (Benkerroum *et al.*, 2003; Bhatti *et al.*, 2004). The mechanism behind the observed resistance is unknown, although hypotheses include cell wall modulation.

Since LAB bacteriocins alone in a food cannot guarantee safety, it is therefore essential to use them with other preservation methods to inactivate *Listeria* or undesirable bacteria. Thus, the food industry is showing an increasing interest in synergistic approaches using LAB bacteriocins as part of hurdle technology to enhance food safety and product shelf life (Table 23.3). Different studies have indicated that the presence of organic acids (like lactate, sorbate, diacetate or acetic acid) increase the anti-*Listeria* activity of nisin (Buncic *et al.*, 1995; Nykänen *et al.*, 2000), pediocin AcH (Schlyter *et al.*, 1993; Maks *et al.*, 2010) or enterocin AS-48 (Ananou *et al.*, 2010a), *in situ* and in food systems. Other licensed preservative compounds such as lysozyme (Mangalassary *et al.*, 2008), the lactoperoxidase system (Zapico *et al.*, 1998; Elliot *et al.*, 2004) or essential oils (Yamazaki *et al.*, 2000; Molinos *et al.*, 2010) can also increase the susceptibility of *Listeria* cells to LAB bacteriocins in food systems. Interestingly, these studies also indicate that inactivation efficacy can be affected by interaction of LAB bacteriocins with food ingredients (Aasena *et al.*, 2003). Therefore, it is believed that practical application of those natural antimicrobial compounds depends upon the conservation of the organoleptic and nutritional attributes of the final food product.

In addition, another strategy for food preservation may be the use of the bacteriocin-producing strains as starter cultures or co-cultures (McAuliffe *et al.*, 1999; Bogovic Matijasic *et al.*, 2007). For example, Liu *et al.* (2008) used a *Lactococcus lactis* strain of dairy origin for heterologous expression of enterocin A, a class IIa bacteriocin. The transformant was used in a co-culture for the preparation of cottage cheese. The results showed that *L. monocytogenes* was eradicated within 2 days in cheese samples prepared with the *Lc. lactis* $_{\text{ENT}}{}^{+}$ strain, while in control samples prepared without the enterocin A-producing strain *L. monocytogenes* could still be detected even after 10 days. This illustrates the need to construct new LAB strains by genetic engineering adapted to the food system and with improved protective properties. For commercial applications some starter cultures with anti-listerial activity are available. For example, Bactoferm F-Lc from Christian Hansen (Hørsholm, Denmark) is a mixed culture of *Pediococcus acidilactici* and *Lactobacillus curvatus*, producing pediocin PA-1 and sakacin A, respectively. It has been patented as an anti-listerial culture for fermented

Table 23.3 Enhanced bactericidal effects of LAB bacteriocins in combination with hurdle technology.

Bacteriocin	Treatment combination	Product tested and results	References
Nisin	Sodium diactetate (0.3 g/l)	Nisin and sodium diactate combined as a dipping solution inhibited *Listeria* growth on sliced cured pork.	Samelis *et al.* (2005)
Nisin	EDTA treatment and storage at 4°C	Nisin (1500 IU/g) and EDTA(50 mM) treatments enhanced the shelf life of chicken meat stored under modified-atmosphere packaging even up to 20 days.	Economou *et al.* (2009)
Nisin	HHP and heat	Combination of nisin and HHP with moderate heat is an interesting approach to inactivate endospores in processed foods.	Gao and Ju (2008)
Enterocin AS-48	HHP, 400 MPa	Enterocin AS-48 combined with HHP treatment caused an important decrease in *Listeria* and *Salmonella* populations in fuet sausage during ripening and afforded protection against regrowth during storage.	Ananou *et al.* (2010a)
Enterocin AS-48	Nitrite/nitrate, sodium benzoate, potassium sorbate or pentasodium tripolyphosphate	Combination of enterocin AS-48 (40 µg/g) with chemical preservatives and/or heat improved the anti-*Listeria* and the anti-*Staphylococcus* effects during storage at 5°C.	Ananou *et al.* (2010b)
Enterocin AS-48	Sodium hypochlorite, hexadecylpyridinium chloride or peracetic acid	Washing treatments containing enterocin AS-48 in combination with other antimicrobials completely inhibited *Bacillus* survival in raw vegetables at 15°C for 1 week.	Molinos *et al.* (2008a)
Enterocin AS-48	HIPEF (35 kV/cm, 150 Hz, 4–1000 µs) and heat (40°C)	Combined treatment of enterocin AS-48 (60 µg/ml) and HIPEF increased the microbial inactivation of *Salmonella* in fruit juice, decreasing the risks for transmission of this pathogen through fresh fruit juices.	Martınez-Viedma *et al.* (2008)
Enterocin AS-48	Polyphosphoric acid (1–2%)	Enterocin AS-48 (25 µg/ml) combined with polyphosphoric acid as a treatment for decontamination of raw vegetables was effective against *Salmonella* and other Gram-negative bacteria.	Molinos *et al.* (2008b)

(Continued)

Table 23.3 (*Continued*)

Bacteriocin	Treatment combination	Product tested and results	References
Enterocins A and B	HHP (400 MPa, 10 min)	Addition of semi-purified extract of enterocins A and B (2000 AU/g) to raw sausages spiked with 3 log CFU/g of *Salmonella* and application of an HHP treatment at the end of ripening reduced the count of *Salmonella* (over 2 log reduction).	Jofré *et al.* (2009)
Nisin and enterocin AS-48	HIPEF	An anti-*Staphylococcus* synergic effect was observed when nisin (20 IU/ml) and enterocin AS-48 (28 AU/ml) were combined with HIPEF in milk.	Lopez *et al.* (2009)
Enterocin EJ97	EDTA	Films activated with a combination of EJ97 and EDTA reduced the concentration of viable *Bacillus* cells in liquids from canned corn and peas at 4°C.	Martınez-Viedma *et al.* (2010)

AU, arbitrary units; CFU, colony-forming units; EDTA, ethylenediaminetetra-acetic acid; HHP, high hydrostatic pressure; HIPEF, high-intensity pulsed-electric field.

sausages. There are already other commercial anti-listerial cultures proposed by Danisco (Copenhagen, Denmark) for sausages and for cooked ham (ALCMix1) or for minced meat and raw sausages (COX1 and XPA1).

There is also the possibility of improving safety in perishable foods through the association of LAB bacteriocins with non-thermal inactivation technologies such as HIPEFs or HHP (Table 23.3), which will act in synergy to overcome *Listeria* growth in food, as studies have shown for nisin (Calderon-Miranda *et al.*, 1999; Black *et al.*, 2005), lactacin 3147 (Morgan *et al.*, 2000) and enterocin AS-48 (Ananou *et al.*, 2010b). Furthermore, active packaging (e.g. renewable biopolymers) coated with LAB bacteriocins prevents *Listeria* growth as well as other pathogens on the surface of processed food such as meats and cheese by direct contact of the package with the surface of the foods (Scannell *et al.*, 2000; Mauriello *et al.*, 2004; Cao-Hoang *et al.*, 2010; Pintado *et al.*, 2010). Moreover, these edible and biodegradable active films may thus be a good, environmental friendly method for eradicating post-process contamination of RTE foods.

23.3.2 Biocontrol of *Clostridium botulinum* and *Clostridium perfringens*

Bioprotection against pathogenic bacteria other than *Listeria* has been the subject of far fewer research studies. *C. botulinum* and *C. perfringens*, anaerobic Gram-positive spore-forming rods, have been implicated in several cases of foodborne poisoning caused by the ingestion of foods containing the potent neurotoxin formed during growth of these pathogens (Wen and McClane, 2004). The spores of those bacteria are heat resistant and can survive in foods that are incorrectly or minimally processed (Brynestad and Granum, 2002), which increases the risk of foodborne poisoning. Table 23.2 gives a list of bacteriocins to which an inhibiting action against those pathogens has been attributed, although some of the described anti-clostridial compounds were not effective against *Clostridium* spores (Okereke and Montville, 1991).

Various examples of biocontrol of *Clostridium* by bacteriocins are described in the literature (Thomas *et al.*, 2002; Anastasiou *et al.*, 2009; Nieto-Lozano *et al.*, 2010). In cheese, nisin has been studied for its action against *C. botulinum* and it is approved as a food additive by the US Food and Drug Administration for this purpose (Federal Register, 1988). This lantibiotic, marketed commercially as Nisaplin™ (Danisco) or Chrisin™ (Christian Hansen), impedes the growth of *Clostridium* in dairy food (Wessels *et al.*, 1998) and is also effective in preventing the outgrowth of *Clostridium* spores (Scott and Taylor, 1981). However, it was appropriate to take into account – in addition to the amount of nisin – the proportion of organic acids (lactate, citrate, etc.), spore load, heat treatment and storage temperature of the dairy product. In addition, growth of the bacterium or spores can be prevented by application of nisin in association with other bacteriocins or by bacteriocin-producing protective cultures as natural food ingredients (Bogovic Matijasic *et al.*, 2007).

In meat products, Nieto-Lozano *et al.* (2010) studied the effects of pediocin PA-1 (class IIa) produced by *P. acidilactici* against *C. perfringens* in Spanish dry-fermented sausages and frankfurter sausages. The authors indicated that pediocin PA-1 (5000 AU/ml; AU means arbitrary units) reduced the counts of the pathogen by 2 and 0.8 orders of magnitude after storage at 10°C for 60 days and at 15°C for 30 days, respectively. Similar results were obtained in raw meat (Nieto-Lozano *et al.*, 2006).

These research results indicate that pediocin PA-1 was the most interesting bacteriocin for the meat industry. Furthermore, application of pediocin PA-1 in dairy products has been

evaluated due to its interesting traits (e.g., activity spectrum, wide pH range activity, solubility and thermostability). This bacteriocin has been successfully co-expressed with nisin (class I) in an *Lc. lactis* strain of dairy origin at levels comparable to the respective parental strains (Reviriego *et al.*, 2007). Pediocin PA-1 has also been expressed in the yeast *Saccharomyces cerevisiae* to improve preservation of wine, bread and other food products where yeast is used (Schoeman *et al.*, 1999). Actually, a formulation produced through a fermentation process of a bacteriocinogenic *P. acidilactici* strain containing pediocin PA-1 (Alta 2341®, Quest International) is commercially exploited as a shelf-life extender in a variety of meat products in many countries worldwide.

23.3.3 Biocontrol of *Staphylococcus aureus*

Staph. aureus is a coccal Gram-positive bacterium that contaminates different food products during preparation and processing (Desmarchelier *et al.*, 1999; Holeckova *et al.*, 2002). Unlike *C. perfringens* and *C. botulinum*, *Staph. aureus* does not form spores. Thus, *Staph. aureus* contamination can be readily avoided by heat treatment of food. Nevertheless, the use of LAB bacteriocins alone or in combination with other antimicrobial processes offers the advantages of using low processing temperatures (<50°C), low energy consumption and conservation of the organoleptic and nutritional attributes of the final food product (Morgan *et al.*, 2000; Sobrino-López *et al.*, 2006).

The purpose of the work published by Sobrino-Lopez *et al.* (2009) was to evaluate the combined effect of enterocin AS-48 (Gálvez *et al.*, 1989) with nisin or lysozyme, or both together with the use of HIPEFs on milk previously inoculated with *Staph. aureus*. The results of that work showed that addition of enterocin AS-48 (28 AU/ml) and nisin (20 IU/ml) to the milk with the use of HIPEF (800 μs) revealed a significant synergistic bactericidal effect (over 6 log reduction) and could be of great interest to the dairy industry. Additionally, the persistence of the bacteriocin during food storage will allow the food products to be preserved safely. However, additional aspects such as resistance to HIPEF and spore inactivation require consideration.

In a more recent publication, Ananou *et al.* (2010b) tested enterocin AS-48 alone or in combination with chemical preservatives and/or heat against *Staph. aureus* in a cooked ham model system. The authors observed and described a synergetic effect of enterocin AS-48 and physicochemical treatments on *Staph. aureus* growth. The combination of enterocin AS-48 (60 μg/g) plus sodium pyrophosphate and heat (60°C, 2 min) resulted in a more efficient treatment to reduce the number of viable cells of *Staph. aureus*. Enterocin AS-48 is a cyclic bacteriocin produced by *E. faecalis* isolated from human wound exudates. The efficiency of this bacteriocin to control bacteria associated with the deterioration of the hygienic quality of food has been assayed (Ananou *et al.*, 2005; Muñoz *et al.*, 2007; Ananou *et al.*, 2010b). The majority of data generated to date indicate that this bacteriocin has considerable potential as a biopreservative in various foods.

23.3.4 Biocontrol of Gram-negative bacteria

Several Gram-negative bacteria, such as *Salmonella*, *Shigella* spp. and *E. coli* O157:H7, are continually linked to food poisoning outbreaks (Hald *et al.*, 2003; Gleeson *et al.*, 2005; CDC, 2010) and therefore the biocontrol of these pathogens represents an important aspect of food safety. In general, Gram-negative bacteria were resistant to LAB bacteriocins because they are protected by an outer membrane. Thus, it is necessary to associate bacteriocins with other

preservation methods to induce sublethal injuries and disruption of the protective outer membrane (Table 23.3). Various researchers have demonstrated that combination of LAB bacteriocins with food-grade permeabilizers (EDTA, citrate, lactic acid, peracetic acid, hexametaphosphate, hydrocinnamic acid, polyphosphoric acid, sodium hypochlorite, etc.) results in inhibition of *Salmonella* as well as other Gram-negative pathogenic bacteria (Boziaris and Adams, 1999; Ukuku and Fett, 2004; Belfiore *et al.*, 2007; Molinos *et al.*, 2008b; Economou *et al.*, 2009). The pH and temperature of the environment also have an influence on the degree of inhibition of microorganisms. Reductions of bacteria obtained by these treatments mainly ranged from about one to two orders of magnitude. Therefore, further treatments as in the hurdle theory proposed by Leistner (2000) may enhance the reductions obtained. On the other hand, the application of both LAB bacteriocins and plant essential oils to meat products showed only a small synergic effect (Solomakos *et al.*, 2008; Molinos *et al.*, 2009), which was more appreciable at higher storage temperatures than at refrigeration temperatures. The reason for this behaviour is unknown. Certain food additives have been shown to be antagonistic to bacteriocin and therefore more research is necessary to clarify the role of different antimicrobial compounds.

In contrast, many bacteriocins isolated from non-LAB are of particular interest to the food industry due to their significant traits (e.g. activity spectrum, solubility, wide pH range). The efficacy of cerein 8A (3200 AU/ml), a bacteriocin produced by *Bacillus cereus* 8A, in combination with EDTA (0.02, 0.05 or 0.1 mol/l) and sodium lactate (0.2 mol/l) in inhibiting *Salmonella enteritidis* has been investigated (Lappe *et al.*, 2009). Cerein 8A was bactericidal against *E. coli* and *S. enteritidis*. The addition of cerein 8A plus EDTA resulted in higher inhibition in comparison with the bacteriocin alone. The greatest inhibition result, more than 2 log reduction of the initial *S. enteritidis* content, was obtained when cerein 8A was combined with EDTA 0.1 mol/l. Despite the bacterial strain producing this compound, these results suggest that cerein 8A may be useful as a preservative in the food industry. A precise knowledge of cerein 8A's features and biosynthetic pathway should lead to novel, effective biocontrol treatments and a resulting improvement in food safety.

Newer mild treatments devised for the biocontrol of Gram-negative bacteria in liquid foods include the combination of HIPEF treatment with LAB bacteriocins (Terebiznik *et al.*, 2002; Martınez-Viedma *et al.*, 2008). It is well established that many variables influence the inactivation effect, with the intensity of HIPEF treatment and the target cells being decisive; temperature and length of the period of contact are also important (Mosqueda-Melgar *et al.*, 2007). Factors to consider in future experiments, to increase the antibacterial activity, would be the conductivity of the medium and the pH. Therefore, more research is needed to obtain the optimal values of treatment required to eradicate pathogenic microorganisms in each food product by HIPEF and bacteriocins.

HHP in combination with bacteriocins (Table 23.3) has also been shown to be effective in inhibiting Gram-negative pathogenic bacteria in food products (Aymerich *et al.*, 2005; Black *et al.*, 2005; Jofré *et al.*, 2009; Ananou *et al.*, 2010a; Jaesung and Gönül, 2010; Lee and Kaletunç, 2010). However, in some studies it was observed that Gram-negative bacteria were more sensitive to HPP, either alone or in combination with bacteriocin, than Gram-positive bacteria. The reason for this behaviour is unknown. However, the synergistic effects could be attributed to a high penetration of bacteriocins into the target cells. The application of bacteriocins with HHP is an effective and promising post-processing intervention to ensure hygienic quality, especially in RTE products (Rodriguez *et al.*, 2005). However, as pointed out by Devlieghere *et al.* (2004), occurrence and regrowth of pressure-resistant and/or

bacteriocin-resistant strains during further storage of food products cannot be avoided. This is the major drawback for sterilization treatment and therefore must be considered as part of an integral food safety system. In addition, elucidation of the mechanisms behind these modes of resistance will provide insights into novel strategies for long-term preservation of food products.

23.4 BIOPROTECTION AGAINST SPOILAGE MICROORGANISMS

23.4.1 Biocontrol of *Bacillus* spp.

Some species in the genus *Bacillus* are frequently isolated from spoiled fruit, vegetable juice or low-acid canned vegetables, e.g. *Bacillus coagulans*, *Bacillus licheniformis* or *Bacillus cerus* (Mallidis *et al.*, 1990; Larpin *et al.*, 2002; Thomas *et al.*, 2002). In addition, these Gram-positive rod bacteria produce endospores, which are resistant to chemical and physical treatments and can be found in the final products, even during storage at low temperature. Moreover, *B. coagulans* is able to increase a food's pH to values that may allow germination of surviving *C. botulinum* spores, which can cause foodborne outbreaks. Thus, inhibition of these undesirable bacteria by bacteriocins has a relevant significance. Thomas *et al.* (2002) showed in a laboratory study that nisin (6.25 µg/g) successfully controlled the growth of *Bacillus* spp. strains in potato-based products for at least 27 days at 8°C. Similar results were obtained by Grande *et al.* (2005) with enterocin AS-48 (20–35 µg/ml). In addition, this bacteriocin prevents spore outgrowth and enterotoxin production. Furthermore, decontamination strategies using enterocin AS-48 as a sanitizing solution with other antimicrobial compounds have been successfully applied for inactivation of *B. cereus* and *Bacillus weihenstephanensis*. With enterocin AS-48 alone treatment yielded reductions on alfalfa sprouts previously contaminated with *Bacillus* by more than two orders of magnitude, especially at refrigeration temperature (Molinos *et al.*, 2008a). By combination of enterocin AS-48 and sodium hypochlorite, peracetic acid or hexadecylpyridinium chloride, Molinos *et al.* (2008a) reported that *Bacillus* cells were completely eliminated or remained at very low concentrations in treated samples during a 1 week storage period at 15°C.

23.4.2 Biocontrol of yeasts and moulds

Several papers have reported that LAB produces a wide range of antifungal metabolites able to control mould growth in food, especially mycotoxinogenic moulds (Dalié *et al.*, 2010). It was also reported that some of these antifungal metabolites are sensitive to proteolytic enzymes, suggesting that the molecule involved in this antifungal activity was a bacteriocin-like peptide (Dalié *et al.*, 2010). The application of LAB cultures and their cell-free extracts has also been reported to selectively inhibit different spoilage moulds on the surfaces of food products (Mauch *et al.*, 2010). Lopes *et al.* (2009) assessed the antimicrobial activity *in vitro* of a synthetic antimicrobial peptide analogue of plantaricin 149 (Pln149a) against *S. cerevisiae*; its interaction with biomembrane model systems was also investigated. Pln149a was shown to inhibit *S. cerevisiae* growth by more than 80% in YPD medium, causing morphological changes in the yeast wall and remaining active and resistant to the yeast proteases even after 24 h of incubation.

23.5 MEDICAL AND VETERINARY POTENTIAL OF LAB BACTERIOCINS

LAB bacteriocins are recognized as safe food additives and are used as preservative agents. They are now being considered as a potent antimicrobial compounds for medical and veterinary use. One important class of bacteriocins that are a potential target for such therapeutic approaches is the lantibiotics. Some of these have broad specificity and insignificant cytotoxicity. Goldstein *et al.* (1998) reported that nisin was more effective than vancomycin in suppressing *Streptococcus pneumoniae* in a murine model. These authors also noted the rapid clearance of nisin from the blood (0.9 h). However, they noted that nisin is very effective despite its short half life. It was further determined that nisin was able to inhibit the growth of *Helicobacter pylori*, the organism that causes chronic gastritis, and reduced gastric mucosal inflammation (Kim *et al.*, 2003). Similar results have been shown in a mouse infection model in which nisin was able to control respiratory infection caused by *Staph. aureus* (De Kwaadsteniet *et al.*, 2009). Fernández *et al.* (2008) have also investigated the ability of nisin in the treatment of staphylococcal mastitis during lactation in women. However, treatment with nisin is problematic due to its low solubility at physiological pH values. In this regard, the use of the lantibiotic two-peptide lactacin 3147 could be more profitable. Carroll *et al.* (2010) used a microtitre alamarBlue assay to show that lactacin 3147 has superior activity compared with nisin at physiological pH against clinically significant mycobacteria strains. Moreover, Ryan *et al.* (1999) found that lactacin 3147 has a considerable potential for the prevention of mastitis in dairy cows. In addition, this two-component bacteriocin is highly active against *Clostridium difficile* and may have potential as a therapeutic agent in the treatment of *C. difficile*-associated diarrhoea (Rea *et al.*, 2007). Mutacin 1140 has also proved effective for the treatment of infectious diseases caused by Gram-positive bacteria (Smith and Hillman, 2008). Gallidermin shows considerable potential for the treatment of gastrointestinal infections and has low cytotoxicity (Maher and McClean, 2006). Other potent applications of lantibiotics include as a vaginal contraceptive for women, application as a feed additive for farm livestock and for prevention of dental caries and periodontal disease.

Irrespective of the techniques used, these studies highlight the potential usefulness of lantibiotics for the treatment of Gram-positive infectious diseases. Other important investigations, e.g. cost-effectiveness, immunogenicity, maximum tolerated dose, specificity, safety aspects and resistance, need to be carried out.

23.6 CONCLUSION

Several LAB produce metabolites such as the bacteriocins, which have potent antimicrobial activities towards foodborne pathogens such as *Listeria*, *Staphylococcus*, *Clostridium*, *E. coli* and *Salmonella*, and towards other health-threatening human pathogens such as *H. pylori*. The inhibition activity seems to be related to the permeabilization of the cell membrane by pore formation. These antimicrobial compounds can serve to increase food safety and prolong shelf life. Many bacteriocins have been characterized and tested. However, some aspects are still unknown (specific activity, physicochemical properties, biosynthetic pathway, interaction with food components) and require consideration.

For the practical application of LAB bacteriocins some limitations may hinder their utilization in many food products, such as an ineffective protection against pathogenic

Gram-negative bacteria, limited diffusion in solid matrices, narrow pH range, possible inactivation by natural proteases or inhibitors (especially in cheese), spontaneous loss of bacteriocinogenicity, low production and emergence of bacteriocin-resistant bacteria. The development of combined biopreservative models that take into account the interaction of bacteriocins with food ingredients and the impact on human health will help to determine optimal operational parameters and lead to knowledgeable decisions regarding the future management of food safety.

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24 Application of Botanicals as Natural Preservatives in Food

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Abstract: The deterioration of food quality occurs due to various physical, chemical, enzymic, or microbiological factors. A number of preservation techniques are available for maintaining food quality, which act by slowing or reducing microbial growth. Addition of preservative agents, either chemical or natural, is one such method. Currently there is an emphasis in the food industry on procedures that deliver food with no chemical preservatives, that is of good quality, that is nutritionally healthy, and which provides a high assurance of microbial safety. All of these can be met by use of natural preservatives which are generally derived from plants and their metabolites.

Keywords: antifungals; antimicrobials; antioxidants; biopreservatives; botanicals; essential oils; food products; herbs; natural preservatives; spices

24.1 INTRODUCTION

Food products which are perishable, once introduced into the market, require protection so that they are not spoiled during storage and distribution. Preservation technologies focus on ways of preserving food to ensure that it is safe and retains its original nutritional qualities. Synthetic preservatives which are currently being used to maintain food quality and extend shelf life are becoming unacceptable to consumers. Their use has raised several concerns in recent years. The need for labeled food products that are free from contamination is driving a trend towards natural preservatives. Even in the food industry there are now investigations into the advantages of new preservation techniques over traditional ones, which can meet the demand for nutritious and natural food products. The main purpose of natural preservatives is to prevent the growth of microorganisms, retard oxidation of fats, and inhibit natural aging and discoloration. Traditionally many natural substances, such as sugar, salt, vinegar, and alcohol, have been used as natural preservatives. Many herbs and spices also have been identified to possess antimicrobial and antioxidant activity. Many botanicals have been screened for both types of activity but only few have been exploited as food preservatives on a commercial basis. In this chapter an attempt has been made to provide an overview of the use of plants or plant-derived materials as natural preservatives in food. It throws light on botanicals and on essential oils that possess preservative action in terms of antibacterial, antifungal, and antioxidant effects. It also details about their efficacy in extending the shelf life of food products.

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24.2 ANTIBACTERIALS

Infection by microorganisms in plants results in defense mechanisms which are enhanced by the presence of antimicrobial compounds found in leaves, fruits, bulbs, and seeds. These compounds are found in intact and infected or injured plants. Antimicrobial components present in intact plants include alkaloids, dienes, flavanols, flavones, glycosides, lactones, organic acids, phenolic compounds, and protein-like compounds (Lopez-Malo *et al.*, 2002). Post-infection inhibitors include isothiocyanates, phenolic compounds, phytoalexins, and sulfoxides (Lopez-Malo *et al.*, 2002). Many botanicals for natural sources of antimicrobial agents have been identified but still many more remain undiscovered. A lot of attention has been given to herbs, edible plants, and medicinal plants (Kinjal *et al.*, 2010; Priyanka *et al.*, 2010) in terms of their antimicrobial compounds. Of greatest potential as food antimicrobials are volatile oils or essential oils obtained from spices. Even members of the Cruciferae and Liliaceae families have been screened in detail for metabolites with activity against microorganisms.

24.2.1 Spices and their essential oils

Spices are plants with distinctive flavors and aromas used in fresh or dry form (Belitz and Grosch, 1987). Traditionally, spices have been used as flavoring and seasoning agents in foods. Many possess significant antimicrobial activity due to presence of specific chemicals or essential oils (Jay and Rivers, 1984; Shan *et al.*, 2007). Usually compounds with phenolic groups are most effective (Dorman and Deans, 2000; Shan *et al.*, 2007). A large-scale systematic investigation of the relationship between phenolic contents and bacterial inhibition was examined in 46 extracts of dietary spices and herbs (Shan *et al.*, 2007). There was a positive relationship which suggested that the antibacterial activity is closely related with phenolic constituents of herbs and spices. The oils of cloves, cinnamon, oregano, thyme, sage, rosemary, and vanillin have been found to be most consistently effective against microorganisms. It was postulated that either individual components of essential oil (Dorman and Deans, 2000) or whole essential oils (Gill *et al.*, 2002; Mourey and Canillac, 2002) have antibacterial activity. It is therefore possible that variation in composition of essential oils is sufficient to cause variability in the degrees of susceptibility of Gram-negative and Gram-positive bacteria. Oils with high levels of eugenol (allspice, clove, bay, cinnamon), cinnamic aldehyde (cinnamon, cassia), and citral usually have strong antimicrobial activity (Davidson *et al.*, 1983; Davidson and Naidu, 2000). The volatile terpenes – carvacrol, *p*-cymene, and thymol – are probably responsible for the antimicrobial activity of oregano, thyme, and savory (Beuchart, 1976; Lambert *et al.*, 2001; Baydar *et al.*, 2004). Terpene thujone in sage and a group of terpenes (borneol, camphor, 1,8-cineole, α-pinene, camphone, verbenonone, and bornyl acetate) in rosemary are responsible for bactericidal activity (Davidson and Naidu, 2000).

A large number of *in vitro* studies have examined antimicrobial activity of spices and their essential oils (Alzoreky and Nakahara, 2002; Packiyasothy and Kyle, 2002). For example, essential oil of clove and cinnamon showed inhibition against *Campylobacter jejuni*, *Escherichia coli*, *Salmonella enteritidis*, *Listeria monocytogenes*, and *Staphylococcus aureus* (Smith-Palmer *et al.*, 1998; Sofia *et al.*, 2007). The oils extracted from the genus *Origanum* showed antimicrobial activity *in vitro* and in food (Aligiannis *et al.*, 2001; Souza *et al.*, 2007). Farag *et al.* (1989) examined the antimicrobial activity of the oils of sage, thyme, rosemary leaves, caraway fruits, clove flower buds, and cumin fruits against

Gram-negative bacteria (*Pseudomonas fluorescens*, *E. coli*, and *Serratia marcescens*) and four Gram-positive bacteria (*Staph. aureus*, *Micrococcus* spp., *Sarcina* spp., and *Bacillus subtilis*). They found that essential oils from sage, cumin, rosemary, and active constituents from them had little effect on Gram-negative bacteria. However, oil of caraway had a moderate effect on this group of bacteria. Oils from clove and thyme were highly effective at concentrations of 0.75–1.5 mg/ml against *Staph. aureus* and *Micrococcus* spp. Delaquis *et al.* (2002) demonstrated that oil of cilantro (leaves of coriander) was effective in inhibiting the growth of *L. monocytogenes* due to the presence of alcohol and aldehydes. Many other spices and their oils have also been tested, including anise, bay, laurel, pimento, caraway, sabadilla, black pepper, red pepper, celery seed, chili powder, cumin, curry powder, dill, fenugreek, ginger, juniper oil, mace, marjoram, nutmeg, orris root, sesame, spear mint, tarragon, and white pepper (Marth, 1966; Davidson and Naidu, 2000). Allahghadri *et al.* (2010) characterized the antimicrobial and antioxidant properties of cumin and ginger with respect to *E. coli*, *Staph. aureus*, and *Streptococcus faecalis.* They showed that bacteria were sensitive to various oil dilutions. Even combinations of some of these spices yielded a positive effect against microorganisms. Ginger and turmeric showed good inhibitory activity against Gram-negative bacteria (Sunilson *et al.*, 2009). In general, Gram-negative bacteria are more resistant to essential oils. In a similar kind of work, it was found that mint oil was more effective against Gram-positive bacteria (Sivropoulou *et al.*, 1995; Iscan *et al.*, 2002) and so were the oils from dill, cilantro, coriander, and eucalyptus (Delaquis *et al.*, 2002). However, there are some volatile oils which are effective against both groups, such as oregano, clove, cinnamon, and citral (Skandamis *et al.*, 2002). Of the Gram-negative bacteria, pseudomonads, which are frequently responsible for spoilage of food stored at low temperature, have been reported to be least sensitive to the action of essential oils (Ruberto *et al.*, 2000; Pintore *et al.*, 2002; Wilkinson *et al.*, 2003) and showed highest resistance to linalool, chavicol (Smith-Palmer *et al.*, 1998), oregano (Skandamis *et al.*, 2002), capsicum, or bell pepper (Careaga *et al.*, 2003). However, at higher concentrations some volatile oil components have been effective (Careaga *et al.*, 2003). Oils of some herbs and spices, such as marigold, ginger root, jasmine, and patchouli, were found to have bactericidal activity against *Campylobacter*, responsible for contamination of poultry products and raw milk (Friedman *et al.*, 2002; Park, 2002; Yin and Cheng, 2003). Although the potential of essential oils having antimicrobial action is evident, very few studies have been carried out in model systems or real food (Davidson, 1997; Skandamis *et al.*, 1999a, 1999b; Nychas *et al.*, 2003). For example, the essential oil of mint has been shown to inhibit the growth of *S. enteritidis* and *L. monocytogenes* in culture media for 2 days at 30°C; however, the effect of mint oil in traditional appetizers was variable (Tassou *et al.*, 1995, 2000).

24.2.2 Allium species

The Liliaceae family members onion (*Allium cepa*) and garlic (*Allium sativum*) have shown inhibitory effects on growth and toxin production of microorganisms including *Bacillus cereus*, *Clostridium botulinum* type A, *E. coli*, *Lactobacillus plantarum*, *Shigella*, *Salmonella*, *Aspergillus flavus*, *Aspergillus parasiticus*, *Candida albicans*, *Torulopsis*, *Trichosporum*, and *Sacharomyces* (Yin and Cheng, 2003; Santas *et al.*, 2010). Antimicrobial activity of proteinic extract of *Allium roseum*, a wild edible species endemic to North Africa, was tested against *C. albicans* and *E. coli*. It was suggested it can be used as a preservative in the food industry (Najjaa *et al.*, 2009).

24.2.3 Citrus fruits

Fruits belonging to six genera (*Fortunella*, *Eremocitrus*, *Clymendia*, *Poncirus*, *Microcitrus*, and *Citrus*) are native to the tropical and subtropical regions of Asia. The major commercial fruits belong to genus *Citrus* which includes several important fruits such as oranges, mandarins, lime, lemons, and grapefruits. Citrus essential oils are present in fruit flavedo in great quantities. This layer consists of the epidermis covering the exocarp and is made up of irregular parenchymatous cells with numerous oil glands. Citrus essential oils are a mixture of volatile compounds and mainly consist of monoterpene hydrocarbons, oxygenated compounds, and non-volatile compounds (Sawamura *et al.*, 2004). It is well known that essential oils from *Citrus* spp. have pronounced antimicrobial effects against both bacteria and fungi (Dabbah *et al.*, 1970; Caccioni *et al.*, 1998; Lanciotti *et al.*, 2004). Citrus essential oils could therefore represent good candidates to improve the shelf life and the safety of minimally processed fruits (Lanciotti *et al.*, 2004), and non-fat and low-fat milk (Dabbah *et al.*, 1970). However, most studies have focused on essential oils from subtropical citrus. The essential oils from two cultivars of tropical citrus, including *Citrus hystrix* DC and *Citrus aurantifolia*, exhibited antimicrobial activity against *B. cereus*, *Staph. aureus*, and *Salmonella typhi* (Chaisawadi *et al.*, 2003). Antibacterial and antifungal activities of essential oils and ethyl acetate extracts from various tropical citrus cultivars available in Thailand were compared and evaluated against both foodborne and food spoilage microorganisms. A representative from each group of food-related microorganisms was selected as the test microorganisms, including Gram-positive bacteria (*Staph. aureus* and *L. monocytogenes*), Gram-negative bacteria (*Salmonella* spp. and *E. coli* O157:H7), spore-forming bacteria (*B. cereus*), yeast (*Saccharomyces cerevisiae* var. *sake*), and mold (*Aspergillus fumigatus*). Additionally, the chemical components of the extract exhibiting high antimicrobial activity were also determined (Chanthaphone *et al.*, 2008).

24.2.4 Cruciferae family

Mustard seeds have a pungent component called allyl isothiocyanate. Isothiocyanates are derivatives from glucosinolates in cells of plants of the Cruciferae family (cabbage, turnip, brussel sprout, cauliflower, broccoli, horseradish, and mustard). Many vegetables of this family have been found to possess antimicrobial properties against several microorganisms of clinical importance. Allyl isothicyanate is inhibitory to fungi, yeasts, and bacteria in the range of 16–110 μg/ml in vapor phase (Isshiki *et al.*, 1992) and 10–600 μg/ml in liquid media (Mari *et al.*, 1993). In the case of bacteria, inhibition varies but generally Gram-positive bacteria are less sensitive to allyl isothiocyanate than Gram-negative ones. Ward *et al.* (1998) prepared horseradish essential oil distillate and applied it to the head space (the area above the agar surface) of cooked roast beef inoculated with *E. coli* O157:H7, *L. monocytogenes*, *Salmonella typhimurium*, *Staph. aureus*, *Serratia grimeseii*, and *Lactobacillus*. Allyl isothiocyanate at 20 μg/l of air inhibited the pathogens on the beef. Delaquis *et al.* (1999) added 20 μl horseradish allyl isothiocyanate on pre-cooked roast beef slices. The beef was stored for 28 days at 4°C and inoculated with spoilage bacteria. *Pseudomonas* and *Enterobacteria* were inhibited to the greatest extent while lactic acid bacteria were more resistant. The development of off-odors and off-flavors was delayed and cooked meat color was preserved in the treated roasts. In an another study, the antibacterial effect of *Brassica oleracea* juice was found to be effective in inhibiting the growth of *S. enteritidis*, *E. coli*, and *L. monocytogenes*, but not *Enterococcus faecalis*, and indicated the

role of glucosinolate-derived isothiocyanates. Also the antimicrobial effect of the juice was reduced in the presence of cysteine, suggesting that action of the juice involves blocking bacterial thiol groups (Brandi *et al.*, 2006).

24.3 ANTIFUNGALS

Many microorganisms are found on foods of intermediate moisture, including *A. flavus* and *Penicillium roqueforti* on bread (Nielsen and Rios, 2000), *Eurotium* spp. on bakery products (Suhr and Nielsen, 2004), *Debaryomyces hansenii* and *Zygosaccharomyces rouxii* in syrups, fruit concentrates, and jams (Andrews *et al.*, 1997), red halophilic cocci on salt cured fish (Prasad and Seenayya, 2000), *Pichia membranifaciens* and *Mucor plumbeus* on cheeses (Taniwaki *et al.*, 2001), and *C. albicans* on dried meat (Pitt and Hocking, 1997). The most important feature of molds from a food safety perspective is their ability to produce mycotoxins such as citrinin, aflatoxin, and roquefortine C (Taniwaki *et al.*, 2001). Aflatoxins, the most dangerous mycotoxins, are secondary metabolites of fungi produced by certain strains of *A. flavus*, *A. parasiticus*, and *Aspergillus nomius* (Fente *et al.*, 2001). Due to their ability to grow in almost all food products, yeasts and molds generate off-flavors, produce toxin, cause discoloration, and cause proteolysis by enzymes like lipases and proteases. Currently, the control measures used are physical methods (aeration, cooling, and modified atmospheres), chemical treatments (Jackson and Bullerman, 1999), and biological methods (Blackwell *et al.*, 1999). These methods require sophisticated equipment and expensive chemicals or reagents. Plant-derived essential oils are also known to possess antifungal activity (Soliman and Badeaa, 2002) and generally they are more active against fungi than against Gram-positive bacteria (Shelef, 1983). Azzouz and Bullerman (1982) evaluated 16 herbs and spices at 2% (w/v) against nine mycotoxin-producing *Aspergillus* and *Penicillium* spp. The most effective antimicrobial spice evaluated was clove, which inhibited growth of all species, and cinnamon was the next effective spice, inhibiting bacterial species. Soliman and Badeaa (2002) found that 500 ppm or less of cinnamon oil can inhibit *A. flavus*, *A. parasiticus*, *Aspergillus ochraceus*, and *Fusarium moniliforme* on potato dextrose agar. Matan *et al.* (2006) tested mixtures of cinnamon and clove oils for inhibitory activity against important spoilage microorganisms: four fungal species (*A. flavus*, *P. roqueforti*, *Mucor plumbeus*, and *Eurotium* spp.), four yeast species (*D. hansenii*, *P. membranifaciens*, *Z. rouxii*, and *Candida lipolytica*), and two bacterial species (*Staph. aureus* and *Pediococcus halophilus*). *A. flavus* and *Eurotium* spp. proved to be the most resistant microorganisms. Thymol has significant effect against fungi. Buchanan and Sheperd (1981) found out that, at 100 ppm, thymol was 99% effective after 7 days. However, aflatoxin production was resumed in 10 days at lower concentrations. Souza *et al.* (2007) reported the effectiveness of *Ocimum vulgare* volatile oil in inhibiting the growth/survival of various food-spoiling microorganisms. It showed effectiveness in inhibiting the growth of all assayed yeasts. Lopez-Malo *et al.* (2002) prepared fruit-based agars containing mango, papaya, pineapple, apple, and banana with up to 2000 μg/ml vanillin and inoculated each with *A. flavus*, *Aspergillus niger*, *A. ochraceus*, and *A. parasiticus*. Vanillin at 1500 μg/ml significantly inhibited all strains of *Aspergillus* in all media. Cerrutti and Alzamora (1996) demonstrated complete inhibition of growth for 40 days at 27°C of *D. hansenii* in laboratory media and apple purée. Ochratoxin A accumulation by *A. ochraceus* was inhibited by eugenol (Basilico and Basilico, 1999), which also prevented citrinin formation by *Penicllium citrinum* in cheese (Vazquez *et al.*, 2001). Chao *et al.* (2000) evaluated antifungal activity of 45 plant oils against *C. albicans*, *A. niger*, and *Rhizopus*

oligosporus. Coriander, cinnamon bark, lemongrass, savory, and rosewood oils were effective against all three microorganisms. Thyme, anise, and cinnamon oils were able to inhibit production of aflatoxin, ochratoxin A, and fumonisin in broth at 2%. Soliman and Badeaa (2002) showed that anise, fennel, and caraway oils were having fungicidal action against *A. flavus*, *A. parasiticus*, *A. ochraceus*, and *F. moniliforme*. The oils of thyme, oregano, rosemary, lavender, sage, and marjoram totally inhibited mycelial growth and conidial germination of *Penicillium digitatum*. Atanda *et al.* (2007) evaluated oils of sweet basil, cassia, coriander, and bay leaf for their potential in the control of aflatoxigenic fungus *A. parasiticus* and aflatoxin production. Studies with essential oil from the leaves of *Chenopodium ambrosioides* under *in vivo* investigation showed that stored wheat could be protected from different storage fungi for 1 year (Kumar *et al.*, 2007).

The essential oil extracts from *Cymbopogon citratus*, *Monodora myristica*, *Ocimum gratissimum*, *Thymus vulgaris*, and *Zingiber officinale* have also been investigated (Nguefack *et al.*, 2004). Addition of neem extract was reported to inhibit aflatoxin production by 50% (Zeringue and Bhatnagar, 1990; Allameh *et al.*, 2001). Another study examined the effect of volatile components of citrus fruit oils on *P. digitatum* and *Penicillium italicum* growth. The hydrodistilled essential oils of orange (*Citrus sinensis* cvv. Washington navel, Sanguinello, Tarocco, Moro, Valencia late, and Ovale), bitter (sour) orange (*Citrus aurantium*), mandarin (*Citrus deliciosa* cv. Avana), grapefruit (*Citrus paradisi* cvv. Marsh seedless and Red Blush), citrange (*Citrus sinensis* × *Poncirus trifoliata* cvv. Carrizo and Troyer), and lemon (*Citrus limon* cv. Femminello) were characterized by a combination of gas chromatography and gas chromatography/mass spectrometry analyses. The antifungal efficacy of the oils was then examined at progressively reduced rates. Findings showed a positive correlation between the monoterpene content of oils and the inhibition of pathogenic fungi. Furthermore, *P. digitatum* was found to be more sensitive to the inhibitory action of the oils (Caccioni *et al.*, 1998).

24.4 ANTIOXIDANTS

Foods containing fat, lipids, terpenes, and branched hydrocarbons are not stable in long-term storage or on intensive heating. Unsaturated and polyunsaturated fatty acids bound in lipids are oxidized by different mechanisms, with formation of free radicals, which are further converted into hydroperoxides. These are odorless and tasteless but they decompose, with formation of volatile compounds such as alkanals, alk-2-enals, alka-2,4-dienals, various ketones, alcohols, and hydrocarbons, giving rise to rancid flavors. Also, free radicals result in food sourness, rottenness of oils, and product aging. Rancidification can be prevented by either using fat materials low in polyunsaturated fatty acids or protecting food products against the access of oxygen or adding inhibitors of oxidation (antioxidants, which are able to inactivate free radicals). Antioxidants act as radical scavengers and inhibit lipid peroxidation and other free radical-mediated processes. Thus they are able to protect the human body from several diseases attributed to the reactions of radicals (Takao *et al.*, 1994). Synthetic antioxidants can be used to prevent free radical damage but have been reported to have toxic effects, such as the well-documented butylated hydroxytoluene and butylated hydroxyanisole (Cornwell *et al.*, 1998). Hence there is growing interest in studies of natural additives as potential antioxidants. The screening of plant extracts and natural products for antioxidant activity has revealed the potential of higher plants as a source of new agents (Rios *et al.*, 1988). Antioxidants from plant materials are associated with a lower incidence of

conditions such as tumors, inflammation, and diabetes (Leong and Shui, 2002). Natural antioxidants has been a major research interest for the past two decades, especially botanicals for possible antioxidant properties. Phenolic compounds, the most important group of natural antioxidants, possess biological and chemical properties in common: they have a reducing character, a capacity for sequestering reactive oxygen species and several electrophiles and for chelating metallic ions, a tendency for self-oxidation, and a capacity for modulating the activity of some cellular enzymes (Robards *et al.*, 1999). The most common antioxidants present in plants are tocopherols, tocotrienols, synergists (citric acid, tartaric acid, phospholipids), and chelates (phytates).

24.4.1 Cereals and legumes

Cereals make up a large part of the human diet. They contain phenolic compounds possessing antioxidant activity (Zielinski, 2002). Buckwheat and oat flour (Xing and White, 1997) are most active sources of antioxidants and phenolics, the majority of which are concentrated in the hulls and in bran. In cereals, melanoidins possess moderate antioxidant activities which are important in baked or roasted products (Elizalde *et al.*, 1991). Grain legumes are less important food components than cereals as their content of phenolic substances is relatively low. They still may help in stabilizing food if added in substantial amounts as an ingredient.

24.4.2 Fruits

Fruits are rich in flavones and anthocyanins that protect against oxidative degradation. In red wine and deep-red-colored fruit juices, various anthocyanins are present which are soluble in the aqueous phase and possess moderate antioxidant activity (Stahl and Sies, 1998). The best investigated fruits are from members of the citrus family. These fruits possess antioxidant activity that is stronger in aqueous emulsions because of the compounds' prevailing hydrophilic character. The antioxidant activity found in fractions of *Citrus sinensis* is attributed to the presence of flavonoids and other phenolic compounds (Anagostopoulo *et al.*, 2006). Nevertheless, the possibility for safe application of these inhibitors to food products must be investigated.

24.4.3 Herbs and spices

Since ancient times, spices have been well known for their antioxidant capacities (Madsen and Bertelsen, 1995). The most important herbs belonging to this group are tea leaves obtained from green tea or fermented tea (*Camellia sinensis* or *Camellia assamica*). Green tea is rich in catechins and related compounds (Yamamoto *et al.*, 1997). It also contains epicatechin, gallocatechin, and gallates. It has been shown to actively protect meat lipids from oxidation (Shahidi and Alexander, 1998). The effect of green tea and grape seed extracts on bonito fillets (*Sarda sarda*) was a delay in lipid oxidation; the effect was enhanced when fillets were treated before freezing (Yerlikaya and Gokoglu, 2010). Efficient inhibitors are also present among oregano, spearmint, savory, lavender, nutmeg, and allspice. The most active inhibitor is present in rosemary and sage. Both of them contain carnosol and carnosic acid as active constituents (Cuvelier *et al.*, 1996; Richhemier *et al.*, 1999). Even ginseng and sweet grass possess antioxidant activities which are used as spices in alcoholic beverages. A comparative study on the chemical compositions of ginger and cumin was carried out by El-Ghorab *et al.* (2010). They analyzed fresh and dried ginger and showed its oil to consist of

camphene, *p*-cineole, α-terpineol, zingiberene, and pentadecanoic acid as major components. In the case of cumin, its volatile oil consisted of cuminal, γ-terpinene, and pinocarveol. The authors concluded that both ginger and cumin can be used as natural antioxidants in foods. Essential oils of carvacrol and thymol were shown to prevent microbial and chemical deterioration when added to food (Ultee *et al.*, 1999; Burt, 2004; Mahmoud *et al.*, 2004a, 2004b). Analysis of components of thyme, mainly thymol and carvacrol, were also suggested to have antioxidant activity (Nakatani, 2000; Lee and Shibamoto, 2001; Miura *et al.*, 2002). Many researchers have observed that the antimicrobial and antioxidant effects of these phenolic compounds can be enhanced by combining them with other natural preservatives (Ramanathan and Das, 1992; Yamazaki *et al.*, 2004). Mentha and pepper oil screened for antioxidant activities showed greater antioxidant activity than the individual oils (Yadegari *et al.*, 2006). Radical-scavenging and antioxidant properties of essential oils from *Rosmarinus officinalis* and *Cuminum cyminum* were better (Gachkar *et al.*, 2007). The antioxidant activity of *Ruellia tuberosa* was investigated by Chen *et al.* (2006) and results revealed that *R. tuberosa* possessed potent antioxidant activity. Eleven volatile oils, namely *Cananga odorata* (Annonaceae), *Cupressus sempervirens* (Cupressaceae), *Curcuma longa* (Zingiberaceae), *Cymbopogon citratus* (Poaceae), *Eucalyptus globulus* (Myrtaceae), *Pinus radiata* (Pinaceae), *Pipercrassinervium* (Piperaceae), *Psidium guayava* (Myrtaceae), *Rosmarinus officinalis* (Lamiaceae), *Thymus* × *citriodorus* (Lamiaceae), and *Zingiber officinale* (Zingiberaceae) were evaluated for their functional properties related to food. These properties were compared to those of *Thymus vulgaris* volatile oil, used as a reference ingredient. Antioxidant and radical-scavenging properties were tested by means of 1,1-diphenyl-2-picrylhydrazyl assay, β-carotene bleaching test, and luminol-photochemiluminescence assay (Sacchetti *et al.*, 2005; Amin *et al.*, 2006). Similarly, Kartal *et al.* (2007) examined *Ferula orientalis* (Apiaceae) and Orhan *et al.* (2007) studied 14 *Salvia* species. Mate (*Ilex paraguariensis*) leaves contain many bioactive compounds responsible for antioxidant activity (Bracesco *et al.*, 2003; Markowicz-Bastos *et al.*, 2006). The antioxidative effect of dietary oregano essential oil and α-tocopheryl acetate supplementation on susceptibility of chicken breast and thigh muscle meat to lipid oxidation during frozen storage at −20°C for 9 months was established (Botsoglou *et al.*, 2003). Sage (*Salvia officinalis*) and rosemary (*Rosmarinus officianalis*) are popular Labiatae herbs with a verified potent antioxidant activity (Dorman *et al.*, 2003; Ibanez *et al.*, 2003).

24.5 APPLICATIONS

In food products, essential oils have been used in bakery products (Nielsen and Rios, 2000), cheese (Vazquez *et al.*, 2001), meat (Quintavalla and Vicini, 2002), and fruit (Lanciotti *et al.*, 2004). Their bioactivity in the vapor phase makes them useful as possible fumigants for protection of stored commodities. Shelf life can even be extended by inhibiting or retarding the growth of pathogenic microorganisms in packed foods and packaging material, which can be achieved by incorporating antimicrobial agents into polymers or coatings, or by adsorbing antimicrobials onto polymer surfaces (Appendini and Hotchkiss, 2002; Oussallah *et al.*, 2004). It is generally found that a greater concentration of essential oil is needed to achieve the equivalent *in vitro* effect in foods (Smid and Gorris, 1999). The ratio has been recorded to be approximately twofold in semi-skimmed milk (Karatzas *et al.*, 2001), 10-fold in pork liver sausage (Pandit and Shelef, 1994), 50-fold in soup (Ultee and Smid, 2001), and 25- to 100-fold in soft cheese (Mendoza-Yepes *et al.*, 1997).

24.5.1 Meat products

The major problem with meat products is their high fat content, which is responsible for the weak action of essential oils on these products (Mejlholm and Dalgaard, 2002). For example, in high-fat products mint oil exhibited little antibacterial effect against *L. monocytogenes* and *S. enteritidis*, whereas in low-fat food the same oil was much more effective (Tassou *et al.*, 1995). In one study, cilantro essential oil was immobilized in a gelatin gel and was found to be a more effective antimicrobial agent against *L. monocytogenes* in ham than essential oil that had not been immobilized (Gill *et al.*, 2002). A similar study with encapsulated rosemary oil showed better response than standard rosemary essential oil against *L. monocytogenes* in pork liver sausage (Pandit and Shelef, 1994). Skandamis and Nychas (2001) and Menon and Garg (2001) observed that the active constituent eugenol isolated from coriander, clove, oregano, and thyme oils was effective at levels of 5–20 µl/g in inhibiting *L. monocytogenes* and *Aeromonas hydrophila* in meat products. In contrast, mustard, cilantro, mint, and sage oils were less effective or were ineffective (Tassou *et al.*, 1995; Gill *et al.*, 2002).

In the case of fish products too, a high fat content appears to reduce the effectiveness of antibacterial essential oils. For example, oregano oil at 0.5 µl/g was more powerful against the spoilage organism *Photobacterium phosphoreum* on cod fillets than on salmon, which is a fatty fish (Mejlholm and Dalgaard, 2002). The potential of oregano oil along with sodium nitrite (chemical preservative) was tested in vacuum-packed and pasteurized minced (ground) pork against *C. botulinum* spores. In the presence of low levels of sodium nitrite only, bacterial growth was delayed to a certain extent but when oregano oil was applied the delay was enhanced (Ismael and Pierson, 1990). Application of allyl isothiocyanate (from mustard and horseradish) as a antimicrobial agent in active packaging of rye bread was found to be very useful in controlling growth of *A. flavus*, *Penicillium commune*, *Penicillium corylophilum*, *Penicillium discolor*, *Penicillium polonicum*, *P. roqueforti*, and *Endomyces fibuliger* (Nielsen and Rios, 2000). These results illustrated the efficient usage of active packaging in delivering inhibitors. Garlic and onion from the Liliaceae family were also shown to have potential for keeping pork belly and loin fresh when refrigerated (Park *et al.*, 2008). The content of oxidative products was reduced and antioxidant activity was comparable to that of sodium ascorbate. Even in pork patties and minced salmon flesh (using garlic extract) and fresh cut salmon (using clove oil) plant extracts were found to be useful in controlling lipid oxidation and microbial growth (Pakawatchai *et al.*, 2009; Miladi *et al.*, 2010; Park and Chin, 2010).

24.5.2 Dairy products

The reaction between the volatile oil carvacrol (oreganum, thyme, savory) and proteins is a limiting factor in the antibacterial activity against *B. cereus* in milk (Pol *et al.*, 2001) and *S. enteritidis* in low-fat cheese (Smith-Palmer *et al.*, 2001). Mint oil at 5–20 µl/g was found to be effective against *S. enteritidis* in low-fat yogurt (Tassou *et al.*, 1995). Similarly Smith-Palmer *et al.* (1998) reported that the oils of clove, cinnamon, and thyme have potential to control growth of *L. monocytogenes* and *S. enteritidis* in tryptone soya broth and concluded that plant essential oils could be used as natural antimicrobial agents in dairy products. Essential oils of cinnamon bark, cinnamon leaf, and clove (Cava *et al.*, 2007) were examined for antimicrobial activity against *L. monocytogenes* in semi-skimmed milk incubated at 7 and 35°C. There was an increase in activity at the lower temperature due to enhancement of membrane fluidity and membrane-perturbing action of essential oils. Also the influence of the

fat content of milk was tested in whole and skimmed milk. In milk samples with higher fat content the antimicrobial activity of the essential oils was reduced. These results indicate the possibility of using these three volatile oils in milk beverages as natural antimicrobials, especially because milk beverages flavored with cinnamon and clove are consumed worldwide. In some cases, addition of garlic extract to butter was found to enhance the rates of inactivation of *Salmonella* spp., *E. coli*, and *L. monocytogenes* at 21 or 37°C (Adler and Beuchat, 2002) and vanillin oil was shown to be active against *L. monocytogenes* (Tipparaju *et al.*, 2004) in yogurt.

24.5.3 Vegetables and fruits

The potential of essential oils as antimicrobial agents in vegetable dishes is increased by a decrease in storage temperature and/or a decrease in the pH of the food (Skandamis and Nychas, 2000). All volatile oils and their components that have been tested on vegetables appear effective against the natural spoilage flora and foodborne pathogens at levels of 0.1–10 µl/g in washing water (Singh *et al.*, 2002). Oregano oil from the Lamiaceae family was efficacious at inhibiting *E. coli* O157:H7 in eggplant salad as compared to the untreated control (Skandamis and Nychas, 2000). A study was conducted to develop a method for prolonging the shelf life of ground fresh tomato using fresh *Allium sativum* and *Eugenia aromatica* by Adekalu *et al.* (2009). They showed an increase in shelf life by 10 days. Similarly, cinnamaldehyde and thymol were found to be effective against six *Salmonella* serotypes on alfalfa seeds when applied in hot air at 50°C as a fumigation method (Weissinger *et al.*, 2001). This may be due to the volatility of the antibacterial compounds. Fruits such as sweet cherry face severe problems of commercialization due to fast decay and loss of quality, both for fruit and stem. Serrano *et al.* (2005) developed a package based on the addition of eugenol, thymol, menthol, or eucalyptol pure volatile oil separately to trays sealed with polypropylene bags to generate a modified atmosphere. When fruit quality parameters were determined those treated with eugenol, thymol, or menthol showed benefits in terms of reduced weight loss, delayed color changes, and maintenance of fruit firmness compared with controls. Stems remained their green color in treated cherries while they became brown in control treatments. Kisko and Roller (2005) attempted to use the natural antimicrobial components carvacrol and *p*-cymene from spices to increase the shelf life of raw fruit juices. Roller and Seedhar (2002) revealed the active components carvacrol and cinnamaldehyde to be effective in reducing the viable count of the natural flora on kiwi fruit when used at 0.15 µl/ml in dipping solution, but they were less effective on honeydew melon. This difference between the fruits was explained by the difference in pH. In one study vanillin herb in combination with calcium lactate, ascorbic acid, and citric acid was utilized to produce a shelf-stable strawberry purée (Cerrutti *et al.*, 1997).

24.5.4 Synergistic effects

Combination of essential oils with other antibacterial agents and with a variety of treatments such as mild heat (Karatzas *et al.*, 2001), hydrostatic pressure (Ogawa *et al.*, 1998), and sodium citrate and monolaurin (Blaszk and Holley, 1998) has been found to very useful in food preservation studies. Ettayebi *et al.* (2000) reported the synergistic effects of volatile oils and nisin on *B. cereus* and *L. monocytogenes*. Pol and Smid (1999) found that the addition of lysozyme as a third preservative factor enhances the synergistic effect between carvone and nisin. Fractions of cilantro, coriander, dill, and eucalyptus oils, when mixed in various

combinations, resulted in additive, synergistic, or antagonistic effects (Delaquis *et al.*, 2002). Combinations of oregano essential oil with sodium nitrite have been examined for their effect on growth and toxin production by *C. botulinum*. Oregano oil acted synergistically with nitrite to inhibit growth in broth, whereas oregano oil applied alone at up to 400 ppm had no significant inhibitive effect on growth. The proposed mechanism of synergism depends on oregano oil reducing the number of spores which germinate and sodium nitrite inhibiting the outgrowth of spores, affecting vegetative growth (Ismael and Pierson, 1990). In another study, the simultaneous application of nisin (0.15 μg/ml) and carvacrol or thymol (0.3 mmol/l or 45 μg/ml) caused a larger decline in viable counts for strains of *B. cereus* than was observed when the antimicrobials were individually applied. The maximum reduction of viability was achieved in cells that had undergone prior exposure to mild heat treatment at 45°C (Periago *et al.*, 2001). At pH 7, the synergistic action of nisin and carvacrol was significantly greater at 30°C than at 8°C, which would appear to indicate temperature-induced changes in the permeability of the cytoplasmic membrane (Periago and Moezelaar, 2001). Thymol and carvacrol have been shown to have a synergistic effect with high hydrostatic pressure. The viable numbers of mid-exponential-phase *L. monocytogenes* cells were reduced more by combined treatment with 300 MPa high hydrostatic pressure and 3 mmol/l thymol or carvacrol than by the separate treatments. Since high hydrostatic pressure is believed to cause damage to the cell membrane, it is suggested that this common target is the root of the observed synergism (Karatzas *et al.*, 2001). These studies indicate that the presence of fats, carbohydrates, proteins, and salt, and the pH value, influence the effectiveness of these agents in foods. Antimicrobial potency is also reduced in foods with lower water activity. There are two examples where spice/herbal materials have been successfully used as either a dip for poultry carcasses (Dickens *et al.*, 2000) or as a surface coating on the saltwater fish Asian sea bass (Harpaz *et al.*, 2003) to extend shelf life. Volatile oils were also found to be useful as anti-listerial agents, i.e., reducing undesirable flavors caused from *L. monocytogenes*. Mourey and Canillac (2002) showed carvacrol and thymol to have the strongest anti-listerial properties, followed by eugenol, cinnamaldehyde, and isoeugenol. A combined anti-listerial effect was observed between nisin and the essential oils (carvacrol, thymol, and eugenol); moreover, the addition of diglycerol monolaurate as a third preservative factor led to further enhancement of anti-listerial activity. These results indicate that nisin and diglycerol monolaurate can be used to enhance the anti-listerial activity of volatile oils, allowing for a reduction in the dosage used in food preservation and thereby resulting in a reduction of undesirable flavors. Other appropriate preservatives in combination with essential oils from spices/herbs and nisin can therefore reasonably be expected to have a similar additive or synergistic effect.

24.6 CONCLUSION

An in-depth understanding of the various properties related to natural antimicrobials, antifungals, and antioxidants is needed to answer many queries related to food preservation despite the burgeoning literature on the subject. There is little doubt about whether aromatic compounds represent a useful tool to increase the shelf life and the safety of processed foods and fruits. Nevertheless, investigations are necessary to identify the conditions that maximize their activity without detrimental effects on the organoleptic properties of the food product. Identification of antimicrobial and antioxidant molecules and their interactions in complex mixtures of essential oil compounds should be addressed, which will help in controlling microbial growth and reducing rancidity. Fluctuations in activity of volatile oil due to

meteorological, seasonal, and geographical factors as well as different compositions due to the plant type need further study. There is also a need to explore the appropriate mode of application of essential oils in foodstuffs. Immersion, mixing, encapsulation, surface spraying, and evaporation onto active packaging are some promising methods of adding these compounds to foods that have not been extensively investigated. The interaction between antimicrobials and packaging materials, rather than the food itself, needs research. The future of botanicals to be used in food preservation lies in the careful selection and evaluation of such molecules and its combination with other chemical preservatives, i.e. synergistic effects, so as to maximize the various properties associated with natural food preservation.

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25 Tropical Medicinal Plants in Food Processing and Preservation: Potentials and Challenges

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Abstract: Increasing awareness of the relationship between diet and disease has lead to greater emphasis on foods capable of meeting basic nutritional requirements while providing additional physiological benefits. Sub-Saharan Africa is the source of a large number of the medicinal plants that are currently in use around the world. These plants have great potential due to their prevalence, nutritive value and biologically active components. Despite these potentials, they have not found use in food products. There is a tremendous potential to add value to tropical medicinal plants by promoting them as a source of high-value functional ingredients for use in food processing and preservation.

Keywords: bioactive compounds; food preservation; food safety; medicinal plants; pathogens

25.1 INTRODUCTION

Foodborne illness and food poisoning are still major problems and a threat for both consumers and the food industry. In view of this, there is considerable interest in the improvement of the nutritional quality, safety and shelf life of foods. The conventional method of enhancing food safety involves the use of chemical preservatives to control food spoilage and prevent the growth of foodborne pathogens. However, concerns still exist with the use of chemical preservatives due to an increasing occurrence of chemical residues in food, resistant microorganisms and new foodborne diseases caused by pathogenic organisms (Aslim and Yucel, 2007; Murdak *et al.*, 2007; Nguefack *et al.*, 2007; Arques *et al.*, 2008). Recent reports show that chemical preservatives cannot eliminate food pathogens such as *Listeria monocytogenes* or delay microbial spoilage totally (Gutierrez *et al.*, 2009). Strong consumer demand for safe and high-quality foods and increasing unease regarding the use of chemical preservatives raise considerable challenges and underscore the quest for natural alternatives (Nguefack *et al.*, 2007; Petitclerc *et al.*, 2007; Arques *et al.*, 2008; Demirci *et al.*, 2008; Li *et al.*, 2008). Consequently, there is a great deal of interest in edible plants containing antioxidants and health-promoting phytochemicals as potential therapeutic agents and natural antimicrobials for use in foods and food packaging materials (Devlieghere *et al.*, 2004; Lopez-Rubio *et al.*, 2004; Cutter, 2006; Periago *et al.*, 2006; Gaysinsky and

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Weiss, 2007; Fisher and Phillips, 2008; Patrignani *et al.*, 2008; Ponce *et al.*, 2008). Apart from their antimicrobial properties, phytochemicals also possess health effects, such as anticancer, anti-aging, anti-atherosclerotic and anti-inflammatory activities (Shahidi and Naczk, 2004; Fresco *et al.*, 2006; Han *et al.*, 2007; Shahidi, 2008), making them attractive as potential sources of bioactive components for use in food systems.

Plant-based bioactive compounds like phenolics, organic acids (from berries), terpenoids (from *Croton uracurana*, *Combretum imberbe*) and isothiocyanates (from mustard extracts) and other phytochemicals have been identified as potential alternatives to chemical preservatives for use in the food industry (Rauha *et al.*, 2000; Puupponen-Pimia *et al.*, 2001; Katerere *et al.*, 2003; Mathabe *et al.*, 2008). Plant-based essential oils possess antioxidant and antibacterial activities with low toxicity against mammals and low environmental impact (Kalemba and Kunicka, 2003; Paranagama *et al.*, 2003; Burt, 2004; Burt *et al.*, 2005; Gaysinsky *et al.*, 2005; Gill and Holley, 2006). Thus plant materials have tremendous potential for utilization in diverse food systems.

25.2 SOME TROPICAL MEDICINAL PLANTS WITH POTENTIAL FOOD-PROCESSING VALUE

Medicinal plants have, for many centuries, occupied a very important position in healthcare delivery in various regions of the world. They are gradually assuming increasing importance and relevance in various countries, where large numbers are used for treatment of various medical conditions such as coughs, fever, headaches, beriberi, neuralgia, dysentery, malaria, constipation, haemorrhoids, jaundice and convulsions. Apart from their medicinal properties, many have shown remarkable potential for use in food processing, preservation and supplementation schemes.

25.2.1 *Ageratum conyzoides*

Ageratum conyzoides L. belongs to family and tribe Asteraceae and Eupatoriae, respectively. It is found in several countries in tropical and subtropical regions of the world, including Central America, Caribbean, Florida in the USA, South-East Asia, South China, India, Nigeria, Australia and South America (Okunade, 2002). It is an annual herbaceous plant considered to be a weed and is not presently consumed as food or feed. *A. conyzoides* contains many bioactive components, including flavonoids, alkaloids, coumarins, essential oils, chromenes, benzofurans, terpenoids and tannins. *In vitro* studies show that whole-plant extracts have antibacterial, muscle-relaxing, wound-healing, anticancer and antiradical properties and are non-toxic (Silva, 2000; Jagetia *et al.*, 2003; Oladejo, 2003; Shirwaikar *et al.*, 2003; Nweze and Obiwulu 2009; Adebayo *et al.*, 2010). *A. conyzoides* contains minerals, as well as essential and non-essential amino and fatty acids (Okunade, 2002). The flavones produced by and released from *A. conyzoides* play an important role in the control of pathogens in citrus orchards (Hu *et al.*, 2002). The plant's ethno-medicinal importance, antioxidant property and inhibitory activity against the *Aspergillus* group of fungi (Patil *et al.*, 2010) may add a new dimension to its potential usefulness in the protection of stored products.

25.2.2 *Cymbopogon citratus* (lemongrass)

Cymbopogon, known worldwide as lemongrass, is a genus of about 55 grasses most of which are native to South Asia, South-East Asia and Australia. *Cymbopogon citratus* is the most

widely used species. It is cultivated as a medicinal herb in India and as a culinary herb and spice in South-East Asia, Sri Lanka and Thailand. It has been used in treatment of malaria, cough and sprains as well as a stimulant and diuretic. Lemongrass oil has therapeutic, antibacterial and antifungal activity (Irkin and Korukluoglu, 2009). Dietary supplementation of cattle diet with lemongrass improved rumen ecology, digestibility of nutrients, rumen microbial population and microbial protein synthesis efficiency (Wanapat *et al.*, 2008). *C. citratus* has properties that could be explored for the treatment of inflammatory diseases of the gastrointestinal tract, reduction (or prevention) of atherosclerosis and control of hepatocarcinogenesis, and it shows larvicidal activity against *Anopheles arabiensis* (Puatanachokchai *et al.*, 2002; Orrego *et al.*, 2009; Figueirinha *et al.*, 2010; Karunamoorthi and Sabesan, 2010). The efficacy of the plant's essential oil in preserving the quality of melon seeds in stores was statistically on par with that of a fungicide treatment (Bankole *et al.*, 2005). The diverse properties of *C. citratus* suggest that it may have food-processing, food-preservation and food-storage potential.

25.2.3 *Chromolaena odorata* (Siam weed)

Chromolaena odorata, formerly known as *Eupatorium odoratum*, is classified as a noxious weed and is widespread in West Africa and other tropical areas. Its leaf fraction has high crude protein content (258 $g \cdot kg^{-1}$ dry matter (DM)) and degradable nitrogen content (60.7 $g\,N \cdot kg^{-1}$ digestible organic matter), low neutral detergent fibre (331 $g \cdot kg^{-1}$ DM) and low acid detergent lignin (53.1 $g \cdot kg^{-1}$ DM), total extractable phenolic (37.1 $g \cdot kg^{-1}$ DM), extractable tannin (0.72 absorbance at 550 nm) and extractable condensed tannin (1.4 $g \cdot kg^{-1}$ DM) content, with little or no phenolic-related antinutritive factors (Apori *et al.*, 2000). *C. odorata* leaves thus have high nutritive value and may have potential for use in ruminant protein supplementation schemes (Apori *et al.*, 2000). The flavonoid components of the plant (Pisutthanan *et al.*, 2006), coupled with the presence of biologically active constituents like coumarins, phenols, tannins, and sterols (Ngono *et al.*, 2006), and its demonstrated radical-scavenging activities (Akinmoladun *et al.*, 2010), suggest that it has significant potential for use as a natural antioxidant and chemoprophylactic agent (Akinmoladun *et al.*, 2010; Srinivasa *et al.*, 2010).

25.2.4 *Garcinia kola* (bitter kola)

Garcinia kola has economic value across West African countries where its seeds are commonly chewed and used for traditional ceremonies and folk medicine. *G. kola* seed extracts possess antidiabetic, antioxidant, antihepatotoxic and broad-spectrum antimicrobial properties due largely to its benzophenone, biflavonoid and flavanone contents (Pietta *et al.*, 2000; Akinpelu *et al.*, 2008; Okoko 2009). The extracts are highly potent against β-lactam-resistant Gram-positive cocci (*Enterococcus* sp P054 and *Staphylococcus aureus* U127) and have direct antibacterial effect on *Streptococcus mutans* (Afolabi *et al.*, 2008; Gangoue–Pieboji *et al.*, 2009).

The utilization of blends of *G. kola* and other medicinal plants as a potential hop substitute in sorghum larger beer brewing has been reported (Eleyinmi and Oloyo, 2001; Eleyinmi *et al.*, 2004). The presence of nutritionally valuable components in the seed and hull of *G. kola* (Eleyinmi *et al.*, 2006) suggests that it may find further use in food and feed formulation and as a potential source of nutritionally valuable nutrients and industrial raw materials. The antioxidant activity of *G. kola* seeds (Pietta *et al.*, 2000; Okoko, 2009), coupled with its chemical composition (Eleyinmi *et al.*, 2006), further underscore its nutraceutical and pharmaceutical potential.

25.2.5 *Vernonia amygdalina* (bitter leaf)

Vernonia amygdalina is a shrub which grows in a range of ecological zones in Africa. Its leaves have an astringent taste due to the presence of alkaloids, saponins, tannins and glycosides (Bonsi *et al.*, 1995). Attempts have been made to improve the taste of *V. amygdalina* via the addition of 6.6% (DM) molasses. It has also been fed to broiler chickens, where it replaced up to $300\,g \cdot kg^{-1}$ of maize-based diet without affecting feed intake, body-weight gain and feed efficiency (Bonsi *et al.*, 1995). It is generally regarded as safe for use as a food and a medicine; however, safety in young children, pregnant or nursing women, or those with severe liver or kidney disease has not been reported. The nutrient profile (Oboh, 2006) and antimicrobial activities of the plant extract against pathogenic food spoilage organisms like *Staph. aureus* and *Escherichia coli* (Okigbo and Mmeka, 2008) attest to it potential value in food processing.

25.2.6 *Allium sativum* L. (garlic)

The medicinal use of garlic (*Allium sativum* L.) dates back thousands of years, but there was little scientific support for its therapeutic and pharmacological properties until recently. Garlic is an important and widely cultivated plant with both culinary and medicinal uses stemming from its biological activities, which include antibiotic, anticancer and antithrombotic activities and lipid-lowering cardiovascular effects (Yeh and Liu 2001; Bhagyalakshmi *et al.*, 2005). Historical experience of garlic consumption satisfied many that the human risk-to-benefit ratio is beneficial and showed that the potential health benefits of garlic are largely dependent on its mode of processing (Staba *et al.*, 2001). Garlic is known to have antimicrobial activity against several spoilage and pathogenic bacteria. Adler and Beuchat (2002) showed that addition of garlic to butter enhanced the rates of inactivation of *Salmonella*, *E. coli* O157:H7 and *Listeria monocytogenes* at 21 and 37°C. It also has the potential to reduce heterocyclic aromatic amine formation in cooked beef patties (Shin *et al.*, 2002). These properties suggest potential food processing and preservation value.

25.2.7 *Gongronema latifolium*

Gongronema latifolium is a tropical rainforest plant primarily used as a spice and vegetable in traditional folk medicine. It contains essential oils, saponins and pregnanes, among others (Morebise *et al.*, 2002). The antimicrobial properties and potential utilization in sorghum larger beer brewing have been reported (Eleyinmi and Oloyo 2001; Eleyinmi *et al.*, 2004). Its amino acid profile, fatty acid contents and antibacterial activity (Eleyinmi, 2007) suggest that it may play a preservative role in food and non-food systems. *G. latifolium* leaves are presently a constituent of the Jobelyn and DM tea blend marketed in the USA for the normalization of blood, maintaining the integrity of lymphocytes and maintenance of healthy blood glucose levels (Ugochukwu *et al.*, 2003).

25.2.8 *Draceana mannii*

Several species of the West African 'soap tree', *Draceana*, are used in traditional medicine for treatment of constipation, dysentery, worms and cough. The presence of biologically active components and its anti-inflamatory properties suggest that the plant may have immunomodulatory properties (Okunji *et al.*, 1990; Tapondjou *et al.*, 2008) with potential food uses.

25.2.9 *Salvia officinalis*

Salvia officinalis has been used in herbal medicine for many centuries. *S. officinalis* extract may be useful in the management of mild to moderate Alzheimer's disease, with the potential for the reduction of patient agitation (Akhondzadeh *et al.*, 2003). The potential utilization of *S. officinalis* for the elimination of germs from minced raw beef to obtain stable and microbiologically safe meat without substantial loss in sensory quality was reported by Hayouni *et al.* (2008). Essential oil from the plant possesses antimicrobial activity, and exhibits fungastatic activity against *Botrytis cinerea* and *Phytophthora citrophthora* (responsible for fruit rot). In view of this, the plant may find use as a natural preservative ingredient in the food and/or pharmaceutical industries (Hayouni *et al.*, 2008; Camele *et al.*, 2010).

The reports outlined here are important in the quest to use plant essential oils, flavonoid, phenolic and other biologically active components as natural preservatives in food products.

25.3 CONCLUSION

In spite of growing efforts aimed at developing effective natural plant-based antimicrobials, their mechanism of action, toxicological and sensory effects have not been fully understood. Hence, the utilization of many tropical medicinal plants in food processing and preservation operations has been severely limited. Current findings and ongoing research interest into the use of medicinal plants suggest that there is considerable potential for the use of plant phytochemicals to improve food quality, nutritional value and overall well-being. Emerging trends in food preservation should therefore focus on a reduction (if not elimination) of chemical preservatives and a shift towards phytochemicals as sources of bioactive components for use in food systems. The availability and low cost of medicinal plants make them an attractive option and may contribute to sustainable food production and preservation. This will, however, require initiatives (at all tiers of government and research institutions) geared towards review of existing food safety programmes with a view to determining areas of possible utilization.

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26 Essential Oils and Other Plant Extracts as Food Preservatives

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Abstract: The safety and management of food during its processing, transport and storage are prerequisites for modern food processing. Despite the worldwide development of new ranges of preservation techniques, the microbiological safety of meat, fruits and vegetables, as well as dairy products, continues to be of major concern to both consumers and the food industry. Several outbreaks of foodborne pathogens during the last two decades have focused public attention on food safety. Environmental and human health concerns with regard to current practices involving synthetic compounds have further compelled the food industry to develop improved strategies. Natural products have been investigated as alternative biocides and antioxidants in an effort to rule out the use of synthetic preservatives, which are regarded as detrimental to health and the environment. Various applications of essential oils and plant extracts as natural antioxidants and antimicrobial agents have been reported and the use of such extracts have been validated by many researchers in several countries. This chapter describes the use of essential oils and other plant extracts as food preservatives, with emphasis on the fruit and vegetable post-harvest environment. The antifungal effects of these extracts on the control of fruit and vegetable pathogens are covered in detail. In addition, the application of plant extracts in the control of bacterial biofilms, which are more resistant than their planktonic counterparts to therapeutic intervention such as antibiotics, is also summarized. Finally, an overview regarding the importance of medicinal indigenous plants from developing countries as natural biocides and antioxidants is provided.

Keywords: antimicrobial agent; essential oil; food preservatives; food safety; natural products; post-harvest decay

26.1 BACKGROUND

One of the greatest challenges of the twenty-first century is the paradoxal relationship between food security and the world's vast population. The unknown threats posed by global warming, as well as the conversion of food into biofuels, driven by an insatiable appetite for energy, has exacerbated concerns over food security. In developing countries, food losses resulting from fungal decay may exceed 40% of the harvest (Pittet, 1989). The proliferation of moulds on crops during storage reduces their shelf life and market value and may even render the products unfit for consumption. Many fungi produce mycotoxins that pose serious threats to human and animal health, with some causing acute human mycotoxicoses. Cases of chronic exposure have also been documented to result in the development of cancer (Sherif

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et al., 2009). Food safety and security is therefore closely linked to the use of fungicides and preservatives to prevent decay and subsequent losses. Although many products for pest and disease control are applied before harvest to the foliage of the growing crop, consumers may be directly exposed to fungicides that are applied to fruit and vegetables for post-harvest protection. Exposure to agrochemicals has been associated with an enhanced susceptibility to diseases such as cancer, spermatotoxicity, hormonal imbalances (Pandey, 2003; Kumar *et al.*, 2007), physiological changes in the liver and adverse effects on the immune system (Ansar, 2000).

Food manufacturers rely heavily on food preservatives to safeguard and extend the shelf life of their products. The intake of food additives, including synthetic preservatives, has been linked to the rising incidence of allergies and attention-deficit hyperactivity disorder in children (Eigenmann and Haenggeli, 2004). Estimations made concerning the average intake of preservatives in the UK vary depending on the statistical procedures used, but were as high as 48.9 mg/person per day for benzoic acid and its salts, 75–85 mg/person per day for nitrate and between 2.4 and 4.2 mg/person per day for nitrite (Massey, 1997). Sulphurous compounds are extensively used in food preservation and were originally classified as generally regarded-as-safe (GRAS) chemicals (Hartman, 1983; Gould and Russel, 2003); however, their use as preservatives has since been reviewed. At the same time, concerns regarding the use of sulphiting agents for the preservation of fresh fruits and vegetables are growing globally (Sivakumar *et al.*, 2010). According to a directive issued by the US Food and Drug Administration, the use of sulphite in/on foods must be declared. Sulphite is thought to be implicated in the downregulation of some biochemical pathways involved with immune-system activation in the cell (Winkler *et al.*, 2006). Moreover, bisulphate is known to trigger allergic reactions in susceptible individuals (Sonneville, 1996). Sulphur dioxide fumigation, commonly adopted to retain the post-harvest qualities of fruits such as litchi and grapes, has also become increasingly unpopular. The treatment results in undesirable residues, alters the taste of fruit (Lonsdale and Kremer-Köhne, 1991) and poses health risks to consumers and pack-house workers in particular (Koeing *et al.*, 1983). For these reasons, consumer demands for natural and safe 'green' preservatives to prevent microbial development and reduce negative effects on health and the environment have increased (Burt, 2004; Tripathi and Dubey, 2004). Adding to the pressure on the food processing industry, the consumer drive coincides with a rise in the recorded number of foodborne diseases related to the consumption of microbiologically contaminated food.

Of particular concern is the occurrence of *Listeria monocytogenes*, which persists in chilled food, thereby increasing the risk of immunocompromised individuals to contract listeriosis (Kleter *et al.*, 2009). *Listeria* and many other bacterial pathogens have the ability to form biofilms that have been proven to be more resistant to antiseptic agents than their planktonic forms (Sandasi *et al.*, 2008). *Salmonella* also remains a primary cause of food poisoning worldwide, and numerous massive outbreaks have been recorded (Mead *et al.*, 1999). The landscape of food safety management in Europe has undergone recent changes, focusing on the early detection of microbiological hazards in food products (Marvin *et al.*, 2009). These measures have served to strengthen public confidence in food safety. In the USA, the Sanitary and Phyto-sanitary Standard regulates food imports in an effort to reduce and eradicate foodborne pathogens and adulteration (Jongwanich, 2009). The Food Quality Protection Act adopted by the USA in 1996 (Dayan *et al.*, 2009) has imposed stringent registration requirements for synthetic pesticides and restricted the use of some in agriculture. This regulation has financial and technical implications for developing countries exporting food products to the USA.

Environmental, health and ethical concerns have culminated in repeated warnings by researchers and consumers that fungicides should be replaced with safer and biodegradable alternatives (Wisniewski *et al.*, 2001). Estimates indicate that annually more than 123 million kg of synthetic fungicides are used to protect freshly harvested fruit and vegetables against about 100 species of fungi that cause post-harvest decay (Eckert and Ratyanake, 1994; Tripathi and Dubey, 2004). Some pesticide molecules that form part of the production cycle are highly stable and therefore persist in the product, ultimately affecting its sales potential (Taube *et al.*, 2002). Fungicides including imazalil, thiabendazole, pyrimethanil (Smilanick *et al.*, 2008), prochloraz (Danderson, 1986) and guazatine (Food and Agriculture Organization, 1998) are applied to fruit crops by dipping the freshly harvested fruit in the fungicide solution. These dip solutions, with volumes often exceeding 500 L, are replaced every few days (Altieri *et al.*, 2005). However, the high costs associated with waste disposal may encourage the irresponsible disposal of this hazardous waste, consequently giving rise to serious environmental problems.

The fate of crop protection products, once incorporated into the soil, is not fully understood (Taube *et al.*, 2002). Fungicides are retained by agricultural soils in the form of non-extractable bound residues, due to interactions with inorganic salts, but may also be retained by other co-sorption processes (Möller *et al.*, 1999). These residues compromise soil microflora that are involved in the degradation chain (Katayama and Kuwatsuka, 1991). The ability of pesticides to migrate through soil into groundwater has been established (Taube *et al.*, 2002), resulting in the compounds gradually seeping into rivers and agricultural lands. Ultimately biomagnification of pesticides in the food chain may take place through bioaccumulation of these xenobiotic substances in organisms. Humans, who are at the pinnacle of the food chain, are particularly prone to such health risks. Pesticides and their metabolic analogues are able to persist in fatty tissues and organs, such as the liver and kidneys (Dórea, 2008). The use of natural antimicrobial agents of low toxicity that are able to equal or improve pathogen control, yet reduce or eliminate disposal problems, would therefore be extremely valuable. Natural products, in contrast to most synthetic antimicrobial agents, break down rapidly in the environment, because they lack persistent, unnatural ring structures and seldom contain halogen atoms. Instead, they tend to be rich in oxygen and nitrogen functionalities and often contain sulphate or phosphate groups that are useful attributes in the quest for novel compounds based on alternative molecular framework structures (Dayan *et al.*, 2009).

The Product Ecological Footprint (PEF) has received considerable attention as a useful gauge within the framework of sustainable development (Van Vuuren and Bouwman, 2005). This indicator is expected to allow consumers to make an informed choice regarding the ecological impact of their diet (Limnios *et al.*, 2009). The productive land required for delivering a specific product is estimated when determining the ecological footprint thereof, while the amounts of wastes and emissions resulting from the production are other major factors contributing to the PEF (Limnios *et al.*, 2009). These considerations have given further impetus to the development of effective, natural antimicrobial agents for use in the supply chains of fresh crops, to reduce wastage caused by decay, while minimizing the environmental impact by eliminating synthetic fungicides.

Apart from environmental concerns, the increasing numbers of fungal and bacterial strains developing resistance to commonly applied antimicrobial agents worldwide (Wild, 1983; El-Goorani *et al.*, 1984), thereby affecting the efficacy and lifespan of protective products (Eckert *et al.*, 1994), is alarming. Research efforts are focused on identifying novel antimicrobial agents from alternative chemical classes that function with different modes

of action, conveying renewed effectivity in combating pathogens that have developed resistance to current chemicals (Wilson *et al.*, 1997, 2001; Wedge *et al.*, 2007).

The challenges associated with fresh food production are daunting. Consumers insist on foods that are safe, have an extended shelf life, are of high quality (Brul and Coote, 1999) and impose a low PEF. Simultaneously, pathogen control is increasingly restricted by legislation and limits imposed on pesticide residues on crops (Kanetis *et al.*, 2007). The use of plant extracts and essential oils as mycobiocides provides potential solutions to the manufacturer's dilemma (Lazar and Jobling, 2009). However, several factors should be taken into consideration when selecting candidates as possible antimicrobial agents. These include the mode of delivery, stability, efficacy against expected undesirable pathogens, safety and cost effectiveness of the agent. In addition, the sensory compatability (Gutierrez *et al.*, 2008) and chemical effects on treated food products must be considered (Wanger and Moberg, 1989; Holley and Patel, 2005). It should be kept in mind that the stereochemistry and hydrophobicity of antimicrobial components affect their antimicrobial action (Veluri *et al.*, 2004). The starting point of any study directed towards the development of a commercial product for post-harvest fungal control remains the *in vitro* determination of activity, allowing both the extract and the required concentration to be selected for the inhibition of a specific pathogen or range of pathogens (Regnier *et al.*, 2008). The success of *in vitro* and subsequent *in vivo* assays to evaluate the antimicrobial action of plant-derived substances depends on factors including the plant strain or chemotype selected, the method of extraction, the density and growth phase of the inoculums tested and the culture medium used. Moreover, intrinsic properties of the food including pH, fat, proteins and water content, and the presence of antioxidants, determine the efficacy of the antagonist evaluated. The incubation time and temperature, packaging method and physical structure of the food are additional factors that may yield inconsistencies in results (Tajkarimi *et al.*, 2010).

26.2 SECONDARY METABOLITES OF PLANTS

Secondary metabolites of plants are volatile and non-volatile chemical compounds produced for a variety of functions other than growth and reproduction (Hartmann, 2007). Functions include the attraction of insects for pollination and protection of foliage against sunburn, insect predators and microorganisms (Bosabilidis, 2002). More than 200 000 secondary metabolites have been identified from the plant kingdom and are recognized as crucial in the survival of plants in an often exigent environment. The diverse biological properties of these compounds have been exploited by humans for centuries, and plants and their extracts have been extensively applied as pharmaceutical agents and as flavourings and preservatives of foods and beverages (Oussalah *et al.*, 2006; Bakkali *et al.*, 2008).

26.2.1 Essential oils

Volatile metabolites are usually isolated from plant material through steam- or hydrodistillation methods; the fragrant mix of compounds obtained is referred to as an essential oil (EO). Approximately 3000 EOs, of which 300 are commercially available, are currently documented (Burt, 2004). Components present in EOs mainly constitute volatile terpenes and hydrocarbons (Dewick, 2002; Bakkali *et al.*, 2008). Although terpenes have extraordinarily diverse structures, they are all derived from the head-to-tail bonding of five-carbon isoprene units. The number of isoprene units contained by the specific terpenoid is used to classify the

compounds as hemiterpenes (C_5), monoterpenes (C_{10}; which are composed of two isoprene units), sesquiterpenes (C_{15}), diterpenes (C_{20}), sesterterpenes (C_{25}), triterpenes (C_{30}) and tetraterpenes (C_{40}; consisting of eight isoprene units) (McMurry, 1996; Dewick, 2002).

Although the chemical compositions of EOs vary significantly between plant species, they may also be highly variable within the same species (Viljoen *et al.*, 2005). These variations in EO profiles result from a variety of factors that influence the plants from which the oils are sourced, including genetic differences, geographical occurrence, soil nutrient status, the plant part utilized, the time of year the plant is harvested and the specific growth stage of the plant (Hussain *et al.*, 2008). The chemical compositions of EOs to be used as antimicrobial agents in/on food products must therefore be ascertained by gas chromatography prior to use. Changes in the relative concentrations of oil constituents may impact severely on the efficacy of the oil against fungal and bacterial pathogens. It should be borne in mind that the action of an EO is seldom due to the presence of only one constituent, although reports have indicated that some terpenoids are more effective antimicrobial agents than others (Burt, 2004). In many cases a synergistic action between oil compounds may yield an EO that is more effective in combating microorganisms than the additive outcomes of individual components tested at the same concentrations (Mourey and Canillac, 2002; Bakkali *et al.*, 2008). For applications in the food industry or in pack-house environments, an EO with a consistent chemical composition, which would infer a consistent antimicrobial activity, is required. Although less economical, this could be achieved by using a single pure terpene or mixtures of pure terpene constituents. Alternatively, a cheaper option for microbial control in a commercial setting is to select an EO of which the main component is largely responsible for the antimicrobial action, since EOs where synergy is the overriding factor would be extremely difficult to control.

The efficacies of EOs depend on the method used to expose fungi or bacteria to the antimicrobial compounds. For example, the effects of vapour application can differ substantially from those of direct exposure (Goňi *et al.*, 2009). In general, a higher concentration (two to 100 times more) of the EO is required to achieve an antibacterial effect in food than indicated in preceding *in vitro* trials (Burt, 2004). The antimicrobial efficacy of commercially available food preservatives containing EOs is often modulated by the prevailing pH, chemical composition (Aureli *et al.*, 1992) and physical structure of the food, the storage temperature and the oxygen concentrations in the packaging (Skandamis *et al.*, 2000). Although the volatility of EOs makes them particularly attractive for use as fumigants against storage pathogens (Tripathi and Dubey, 2004), the *in vitro* antifungal activities of EO vapours are not always reflected *in vivo* (Plaza *et al.*, 2004; Tripathi and Dubey, 2004). The low vapour pressure of the active ingredients at reduced storage temperatures could account for this observation. The use of sub-atmospheric pressures to enhance volatilization of EOs has proved more effective, increasing fungal control by 10-fold (Arras *et al.*, 1999; Plaza *et al.*, 2004). EOs containing alcohol, ketone, ester and hydrocarbon functional groups displayed higher activity in the vapour phase, while those containing aldehyde functions performed better in microbial inhibition assays where diffusion of the compounds was required (Inouye *et al.*, 2003).

26.2.2 Non-volatile secondary metabolites

Non-volatile secondary metabolites are numerous. Van Wyk *et al.* (1997) listed some of these and included amino acids, lectins, glycoproteins, flavonoids, tannins, quinones, coumarins, terpenoids, steroids and alkaloids as some of the most important bioactive compounds. In some cases, compounds are attached to one or more sugar moieties, and are then known as

glycosides. These compounds are usually extracted from the plant by solvent extraction, depending on the nature of the targeted compound (Romanik *et al.*, 2007). In some cases, water may be a suitable solvent, but often organic solvents may be required. Unfortunately, the use of organic solvents has been associated with health risks (Rosenberg *et al.*, 2004; Bushnell *et al.*, 2007).

Many plant secondary metabolites have shown excellent activity *in vitro* against food pathogens. However, in most cases these results were not followed up by extensive *in vivo* trials, thereby wasting funds and opportunities (Devlieghere *et al.*, 2004). The variability of secondary metabolite concentrations from plant to plant, tissue to tissue and even cell to cell makes it necessary to assay as wide a concentration range as possible and to construct dose–response curves, ideally covering 10–90% of the effect (Hadacek, 2002). The discovery, evaluation and development of effective mycobiocides depend on the application of reproducible miniaturized bioassay systems. A practical method to screen complex plant extracts and identify potential antimicrobial agents is to separate compounds (extracted with solvents with a range of polarities) using thin-layer chromatography. Spores of the pathogen under evaluation are suspended in a growth medium and are sprayed over the surface of the plate, which is subsequently incubated to allow microbial growth. A chemical reagent is then used to visualize zones of inhibition, allowing identification of the compounds responsible for inhibition. A case study, detailing the procedure followed to identify antagonists of strawberry anthracnose, was conducted by Wedge *et al.* (2007).

26.3 MODES OF ACTION OF ESSENTIAL OILS AND PLANT EXTRACTS

Little is known with regard to the mechanisms involved in the antifungal activities of plant-derived extracts and compounds. It has been suggested that some compounds, for example allyl isothiocyanate present in mustard, exert their influence along more than one microbial metabolic pathway (Tajkarimi *et al.*, 2010). The properties of the microorganism exposed to the plant extract determine its susceptibility to a particular compound and the mode of action displayed by the antimicrobial agent. In most cases, Gram-negative bacteria are less susceptible to antimicrobial agents, possibly because the diffusion of hydrophobic compounds is restricted by the outer lipopolysaccharide membrane that is characteristic of these organisms.

Many studies have reported that the action of EO components can be attributed to their ability to disrupt the outer membranes of bacteria, due to their accumulation in the lipid-rich cell-membrane structures (Burt, 2004; Goňi *et al.*, 2009). These membranes are disintegrated as a result of damage caused by the EO to the phospholipid bilayer of the cell membrane, thereby disrupting enzyme systems and eventually causing alteration of genomic templates. EOs oxidize unsaturated fatty acids, leading to the formation of fatty acid hydroperoxidases (Goňi *et al.*, 2009; Tajkarimi *et al.*, 2010). To have an effect on bacteria, EOs must be sufficiently polar to dissolve to some extent in the cell contents, but sufficiently non-polar to interact with non-polar cellular structures (Cox *et al.*, 2001). These researchers proposed that the polarity of the EO compounds must be within an optimum range to reach lethal levels in the microbial cellular structures.

The variation in efficacy observed for direct contact and vapour applications of EOs can be explained by the differences in the polarities and volatilities of the individual EO components (Cox *et al.*, 2001). Less volatile hydrophilic (polar) compounds tend to diffuse more easily into aqueous media and therefore display higher activities in direct contact (disc diffusion,

toxic medium) assays than the head-space components. EO constituents reach a state of equilibrium whereby the highly volatile hydrophobic compounds are more prevalent in the head space than in the microbial growth matrix. These observations indicate that *in vitro* experiments should be carefully designed to closely mimic the method of EO application on the foodstuff or crop under investigation.

Carvacrol, one of the major components of oregano and thyme EOs, was investigated to determine its effect on the vegetative cells of *Bacillus cereus*, an important foodborne pathogen (Ultee *et al.*, 1999). Following exposure of the organism to 0.25 and 1 mM carvacrol, the cytoplasmic membrane of the bacterium was observed to become more permeable towards protons and potassium ions. Follow-up studies by the same authors (Ultee *et al.*, 2002) led to the hypothesis that carvacrol destabilizes the cytoplasmic membrane and acts as a proton exchanger, resulting in a reduction of the pH gradient across the cytoplasmic membrane. It was speculated that the collapse of the protonmotive force and depletion of the ATP pool ultimately causes cell death. It appears that the presence of a hydroxyl functionality and a phenolic ring with delocalized electrons is essential for the antimicrobial activities of carvacrol and related compounds such as thymol, cymene, menthol and carvacrol methyl ester.

In their study on the inhibition of white and brown rots by oxygenated aromatic EO compounds, Voda *et al.* (2003) reported that the action of secondary metabolites towards fungi is associated with the inhibition of thiol-containing active sites of fungal enzymes.

Bacterial biofilms, consisting of an ordered community of pathogens that form a protective polysaccharide outer layer, are generally more resistant to EO and other antagonists than the planktonic form of the bacterium (Sandasi *et al.*, 2008). Organisms that form biofilms are therefore more difficult to control in the food chain. Results obtained using EOs against the sessile form cannot be directly compared to those obtained against the biofilm (Leonard *et al.*, 2010). In many cases, the activities of pure EO components differ from those observed when the compounds are combined (Mourey and Canillac, 2002). Sandasi *et al.* (2008) reported that treatment of listerial biofilms with some pure EO components actually promoted the growth of the biofilm and concluded that the antibiofilm activity of EOs is often due to the synergistic effect of various oil components. Very little is currently known about the interaction of antagonists such as EO components, with biofilms of various microorganisms, but it is clear that the interaction is of a complex nature (Lis-Balchin and Deans, 1997). Since biofilm-forming bacteria are abundant in the food chain, these organisms pose a danger to human health. In the past decade, many reports of infections associated with the presence of *L. monocytogenes* have surfaced throughout the world (Salamina *et al.*, 2000), with many cases occurring in Europe (European Food Safety Authority, 2008) and the USA (US Department of Agriculture Food Safety and Inspection Service, 2009).

26.4 SPECIFIC APPLICATIONS OF PLANT EXTRACTS IN THE FOOD INDUSTRY

Various books are available that supply extensive information on post-harvest management and include handling, packaging and storage preservation methods for specific crops. This section is therefore devoted to providing an overview of the current trends and directions of exploratory work being done by researchers and outlines the use of EOs and other plant extracts to control microbial growth and proliferation in fruit, vegetables, fish, meat and dairy products.

26.4.1 Fruits

Consumers in developed countries demand access to high-quality, seasonal fruit throughout the year. Moreover, organic fruit has gained popularity globally. These requirements imply that fruit must be exported from Africa, Australia and other continents to Europe and America to satisfy the demands of consumers. However, the crop must be protected during these long periods of transport and this has traditionally been achieved through the application of post-harvest fungicides. A new drive to reduce synthetic fungicides and promote organic products has given impetus to research concerning the use of natural products as antifungal agents on fruit.

26.4.1.1 Citrus

Originating in the subtropical and tropical south-east regions of the world, the genus *Citrus* encompasses about 16 species (Fisher and Phillips, 2008). Oranges, grapefruit, lemons, lime and tangerines are the most commercially viable species and are important export commodities of several countries. Oranges comprise about half of the total global citrus production (http://citrusfruits.wordpress.com/2007/08/02/world-production/). In 2007–2008 the production of citrus was estimated at 71 million tonnes (www.fas.usda.gov/htp/2008_citrus.pdf) with Brazil (17.1 million tonnes), the USA (11.5 million tonnes) and China (17.6 million tonnes) being responsible for approximately two-thirds of the global production. However, Spain (3.2 million tonnes), South Africa (1.26 million tonnes) and the USA are the top exporters of citrus (www.fas.usda.gov/htp/2008_citrus.pdf).

Citrus is particularly vulnerable to post-harvest decay and the most prevalent pathogens associated with post-harvest losses include green and blue mould (*Penicillium* spp), grey mould caused by *Alternaria citri* Elli and Pierce, *Aspergillus* rot (*Aspergillus niger* Van Tiegh), *Phytophtora parasitica* Dast, *Geotrichum candidum* Link ex Pers., responsible for sour rot, and *Colletotrichum gloeosporioides* Penz. that causes anthracnose (Klieber *et al.*, 2002). The occurrence of these pathogens can in many cases be linked to cultivation and handling practices, the climate and the citrus cultivar (Eckert and Ogawa, 1985). Although post-harvest fungicide treatments and sanitizers have played a significant role in the development of the global trade in citrus fruits, application of many chemical fungicides such as imazalil, mancozeb and benomyl are now being restricted. The development of pathogen resistance against conventionally used fungicides is driving the search for new crop-protection products, particularly since an increasing number of citrus pathogens are emerging that are not only resistant to a single fungicide, but are double- or triple-resistant to the active fungicides on the market (Kanetis *et al.*, 2007). Application of combinations of commercial fungicides, rotation programmes and the interspersed use of natural products may serve to lengthen the useful lifespan of these products. After 20 years of research, several post-harvest biocontrol products have been developed (Droby *et al.*, 2009). Although the effectiveness of some of these products, for example *Pantoea agglomerans* CPA-2 against green and blue mould, have been demonstrated (Plaza *et al.*, 2004; Caňamås *et al.*, 2008), the effectiveness of microbial antagonists is still under scrutiny and their use has in some cases not provided acceptable commercial control.

The *in vitro* use of EOs has proved effective against many pathogens of citrus. One approach is based on the natural mechanisms of the fruit to fight infection. Citral, for example, was selected as an antagonist against citrus pathogens (Klieber *et al.*, 2002; Wuryatmo *et al.*, 2003), because declining citral levels in the flavedo layer of lemon peel were correlated with an increase in susceptibility to *Penicillium digitatum* (Ben-Yehoshua *et al.*, 1992). Klieber

et al. (2002) subsequently found that high concentrations (6000–15 000 μL/L) of citral (a *cis*/*trans*-isomeric mixture of geranial and neral) were able to inhibit the growth of *P. digitatum*, *Penicillium italicum* and *Geotrichum candidum* isolated from lemons. In addition, the citral present in head-space volatiles at concentrations above 16 000 μL/L were able to completely inhibit all three pathogens. More recently an *in vitro* trial conducted by Linde *et al.* (2010) indicated that citral-supplemented growth medium was able to inhibit *P. digitatum* at a concentration of 2000 μL/L, while citral-rich EOs of lemongrass (*Cymbopogon citratus*) and *Lippia rehmannii* were effective at 3000 μL/L. The advantage of using biocides prepared from natural sources such as lemon oil is that these are regarded as food additives, yet are able to control pathogens. Citral has been reported to kill cancer cells without affecting normal cells grown in culture and could be beneficial to consume (Ben-Yehoshua and Ofir, 2009).

Results obtained by Stange *et al.* (2002) contradicted this approach through findings that solvent extracts of orange peels actually stimulated the growth of *P. digitatum* and *P. italicum*, but had little effect on *Penicillium expansum*, a non-pathogen of citrus. They postulated that although many of the compounds present in citrus peel are effective antifungal agents towards a host of pathogenic fungi, *P. digitatum* and *P. italicum* have naturally adapted to this defense strategy and are therefore resistant towards these compounds. This was confirmed by Droby *et al.* (2008), reporting that volatile compounds released after wounding of citrus stimulated the germination and germ-tube elongation of *P. digitatum* and *P. italicum*. Limonene, one of the major components of orange oil, was found to strongly stimulate germ-tube elongation. A similar result was obtained by Du Plooy *et al.* (2009a), finding that limonene stimulated the mycelial growth of *P. digitatum in vitro*. Citrus pathogens such as *Penicillium* are opportunistic wound pathogens (Droby *et al.*, 2008) and for this reason *in vitro* studies are mostly aimed at applying antimicrobials that inhibit spore germination and subsequent germ-tube elongation (Saks and Barkai-Golan, 1995; Arrebola *et al.*, 2010a; Hao *et al.*, 2010). These trials are conducted in culture and the resulting spore suspension is viewed under an inverted microscope to determine the percentage germination in samples comprising of approximately 200 spores (Afek *et al.*, 1995).

Other EOs and their pure components have been evaluated *in vitro* against citrus pathogens and include *R*- and *S*-citronellic acid, *R*-and *S*-citronellal (Wuryatmo *et al.*, 2003), thyme, oregano, clove, cinnamon (Plaza *et al.*, 2004) and oils from *Citrus sinensis*, *Citrus aurantium*, *Citrus deliciosa*, *Citrus paradises* and *Citrus limon* (Fisher and Philips, 2008, referring to Caccioni *et al.*, 1998). Most of these oils proved effective in the vapour phase. However, application of EOs in the pack-house environment is often ineffective (Plaza *et al.*, 2004). Some EOs cause phytotoxic reactions when applied to the rind or incorporated into the packaging, thereby disqualifying these oils as fungicides even if they are able to control pathogenic growth (Arras *et al.*, 1993, 1997; Plaza *et al.*, 2004).

Plaza *et al.* (2004) stressed that the application of EOs in the citrus industry to control post-harvest decay can only be implementable if no additional processing or apparatus is required in the pack-house. Du Plooy *et al.* (2009a) overcame this problem in a commercial trial by supplementing the coating of Tomango with spearmint and *Lippia scaberrima* EOs at a concentration of 2500 μL/L. The fungal dip solution was eliminated, thereby actually reducing the number of processing steps. Results obtained by these researchers with respect to pathogen control and organoleptic parameters such as colour, pH and total soluble sugar were comparable to the conventional treatment using synthetic fungicide. Unexpectedly, superior water retention (juiciness) was measured in fruit treated with EO amended coatings. The increased water retention was proposed to be the result of the affinity of the natural rind terpenoids to those added by supplementation of the coating with EO, leading to improved

film formation. The advantage of using coatings amended with EOs, rather than vapour, is that the close contact between the EO components and the fruit surface allows exposure of each fruit to the same concentration of inhibitor over an extended period (Du Plooy *et al.*, 2009a).

Jojoba oil, although not an EO, has been used in its isomerized form known as *trans*-jojoba oil as a coating to replace synthetic coatings (Ahmed *et al.*, 2007). The *trans* form has a soft waxy texture and displays excellent resistance to oxidation. Application of 20–30% *trans*-jojoba oil yielded the same degree of pathogen control as the commercial treatment and maintained fruit quality during storage.

Fewer reports describing the use of plant fractions other than EOs are available. Table 26.1 is a summary of some of the work done in this regard. Research has also been directed towards eliminating conventional fungicides by heat treatments and other means. Although the mode of action is not yet known, storing fruit at high temperatures (32–36°C) and high relative humidity (94–98%) for 2–3 days enhances the host-defence mechanism of the fruit. This curing action stimulates the release of antifungal compounds (Plaza *et al.*, 2003), favouring wound healing and curbing the development of pathogens. However, cured fruit have a shorter shelf life due to the absence of remedial products that can protect against later pathogen exposure. In addition, curing can cause loss of moisture and firmness and can lead to a reduction in the colour index and citric acid content of the fruit. The use of sodium carbonate and sodium bicarbonate salts (Smilanick *et al.*, 1999) as well as curing and hot-water treatments to reduce pathogen pressure on the fruit (Porat *et al.*, 2000; Tripathi and Dubey, 2004) in combination with treatments incorporating natural products may be an option for citrus.

26.4.1.2 Mango

Cultivated in many tropical and subtropical regions, mango (*Mangifera indica* L.) fruit are a major asset to countries such as India, Pakistan, Brazil and Mexico, from where the fruit is distributed worldwide (Sauco, 2004). Globally, mango is the most frequently eaten fruit. In Indian culinary practices, unripe mango is used as *hat char*, and although India is a major producer of the fruit almost everything is consumed by the domestic market. Once ripe, mango is extensively used in food and for juice, as well as for the preparation of flavourants, fragrances and colouring agents (see www.mango.co.za). Many new cultivars have been developed to satisfy the diverse flavour and aroma requirements of global populations and to produce fruit that are less resistant to transport damage and have a longer shelf life. However, the preservation of fruit that produce a variety of volatiles is one of the most difficult physiological problems facing the industry (Du Plooy *et al.*, 2009b). A build-up of these volatiles during storage increases the occurrence of mango lenticel discoloration. Although regarded as a cosmetic condition, this discoloration is a commercially unacceptable blemish (Bally *et al.*, 1996; Du Plooy *et al.*, 2009b).

Although several biocontrol agents have been developed to control post-harvest diseases of mango (Govender and Korsten, 2006; Kefialewa and Ayalew, 2008), only a few reports on the use of plant-derived compounds are available (Regnier *et al.*, 2008; Yienjit *et al.*, 2010). Fungal pathogens of mango such as *C. gloeosporioides* and *Lasiodiplodia theobromae* are of major concern to the industry (Swart *et al.*, 2002). Currently, pack houses use combinations of hot-water treatments and synthetic fungicide dips (Johnson *et al.*, 1995) or modified-atmosphere packaging (MAP) (Kim *et al.*, 2007) to prevent decay and extend shelf life. Recently, Linde *et al.* (2010) conducted *in vitro* studies to determine the antifungal activities of EOs from *L. rehmannii* and *C. citratus* (lemongrass) against anthracnose and stem-end rot

Table 26.1 Overview of the major citrus pathogens and their control by plant extracts and EOs.

Pathogen	Plant extract	Active compound	Comments	References
Penicillium digitatum, *Penicillium italicum* and *Geotrichum candidum*	Tea	Saponin	In combination with synthetic fungicides with a saponin/fungicide ratio of 8:2 *in vitro* and *in vivo*	Hao *et al.* (2010)
P. digitatum	*Acacia seyal* (del var. *Seyal*) extract combined with wax	Phenolic acids (gallic acid)	Methanol/acetone/water (7:7:1) extract Concentration not specified *In vivo* wound inoculation	Mekbib *et al.* (2007)
P. digitatum	*Withania somnifera* (L.) Extract combined with wax	Phenolic acids (caffeic acid, salicylic acid, 3,4-dihydroxy benzoic acid)	Methanol/acetone/water (7:7:1) extract Concentration not specified *In vivo* wound inoculation	Mekbib *et al.* (2007)
P. italicum	*Acacia nilotica*	Sitosterol, α-amyrin, naringenin-5-methyl ether, kaempferol, kaempferol-3-O-rhamnoside, myrcetine-3-O-rhamnoside	Aqueous extract applied Concentrations of 20 mg/L phenolics required for growth inhibition Shelf life of oranges extended for 6 days	
P. digitatum	*Lippia javanica* *Lippia rehmannii*	Phenyl ethanoids (verbascoside and isoverbascoside)	Verbascoside 600 µg/L *in vitro* and *in vivo* inoculated fruit Isoverbascoside 1000 µg/L *in vitro* *Lippia rehmannii* and *Lippia javanica* 600–1000 µg/L *Lippia javanica* >600 µg/L *in vivo*	Shikanga *et al.* (2009)
Guignardia citricarpa, *P. digitatum* and *P. italicum*	Garlic (*Allium sepa*)		Water and ethanol extracts of garlic cloves were applied to inoculated Valencia and Shamouti cultivars at 0.1–1% (v/v) in cooking oil, in fruit coating or alone. Treatments were sprayed 6 h after inoculation and compared with commercial fungicides.	Obagwu and Korsten (2003)

(*Continued*)

Table 26.1 (*Continued*)

Pathogen	Plant extract	Active compound	Comments	References
Alternaria citri (attacks the navel end)	EOs from *Artemisia afra*, *Lavandula angustifolia*, *Eriocephalus punctulatus* and *Mentha piperita*	Terpenoids	Effective *in vitro*	Poswal (1996)
P. digitatum and *P. italicum*	Citrus paradise (Star Ruby grapefruit flavedo)	7-Geranoxy coumarin	Evaluated *in vitro* and *in vivo*	Agnioni *et al.* (1998); Tripathi and Dubey (2004)
P. digitatum	*Coprosma repens*	Phenolic compounds	Water or 20% aqueous ethanol extract effective	Obagwu (2003)
P. digitatum, *Penicillium expansum* and *Alternaria alternata*	*Aloe vera* gel		10^5 µL/L *in vitro*. *P. digitatum*-inoculated grapefruit displayed significant disease reduction.	Saks and Barkai-Golan (1995)
Botrytis cinerea, *Monilinia laxa* and *P. digitatum*	Oil of laurel (*Laurus nobilis*) extracted by supercritical CO_2	Contains 1,8-cineole, linalool, terpineol acetate and methyl eugenol	*M. laxa* inhibited by 200 mg/mL and *B. cinerea* by 1000 mg/mL *in vitro* Curative and preventative treatments on peaches, kiwi fruit, oranges and lemons artificially inoculated with all three pathogens Applied in the form of spray on the fruit skin at 1, 2 and 3 mg/mL Effective disease control in the absence of phytotoxic reactions or off-flavours	De Corato *et al.* (2010)
P. digitatum and *P. italicum* on the Washington Navel orange	Shiraz thyme (*Zataria multiflora* Boiss.) EO	Main components: carvacrol (63.17%), thymol (15.1%) and *p*-cymene (7.87%)	0, 200 and 400 µL in the forms of spray and dipping for 10 and 20 min (*in vivo*) Fruit inoculated and treatments curative and preventative Not all methods of treatment and concentrations were effective for inhibiting green and blue moulds.	Solaimani *et al.* (2009)

pathogens. Lemongrass oil and its main component, citral, were found to totally inhibit fungal growth at 3000 μL/L in the growth medium. *Areca catechu* Linn (betel nut), a palm tree from the Arecaceae family, widely distributed in South-East Asia, has been reported to have several therapeutic properties such as antimycobacterial and even anti-HIV activity. Yenjit *et al.* (2010) prepared hexane, ethyl acetate and methanol extracts from the pericarp of the fruit, and reported that three triperpenes and lauric acid, present in the extracts, were able to inhibit the mycelial growth of *C. gloeosporioides*. Some of these compounds inhibited spore germination and germ-tube elongation of the pathogen. Regnier *et al.* (2008) exposed *C. gloeosporioides* and *L. theobromae* isolated from mango to vapours and toxic medium supplemented with *L. scaberrima* EO, limonene, 1,8-cineole and *R*- and *S*-carvone. With the exception of limonene, all test substances inhibited fungal growth at 2400 μL/L in toxic medium. In the vapour phase, the two enantiomers of carvone and *L. scaberrima* oil displayed the highest fungistatic activity. Preliminary *in vivo* trials using fruit inoculated with *C. gloeosporioides* and *L. theobromae* indicated that EOs incorporated into coatings have the potential to control post-harvest decay of mango.

26.4.1.3 Pineapple

Avocado, papaya and pineapple (*Ananas cosmosus* L., Merill) are the most important tropical fruits in the world, representing 47, 12 and 7% of the global production of tropical fruit respectively (Ploetz, 2001). In addition to having a pleasant aroma and flavour, pineapples are rich in vitamins and minerals. The fruit is used mainly for canning, but also for juicing, while semi-processed fruits are becoming increasingly popular (Marrero and Kader, 2006). However, pineapples are susceptible to a range of conditions, such as dark heart, yeasty rot and brown spot, that cause the quality of the fruit to deteriorate. The shelf life of the fruit is limited by mesophyllic yeast development and juice leakage (Montero-Calderón, *et al.*, 2008), while fresh fruit production is further restricted by a number of post-harvest fungal diseases. The most severe of these pathogens include *Thielaviopsis paradoxa* (De Seyn.) Holm, teleomorph: *Ceratocystis paradoxa* (Dade) C. Moreau (Reyes *et al.*, 2004; Wijeratnam *et al.*, 2005) and *Fusarium* species (Jacobs *et al.*, 2010). Decay pathogens are suppressed by cold storage below 8°C, but once the fruit temperature reaches 12–15°C the pathogens proliferate rapidly. Research has focused largely on the development of microbial antagonists as biocontrol agents against pineapple post-harvest diseases (Reyes *et al.*, 2004; Wijesinghe *et al.*, 2010). Despite the economic importance of the crop there is a disappointing lack of data with regard to the use of plant extracts for fungal control. This area of research provides extensive opportunities for the development of tailor-made mycobiocides.

26.4.1.4 Avocado

The versatility of avocado (*Persia americana* Mill.) fruit has contributed to the expansion of the market, which is projected to reach 4.7 million tonnes by 2012 (see www.market-researchanalyst.com/2008/01/14/avocado-market-forecast-2008-2012-production-exports-imports/). Currently, Mexico is the world leader, producing almost 30% of avocado fruit worldwide. However, Chile competes with Mexico as an exporter and both enjoy an equal share (30%) of the global market. Most fruit losses are attributed to fungal infections by *C. gloeosporioides* (Sanders and Korsten, 2003), *L. theobromae* (Pat.) Griffon and Maubl. (Maftoonazad *et al.*, 2007) and *Alternaria alternata* (Darvas *et al.*, 1990). Rapid ripening of the fruit takes place due to significant increases in ethylene levels, produced at the onset of

ripening. Shelf life is increased by using MAP to limit oxygen while increasing the levels of carbon dioxide in the atmosphere surrounding the fruit (Yahia and Gonzalez-Aguilar, 1998).

Some *in vitro* studies aimed at fungal control have been conducted using EOs. Linde *et al.* (2010) investigated the *in vitro* antifungal effects of *L. rehmannii* and lemongrass (*C. citratus*) EOs and pure citral against *C. gloeosporioides*, *L. theobromae* and *A. alternata*, isolated from avocado. The mycelial growth of all three pathogens was inhibited using 3000 µL/L of the EOs and 2000 µL/L of citral. In another investigation, the EO of *L. scaberrima*, the *R*- and *S*- enantiomers of carvone, as well as limonene and 1,8-cineole, were found to inhibit fungal growth of all three pathogens at 2500 µL/L, with the exception of limonene (Regnier *et al.*, 2010). *Lippia scaberrima* EO incorporated into coating proved effective in controlling fungal decay in avocado fruit inoculated with virulent strains of *C. gloeosporioides* and *L. theobromae*. Thereafter, a simulated export trial using Carnauba-based coating supplemented with *L. scaberrima* or *Mentha spicata* (spearmint) EOs indicated that fruit quality was maintained equally well when EOs were applied, compared to the conventional synthetic fungicide treatments. Water retention in the fruit was enhanced by the EO treatments.

26.4.1.5 Papaya

The fruit of the papaya (*Carica papaya*) is an important commodity, mainly produced by Brazil and India (http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor). It is widely distributed in the tropics and commercially cultivated in Mexico, Ecuador, Peru, Indonesia, Nigeria, Congo, Ethiopia, Côte d'Ivoire, Ghana, Philippines and China. Unfortunately, the fruit is extremely sensitive to handling, transport and storage; these may result in poor overall acceptability and decay. Diseases are prevalent and may be responsible for losses representing as much as 40% of the crop (Mendoza *et al.*, 2008). Among the many pathogens that flourish on papaya, *Rhizopus stolonifer*, *C. gloeosporioides*, *A. alternata*, *Fusarium oxysporum* and *Botryodiplodia theobromae* are the most common (Alvarez and Nishijima 1987; Cia *et al.*, 2007).

Efforts have been made, and practices are continuously being improved, to manage post-harvest decay. Producers rely mainly on chemical fungicides and heat treatments for the control of post-harvest pathogens. However, these treatments in many cases result in poor post-harvest quality (González-Aguilar *et al.*, 2003). Active packaging systems have been found to reduce the growth of mould, yeast and mesophilic aerobic microorganisms and contribute to extending the shelf life of the fruit. Some natural preservatives, such as carnauba, shellac, chitosan and beeswax, have been evaluated for their abilities to control post-harvest decay, but these applications seem to have lost momentum. Only one recent study by Ali *et al.* (2011) was available, reporting on the efficacy of chitosan to maintain and extend the shelf life of Eksotika II papaya fruit. Although several studies have detailed the success of microorganisms such as *Candida oleophila* (Gamagae *et al.*, 2003) in reducing anthracnose decay of papaya fruit during storage and transport, these treatments have not been embraced by the industry. To date, the incorporation of EOs in coatings and active packaging of papaya fruit has not been taken seriously. However, promising results were reported by Bosquez-Molina *et al.* (2010) on the use of EOs against *C. gloeosporioides* and *R. stolonifer*. *In vivo* trials indicated that the application of mesquite gum supplemented with thyme (0.1%) and Mexican lime (0.5%) oils on papaya effectively controlled the two pathogens without reducing fruit quality. However, more studies are required to validate these findings.

26.4.1.6 Banana

Many people in tropical countries rely on the production of banana fruit (*Musa* spp.). This commodity not only has economic value, but provides a highly nutritious food source to marginalized communities. Several fungal disease complexes are responsible for the loss of the fruit during storage and transport to local or distant markets (Basel *et al.*, 2002; Ranasinghe *et al.*, 2003). *Lasiodiplodia theobromae*, *Colletotrichum musae*, *T. paradoxa*, *Curvularia* spp. and *Fusarium verticillioides* are the most prevalent pathogens on banana and cause crown rot. These fungi therefore pose a significant threat to the industry. In most cases, crown rot-causing pathogens are controlled by the application of thiabendazole. However, the emergence of pathogen resistance to this fungicide has been reported (De Lapeyre de Bellaire and Chilin-Charles, 2008). The use of MAP to control ethylene emissions from the crop has been investigated to control fungal decay (Marchal, 1998).

Although edible coatings consisting of natural antimicrobial compounds such as propolis, alginate/calcium gels (Krauss and Johanson, 2000), chitosan (1%), arabic gum (5–20%) and mixtures of the two substances have been evaluated (Maqbool *et al.*, 2010), there are very few reports regarding the application of plant metabolites. An odour-free precursor of the active substance derived from garlic (*Allium sativum*) was found to inhibit crown rot on bananas. However, the garlic extract was ineffective under high pathogen pressure. The use of most EO components can probably be discounted because of the possibility of absorption of these volatiles by the skin, leading to off-flavours.

26.4.1.7 Stone fruit

The genus *Prunus* encompasses a variety of species, collectively known as stone fruit. Almonds, apricots, cherries, nectarines, peaches, plums and prunes are the most important commercial stone fruit commodities. Iran is the largest producer of stone fruits in the world (http://faostat.fao.org/site/339/default.aspx). Estimates provided by the US Department of Agriculture for the production of cherry and peach/nectarine in 2009/2010 (www.fas.usda.gov/psdonline/circulars/StoneFruit.pdf) indicated that the global production of cherries was set to reach 2.3 million tonnes, with approximately half produced by the USA and Turkey. Peach and nectarine represent the largest trades among stone fruit, with 16.3 million tonnes produced in 2010. A compendium of stone fruit diseases was published in 1995 with the aim of helping field researchers, fieldsmen and women, and growers (Ogawa *et al.*, 1995). Recently, an extensive database containing virtually every stone fruit disease was compiled by Cornell University (www.nysaes.cornell.edu/pp/extension/tfabp/stone.shtml). Table 26.2 summarizes scientific reports dealing with the use of plant-derived extracts and compounds on stone and other fruit.

26.4.1.8 Lychee, rambutan and longan

The family Sapindaceae encompasses important tropical and subtropical fruits such as lychee (*Litchi chinensis* Sonn.), rambutan (*Nephelium lappaceum*) and longan (*Dimocarpus longan* Sour.) that are highly valued as exotic fruits. More than 95% of the world production of these fruits occurs in Asia (China, India, Malysia, Thailand, Taiwan and Vietnam). However, Africa (South Africa, Madagascar, Mauritius and Reunion) also produce and export the commodity to Europe, in competition with Israel. The application of sulphur to lychee fruit, usually in the form of sulphur dioxide, is widespread to prevent spoilage and browning. However, this treatment causes allergic reactions in sensitive individuals and may impact negatively on the

Table 26.2 Overview of some studies reporting on the use of plant extracts and EOs in the preservation of stone and pome fruits, including grapes and strawberries.

Fruit	Major pathogens	Conventional control measure	Mycobiocide	References
Stone	Brown rot (*Monilinia* spp), grey mould (*Botrytis cinerea*) and Rhizopus rot (*Rhizopus stolonifer*)	Intensive pre-harvest application; however, many studies indicate that fungicides act mainly as protectants due to inadequate penetration; *Pantoea agglomerans* was active against *Monilinia* spp. and *B. cinerea*, displaying good penetration into wounds. Curing treatments at 50°C for 2 h and 95–99% relative humidity reduced disease incidence.	A *Bacillus* biocontrol agent combined with lemongrass oils in a MAP application controlled peach pathogens on inoculated fruits. Absence of disease and no off-flavour development at market shelf conditions	Rosslenbroich and Stuebler (2000); Pratella *et al.* (1993); Bonaterra *et al.* (2003); Arrebola *et al.* (2010b); Casals *et al.* (2010); Casals *et al.* (2010); Förster *et al.* (2007)
Pome	Mosaic virus, scab, rot, galls and moulds such as *Aspergillus* spp., *Penicillium expansum* Link., *B. cinerea* Pers. and *Rhizopus nigricans* (Ehrenb.)	Although numerous biocontrol products are registered, their efficacy under semi-commercial environments is inconsistent.	MIC for eugenol *in vitro* against four major European pathogens of apple was 2000 mg/L, while 150 µL/L of volatile eugenol inhibited mycelial growth. A mixture of eugenol 2000 mg/L and soy lecithin (50 000 mg/L) reduced disease incidence on apples without phytotoxic effects. Suppression of lenticel rot (*Neofabraea alba*) was achieved on Golden Delicious apples by volatiles of carvacrol, *trans*-cinnamaldehyde, citral and trans-2-hexenal. A lower inhibition was obtained for carvone, hexanal, *p*-anisaldehyde, 2-nonanone and eugenol.	www.nysaes.cornell.edu/pp/extension/tfabp/pome.shtml; Barkai-Golan (1980); Usall *et al.* (2000); Calvo *et al.* (2007); Sugar and Basile (2008); Arras *et al.* (1995); Carta *et al.* (1996); Anthonov *et al.* (1997); Bouchra *et al.* (2003); Amiri *et al.* (2008); Neri *et al.* (2009)

Grape	*Aspergillus* spp., *B. cinerea* Pers. Fr.	Sulphur dioxide fumigation and cold storage Fumigation with high concentrations of ozone Volatiles of *Muscodor albus* have been evaluated and patented in the USA.	Carvacrol vapour totally inhibited the growth of *B. cinerea in vitro* and *in vivo* and the inhibition proved dependent on carvacrol concentration. Grapefruit seed extract, evaluated *in vitro* inhibited spore germination and mycelial growth of *B. cinerea*. Chitosan and grape seed extract treatments, alone or in combination, lead to reduction of post-harvest fungal infection of Redglobe grapes.	http://fruit.cfans.umn.edu/grape/IPM/botrytis.pdf; Barkai-Golan (1980); Franck *et al.* (2005); Gabler *et al.* (2010a, 2010b); Martinez-Romero *et al.* (2007); Xu *et al.* (2007)
Strawberries	*Aspergillus* spp., grey mould (*B. cinerea*), Rhizopus rot (*Rizopus nigricans*, *R. stolonifer*), anthracnose fruit rot (*Colletotrichum acutatum*) and leather rot (*Phytophthora cactorum* Lib and Cohn)	Copper and sulphur salts, ronilan, benlate, thyram, pyraclostrobin, boscalid, cyprodinil, fludioxonil, azoxystrobin and captan Post-harvest cooling is essential to control post-harvest diseases but not sufficient to eliminate decay.	EO of *Thymus vulgaris* displayed significant antifungal activity against *B. cinerea* and *R. stolonifer* at concentrations of 50–200 ppm. No phytotoxic symptoms were observed. Pre-harvest sprays of chitosan to reduce post-harvest decay on strawberries stored at 3 and 13°C indicated that repeated sprays of chitosan (6 g/L), performed 10 days apart, provided protection, while maintaining quality for 4 weeks under cold storage.	Barkai-Golan (1980); Wedge *et al.*(2007); Bhaskara Reddy *et al.* (1998, 2000)
			The *in vitro* antifungal activities of plant EOs of *Satureja* spp. (*Satureja hortensis*, *Satureja khuseztanica*, *Satureja spicigera*)	Nabigol and Farzaneh (2010)

(*Continued*)

Table 26.2 (*Continued*)

Fruit	Major pathogens	Conventional control measure	Mycobiocide	References
			Thymus spp. (*Thymus danensis* and *Thymus carmanicus*), *Salvia officinalis* and *Artemisia aucheri* were investigated against *R. stolonifer*, *P. digitatum*, *Aspergillus niger* and *B. cinerea*). While *Thymus* spp exhibited some inhibition of *P. digitatum*, *R. stolonifer* and *B. cinerea*, only *Satureja* spp. were able to control all pathogens at 300 µL/L. No fungicidal effect was observed for any of the oils.	

MIC, minimal inhibitory concentration.

health of pack-house workers (Koeing *et al.*, 1983). Many other post-harvest treatments applied to lychee, rambutan and longan have been investigated and are available in the literature. Various active packaging applications, alone or in combination with chitosan, have been tested on lychee and reportedly extend the storage life and quality of the fruit (De Reuck *et al.*, 2009a, 2009b). The limited number of reports on the use of plant extracts on lychee and related fruit can possibly be attributed to the porous nature of the pericarp. Absorption of odiferous components such as EOs through the pericarp could result in off-flavours (Lambert *et al.*, 2001). Sivakumar *et al.* (2002) successfully used blotting sheets impregnated with 30 mg/L cinnamaldehyde in commercial packaging of rambutan to reduce fungal decay. The use of this terpenoid against lychee pathogens was later confirmed *in vitro* by Tunc *et al.* (2007), who evaluated several aroma compounds including cinnamaldehyde and carvacrol, in the vapour phase, individually and in combination, for their effect against *Penicillium notatum*, a common pathogen of lychee.

26.4.1.9 Pome fruit

Pome crops have been cultivated for more than 2000 years. They are produced extensively all over the world, with a steadily increasing consumer demand. Apples (*Malus* spp.) and pears (*Pyrus* spp.) are the most important of the pome fruits and are consumed on a daily basis by the majority of the world's population. Bananas, grapes and apples are the most commonly produced fruit in the world, in addition to citrus. Production figures indicate that China is the world leader, with a 42.8% contribution, followed by the European community and the USA. However, due to local demand, as little as 10% of pome production enters the international market. Furthermore, only a few countries manage to successfully export their fruit over long distances (Huang, 2007). According to the World Apple and Pear Association (www.wapa-association.org/asp/article_2.asp?doc_id=492), the world pear production exceeded 20 million tonnes in 2008, once again with China as the main producer.

Several reports detailing the use of EOs and other plant-derived extracts against pathogens of pome fruit, such as *Botrytis cinerea* (Arras *et al.*, 1995; Carta *et al.*, 1996; Anthonov *et al.*, 1997; Bouchra *et al.*, 2003), have been published and are summarized in Table 26.2.

26.4.1.10 Grapes

Global grape (*Vitis vinifera* L.) production reached 69 million tonnes in 2009. Although Italy is currently the biggest producer, China is rapidly expanding its production, while grape crops in France are steadily declining. The majority of grapes are used for wine (71%), followed by juicing (www.bkwine.com/news/world-wine-production-stable-in-2009/). The list of available cultivars is extensive, with varieties developed for its intended commodities: wines, table grapes, raisins, currants and sultanas. Table grapes are mainly decayed by *B. cinerea* Pers. Fr. which accounts for more than 20% of storage-related losses (see http://fruit.cfans.umn.edu/grape/IPM/botrytis.pdf). Many investigations have documented the use of plant secondary metabolites, mainly EOs, to control fungal pathogens of grapes (Table 26.2).

26.4.1.11 Strawberries

Strawberries (*Fragaria* × *ananassa* Duchesne) are the most widely cultivated berry-type fruit. Commercial plants are propagated from runners and then grown using plasticulture or hydroponics. In 2009, the USA exported 1.3 million tonnes of fresh strawberries and

14.5 million tonnes of frozen produce, while an additional 81% was consumed domestically (www.agmrc.org/commodities__products/fruits/strawberries/commodity_strawberries.cfm). Many diseases flourish on strawberry plants and fruits. Despite the use of disease-free stock with genetic resistance as the preferred option to limit pathogens, fungicides are applied for fruit protection. The use of plant-derived compounds as crop protective products for strawberry is gaining momentum (Table 26.2).

26.4.2 Vegetables, legumes and grains

The consumption of vegetables and grains has increased tremendously throughout the world, due to public awareness of the benefits of including legumes, legume fruits, tubers, rhizomes, roots and grains in the diet (Winston, 1997). Producers and retailers of vegetables must ensure consistent supplies, in addition to safe and nutritious products. The highly perishable nature of fresh produce, due to the susceptibility of the crops to pathogens and insects, provide a continuous challenge. Inadequate harvesting, handling, processing, storage and distribution methods increase the risk of decay and may significantly reduce the shelf life and market value of vegetables. Convenience foods such as salads are considered a high risk for the propagation of foodborne illnesses when contaminated by pathogens such as *Listeria*. Various types of packaging have been developed during the last two decades to reduce decay in ready-to-eat green products. Equilibrium MAP has proved to be the most effective packaging technology for green vegetables (Day, 1996; Jacxsens *et al.*, 2000). Several EOs have been evaluated as antimicrobial control measures and some are already being used. These include thyme verbena (*Thymus baeticus*), thyme red (*Thymus zygis*), Spanish oregano (*Thymbra capitata*), tea tree (*Melaleuca alternifolia*), clove (*Eugenia cryophyllata*), sage (*Sage lavendulifolia*) and rosemary (*Rosmarinus officinalis*) EOs (Molinos *et al.*, 2009; Tajkarimi *et al.*, 2010). However, limited scientific information is available regarding the use of packaging technology in combination with botanical fractions. Data collated mainly from journal articles are summarized in Table 26.3.

Lettuce (*Lactuca sativa*), and other green leafy vegetables form part of the daily diets of millions of people worldwide. Generally used in fresh salads, they are sold soon after harvest. EOs have been applied as mycobiocides on lettuce (Table 26.3).

Podded vegetables such as beans, peas, soybean, groundnut and lentils are widely cultivated in Africa, Latin America and Asia. These vegetables are the main source of income of numerous small-scale farmers and play an important role in the diets of rural households and as animal feed (Zohri *et al.*, 1992; Langyintuo *et al.*, 2003). The seed-like nature of podded vegetables make them susceptible to the same decay pathogens as grains.

Rice, maize, sorghum and wheat dominate as the fastest biomass-producing foods in developing countries, as well as in Europe and the USA. The greatest losses are caused by insects (worms and borers) and fungal pathogens during storage. In developing countries, the control of insect pests is frequently achieved using traditional knowledge. For example, the solarization of a small amount of seed placed on jute bags (www.researchintouse.com/nrk/RIUinfo/PF/CPH28.htm) or the application of dried powdered plants such as paddy husk ash (Andan, 2002) are often the only form of protection used by farmers to reduce infestation by the cowpea beetle. EOs have been the focus of many studies due to their insect-repellent properties (Nerio *et al.*, 2010). Dayan *et al.* (2009) reviewed the use of natural products in insect management and pointed out that three of the five most commonly used insecticide classes are natural products or derived from natural products. Among the botanical insecticides reported, neem-based products, *Pyrethrum*, rotenone, *Ryania speciosa* mixture

and *Sabadilla* are commercially manufactured. Although the purpose of this review is not to discuss the use of natural products as insecticides, we would like to mention some of the EOs and plant extracts listed as permissible for use on organic crops by the Organic Material Review Institute of the USA. These include thyme oil-based products such as Proud 3 (BioHumaNetics) and Organic Yard Insect Killer (Green Light), rosemary oil and peppermint-based insecticides produced by EcoSmart Technologies and extracts of clove oil produced by Green Light and Bioganic Brand that are used in Europe and the USA.

However, insect infestations are not the only source of decay and the development of primary mould infection during storage can be equally detrimental to crops. The production of mycotoxins by moulds, particularly those prevalent on grains, is of major concern to health associations all over the world. It is alarming that only very few of the commercial EO-based products are marketed as fungicides with action against mycotoxin-producing fungi (Table 26.3). As stored food commodities are frequently affected by more than one fungus, an agent with a broad fungicidal action is required. According to Kumar *et al.* (2008), thyme EO exhibited activity over a broad fungal spectrum (antifungal and antiaflatoxigenic efficacy) and should therefore be considered for large-scale applications.

An effectively balanced diet includes regular consumption of tomatoes, carrots and potatoes. In general, carrots and tomatoes are either eaten raw in salads or cooked as part of a vegetable mix, while potatoes are consumed in the cooked form. Harvesting of unripe tomatoes is a common practice, but it forces the producer to preserve the fruit for several days before marketing (Hobson, 1981). This provides the opportunity for pathogens to proliferate. The most significant pathogens, the conventional control methods and plant-derived extracts and compounds used on tomatoes, carrots and potatoes are listed in Table 26.3.

In recent years, the potato has become the most frequently consumed tuber on the globe. After brushing and packing in perforated packaging, potatoes can be stored in a cool, dark, well-ventilated area for more than 3 weeks. Biodegradable MAP packagings have been developed that are able to increase the shelf life of organic potatoes, while reducing greening of the tuber. Stored potatoes are susceptible to sprouting and effective sprout control is an important factor in potato storage. In the USA, chlorpropham (1-methylethyl-3-chlorophenylcarbamate) is the most commonly used anti-sprouting agent, but it is not permitted by the organic market. Several alternatives, such as the exposure of potato tubers to an iodine-saturated atmosphere (Pifferi, 2001; Eolini *et al.*, 2004) have been investigated. Inhibition of sprouting and accumulation of starch were observed after application of hydrogen peroxide plus (HPP) (Afek *et al.*, 2000; López-Delgado *et al.*, 2005). The potent antimicrobial activities of EOs have encouraged many researchers to investigate the efficacies of these compounds against bacteria and fungi isolated from the surface of potato tubers (Table 26.3). As early as 1993, some reports described the antibacterial effect of carvacrol-containing EOs to control *Pectobacterium carotovorum*. The overall benefit of using EOs is that they can serve a dual purpose, acting as anti-sprouting as well as antimicrobial agents (Coleman *et al.*, 2001; De Carvalho *et al.*, 2006).

26.4.3 Seaweed

Considered a delicacy, seaweed is a highly priced sea vegetable that is widely consumed in Asia. An increased demand for seaweed salads and soup has prompted the industry to improve the shelf life of the fresh algal crop. Species of green and brown seaweed are eaten raw, boiled and roasted, and are frequently salted before drying. However, inadequate post-harvest management during cleaning and drying leads to low-quality products and even losses. To our

Table 26.3 Pathogens and control methods of vegetables and grains using mycobiocides.

Vegetable	Major pathogens	Conventional control measure	Mycobiocide	References
Lettuce	Rhizoctonia bottom rot (*Rhizoctonia solani*), downy mildew (*Bremia lactucae*), Sclerotinia drop (*Sclerotinia sclerotiorum*), grey mould (*B. cinerea*), foodborne diseases (*Escherichia coli*, *Salmonella* and *Listeria monocytogenes*)	Washed with peracetic acid mix or hypochlorite and transferred into special bags Electrolysed water	Satisfactory results have been reported using natural compounds and plant extracts, particularly. A sanitizing product known as Fungastop™ containing mint oil as the main active component is available as a mycobiocide.	http://vegdis.cas.psu.edu/Veg-Diseases/Identification_files/lettuce.html; Burt (2004); Tripathi and Dubey (2004); Martinez-Romero *et al.* (2008) Park *et al.* (2008)
Grains	*Aspergilus flavus*, *Fusarium* spp.	Applications of chemical fungicides (Benzimidazoles; Prochloraz; Flutriafol; Morpholines; Strobylurines; Phthalonitriles…)	A range of plant EOs are becoming available as specific fungicides. These include *Ocimum gratissimum* and *Cymbopogon citratus* (lemongrass).	Mishra and Dubey (1994); Nguefack *et al.* (2004); White and Toman (1994)
			Tea tree oil, which is characterized by a broad antimicrobial spectrum of activity, was able to effectively control *A. flavus*.	Dubey and Kishore (1988); Tripathi and Dubey (2004)
			An assessment of *T. vulgaris* L. EO proved the oil to be a safe grain preservative.	Kumar *et al.* (2008)
			EOs of *L. rehmannii* (20 µL/L) and lemongrass (50 µL/L) and pure citral (20 µL/L) were effective against *Fusarium oxysporum* in toxic medium.	Linde *et al.* (2010)
Tomato	*Aspergillus* spp, *Rhizoctonia solani*, *Botrytis cinerea*, *Penicillium* spp and *Alternaria alternata*	Mature-green fruits are dipped in heated (38–42°C) chlorinated water and wax applied	*In vitro* and *in vivo* evaluation of thyme and cassia oils to control *A. alternata* suggested that the integration of the EOs with MAP has the potential for fungal control.	Barkai-Golan (1980); Akhtar *et al.* (1994); Hobson (1981); Feng and Zheng (2007); Liu *et al.* (2007); Rodriguez-Lafuente *et al.* (2010)

		The use of ultraviolet-C light for preservation is well documented Dipping the fruits in a solution of chitosan provided an effective control of *B. cinerea* and *P. exposum* and elicited the biochemical defence system of the tomato.		
Carrot	Grey mould (*Botrytis rot*), watery rot (*Sclerotinia rot*), Rhizopus rot, bacterial soft rot, (*Erwinia carotovora* subsp. Carotovora), sour rot (*Geotrichum rot*)	Good handling, low temperature storage and transportation conditions are the best methods to minimize losses. Products such as Rovral™ and Shemer™ (a yeast-based product) are partially effective fungicides. The synergistic effects of combining various control methods including steam and hydrogen peroxide application have been evaluated. Edible coatings controlled browning and ripening, while providing a barrier to moisture.	The combination of plant extracts with edible coatings in sealed bags is a possibility.	Liew and Prange (1994); Li and Barth (1998); Eshel *et al.* (2009)
Potato	*Rhizoctonia solani*		The use of volatile plant compounds in post-harvest potato storage is an ancient method. EOs including spearmint, peppermint, clove, caraway and clove are applied to certified organic crops. EOs of *L. rehmannii* (20 µL/L) and lemongrass (50 µL/L) and pure citral (50 µL/L) were effective against *R. solani* in toxic medium.	Vaughn and Spencer (1991); Vokou *et al.* (1993); Frazier *et al.* (2004); Costa E Silva *et al.* (2007); Teper-Bamnolker *et al.* (2010); Linde *et al.* (2010)

(*Continued*)

Table 26.3 (*Continued*)

Vegetable	Major pathogens	Conventional control measure	Mycobiocide	References
Sweet potato	*Botryodiplodia theobromae*, *Streptomyces ipomoeae*, *Fusarium* spp., *Epicoccum* spp., *Mucor racemosus*, *Sclerotinia* spp., *Ceratocystis fimbriata*	Crop rotation Application of fungicide (Mertect340-F applied at 237 mL/28 L on seed or root/slip treatment). Maxim 4FS (seed treatment) (active ingredient: Fludioxonil), Botran75-W (post-harvest application to non-stored commodity) (active ingredient: Dicloran) Post-harvest curing at 30–35°C and 85–95% relative humidity for 5–10 days	Chlorogenic acid, pyrogallol, pyrochatecol, phenol and resorcinol	http://en.wikipedia.org/wiki/List_of_sweet_potato_diseases Mohapatra *et al.* (2000)

knowledge, few studies have been done regarding the use of plant extracts to extend the shelf life of seaweed.

26.4.4 Fish and meat

Traditionally, herbs and spices have been applied to meat to preserve and improve their sensory characteristics. Plant extracts that have the ability to act as antioxidants can reduce lipid oxidation, the cause of deterioration in meat qualities such as flavour, colour and texture (Fasseas *et al.*, 2007). Meat and meat products are a major source of foodborne infections and are commonly populated with *Campylobacter* spp., *Salmonella* spp., *Yersinia enterocolitica*, *Escherichia coli* and *L. monocytogenes* (Mayrhofer *et al.*, 2004). A survey conducted by Mayrhofer *et al.* (2004) revealed a thermophilic *Campylobacter* incidence of 51% in 243 chicken samples and 53% in 257 turkey samples, while the prevalence of *Y. enterocolitica* was found to be 43.3% in pork and 45% in chicken. Moreover, *L. monocytogenes* occurred in pork (22% prevalence rate), beef (12%), chicken (26%) and turkey (14%). A disturbing finding was that many of the isolates displayed multidrug resistance to antimicrobials.

It has been established that certain EOs stand out as better antibacterial agents than the commonly used preservatives for meat applications (Tassou *et al.*, 1995; Hammer, *et al.*, 1999). The EOs of oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*) and rosemary (*R. officinalis*) are reportedly among the most active in combating food spoilage and pathogen microorganisms (Table 26.4). However, the efficacy of the EOs depend on the pH, storage temperature, the amount of oxygen present, and the concentration of the EO itself as well as that of its active components (Tajkarimi *et al.*, 2010). EOs with antibacterial properties often contain carvacrol, thymol, citral, eugenol, 1,8-cineole, limonene, pinene, linalool and their precursors. Extracts from herbs and spices have been the focus of many investigations aimed at extending the shelf life of muscle meats (Chouliara *et al.*, 2007). In many cases the antimicrobial activities of these extracts are too low to safeguard food at levels where the taste is not discernable. Combinations of antimicrobials are therefore seen as a more practical option, allowing the substances in the 'preservative system' to play a concerted or even synergistic role in inhibiting bacterial spoilage. Blaszyk and Holley (1998) evaluated the *in vitro* application consisting of a mixture of monolaurin (an antimicrobial fatty acid), eugenol (a phenyl propanoid phenolic) and sodium citrate (chelator) against six meat spoilage organisms and found that the inclusion of eugenol was essential to prevent detectable growth of *Leuconostoc mesenteroides*, *L. monocytogenes* and lactobacilli.

The use of EOs in combination with MAP for meat preservation shows much promise (Table 26.4). Modification of the atmosphere surrounding the food so that it contains less oxygen renders pathogens more susceptible to the EOs (Tajkarimi *et al.*, 2010). For this reason, vacuum packaging combined with EOs has also proved to be successful (Ntzimani *et al.*, 2010).

An interesting aspect of meat preservation is the use of EOs in the diet of animals to help reduce microbial counts in the slaughtered meat (Botsogloua *et al.*, 2002; Govaris *et al.*, 2007; Soultos *et al.*, 2009). Oregano EO exhibits antioxidant and antimicrobial activities. Incorporation of the oil into the diets of chickens, turkeys and rabbits was found to improve the oxidative stability of raw and cooked meat during refrigeration storage (Soultos *et al.*, 2009). These researchers used oregano EO to supplement the diets of rabbits at concentrations of 100 and 200 mg/kg. The latter concentration was found to significantly reduce microbial counts in the refrigerated meat compared to that of the controls. Dietary supplementation with EOs is a good option since the amount of EO that can be applied to the meat surface is usually limited

due to the effects on flavour. In addition, the effectiveness of the EO may be modulated as a result of interaction with other food ingredients (Soultos *et al.*, 2009).

Compared to the use of EOs in meat and fish preservation, fewer reports concerned with the use of other plant extracts are available. Kumudavally *et al.* (2008) reported the use of green tea extract (known to display antibacterial properties) on mutton (Table 26.4). Treatment of the mutton with the tea extract extended the shelf life for up to 4 days. This finding was attributed to a reduction in the formation of degradation products responsible for microbial spoilage.

26.5 MEDICINAL PLANTS AND THE REGULATIONS GOVERNING THE USE OF BOTANICAL BIOCIDES

Most current legislation regards biocides as active substances and preparations containing one or more active fractions (plant extracts) or substances. These preparations are supplied in the form applicable to the user, for the purpose of destroying, rendering harmless or controlling harmful organisms by chemical or biological action (www.hse.gov.uk/biocides/legislationapply.htm). In general, biocides must be approved (registered) before they can be imported, used, stored, sold, supplied or advertised (www.environment.fi/biocides).

The application of a variety of medicinal plants as biocides dates back to antiquity (Dayan *et al.*, 2009). All plants can be regarded as medicinal if we consider that they all produce secondary metabolites, some more useful than others in terms of their action against insect and microbial pests. After almost a century of developing synthetic pesticides, the use of medicinal plants in agriculture is gaining momentum worldwide as the focus of research and development and has led to the establishment of a strong global industry, generating billions of dollars. The success of this industry lies further downstream, depending on the mode of collection, propagation and harvesting of the source materials (http://ec.europa.eu/environment/biocides/pdf/dir_98_8_biocides.pdf). To this end, stakeholders have to be taken into account regarding their intellectual property to plant exploitation.

The largest natural biodiversity of plants occurs in tropical and subtropical regions, the location of many developing countries. Nevertheless, most developing countries have largely ignored the potentially huge contribution that indigenous plants can make to their struggling economies. Brazil has a very rich flora, accounting for 22% of the higher plant species on the planet (Rates, 2001). Although social, cultural and economical problems, lack of well-planned and integrated strategies and poor access to scientific information have negatively affected the development of the pharmaceutical industry in that country, the value of the market was already estimated at US$40 billion in 1996. However, the protection and recognition of stakeholders (Bast *et al.*, 2002) is vital to the economic and social advancement of developing nations. All over the world, nations are legislating access for biosprospecting purposes to protect their biological and genetic resources (Crouch *et al.*, 2008). South Africa, endowed with the smallest, but most diverse plant kingdom, has adopted legislation regulating the bioprospecting of indigenous plants to protect the indigenous flora and ensure stakeholder rights (Crouch *et al.*, 2008). Many international symposia have discussed the necessity of paired legal requirements. However, some poorly defined aspects such as ethics, politics and cultural practices are expected be taken into account, often leading to a bottleneck in the development of new natural-origin plant protection products.

Plant extracts are not automatically recognized as safe and must undergo thorough investigations before being approved, patented and marketed (Kuhlmann, 1997). The overall

Table 26.4 Overview of some studies reporting the use of plant extracts and EOs in meat preservation.

Meat	Natural product	Effective concentrations	Notes	Reference
Listeria monocytogenes isolated from meat	Thyme, rosemary and oregano EOs	Complete inhibition of *L. monocytogenes* with mixture of lactic acid containing 300 and 200 ppm of rosemary and thyme oils, respectively	*In vitro* using the agar-well diffusion method	Dimitrijevic *et al.* (2007)
Chicken	Oregano EO	0.1% (w/w) extended shelf life by 3 to 4 days	EO used in combination with MAP Concentration of 1% (w/w) imparted a strong taste	Chouliara *et al.* (2007)
Poultry patties prepared by mixing ostrich, chicken and turkey	Thymol and carvacrol (main components of oregano oil)	50–300 ppm	EO used in combination with MAP Meat patties mixed with antimicrobials reduced bacterial cell load by 1–1.5 log CFU/g at 0–3°C.	Mastromatteo *et al.* (2009)
Semi-cooked coated chicken fillets	Rosemary and oregano EO	0.2% (v/w)	Samples treated with EOs in combination with vacuum packaging and EDTA/lysozyme extended shelf life by 7–8 days.	Ntzimani *et al.* (2010)
Turkey	Rosemary EO	5–10 g EO/kg as dietary supplement	Rosemary-supplemented groups presented bacterial counts significantly lower compared to controls.	Govaris *et al.* (2007)
Beef	Oregano EO	Not reported Filter paper soaked in EO	EO used in combination with MAP	Skandamis and Nychas (2002)
Beef	*Corydothymus capitatus* EO was the most active. Oregano, *Cinnamomum cassia*, *Satureja* and thyme oils had strong antimicrobial activities	MIC of 0.025% and a MTC of 0.06% MIC values of 0.05%	*In vitro* investigation testing inhibition of *Pseudomonas putida* originally isolated from beef	Oussalah *et al.* (2006)

(*Continued*)

Table 26.4 (*Continued*)

Meat	Natural product	Effective concentrations	Notes	Reference
Beef	Thyme EO	Combination of EO at 0.6% and nisin at 500 or 1000 IU/g indicated additive effect, which was higher during storage at 10°C than at 4°C	*In vitro* against *E. coli* and *in vivo* on inoculated beef	Solomakos *et al.* (2008)
Beef	EOs from *S. officinalis* (mainly 1,8-cineole) and *Schinus molle* (mainly α-phellandrene)	20 µL per Petri dish for *in vitro* studies Antibacterial activities of both EOs in beef were evident, but additions above 1.5% affected taste	*In vitro* disk diffusion method against two *Salmonella* species and *in vivo* in inoculated beef	Hayouni *et al.* (2008)
Beef fillet	Oregano EO	0.8% (v/w)	*Salmonella typhimurium* survived under all storage conditions in controls. Addition of oregano EO resulted in reduction of 1–2 $\log_{10}$ CFU/g of most microbial populations with lactic acid bacteria showing the greatest reductions.	Skandamis *et al.* (2002)
Buffalo	Clove EO	0.1% (v/v)	Reduced microbial counts after treatment with lactic acid and clove with or without vitamin C Better sensory characteristics, colour and odour	Naveena *et al.* (2006)
Fresh Mediterranean swordfish fillets	Thyme EO	0.1% EO	Storage at 4°C after addition of EO extended the product's shelf life under aerobic conditions by 5 days, while the combination of MAP and EO extended shelf life by 7.5 days.	Kykkidou *et al.* (2009)

Sea bream (*Sparus aurata*) fillets	Oregano EO	0.8% (v/w)	EO combined with MAP yielded a pleasant flavour and slowed spoilage.	Goulas and Kontominas (2007)
Octopus	Oregano EO	0.2 and 0.4% (v/w)	Used in combination with vacuum packaging Not effective against *Pseudomonas* species and lactic acid bacteria	Atrea *et al.* (2009)
Carp fillets	Carvacrol and thymol	0.5% of each, mixed	Treatment with electrolysed NaCl solutions in combination with EO compounds had stronger antimicrobial and antioxidant effects than all of the other treatments during drying.	Mahmoud *et al.* (2006)

CFU, colony-forming units; EDTA, ethylenediaminetetra-acetic acid; MIC, minimal inhibitory concentration; MTC, minimum toxic concentration.

process, depending on the target, is regulated by the requirements set out by the food and drug administration of the country where the plant extract will be commercialized. As described by Cordell (2000), the development of such extracts involves numerous pharmacological studies (Phase I) and formulation studies (Phase II), followed by a clinical or human case study (Phase III). A post-marketing phase is often required to ensure the efficacy of the product and to monitor the long-term side effects.

By definition, EOs cannot be patented because they have a long history of use. Nevertheless, any integration of these substances into packaging or coatings may be patented and registered for commercial application. This is an expensive and often time-consuming process that hampers development and may lead to the abandonment of registration. Although GRAS with no limitations, EOs are mainly registered as flavouring agents and adjuvants. However, due to current safety regulations, acute toxicity studies such as developmental and reproductive toxicity, immunotoxicity, genotoxicity, cytotoxicity and even carcinogenicity are mandatory. These studies are complicated by the variability in EO composition and the additive, synergistic and modulation effects caused by the mix of terpenoids present in the oil.

The long and frustrating processes required for registration of natural biocides often discourage stakeholders from registering a potential product. According to the EU-funded project REBECA, the registration of biocontrol organisms, plant extracts and semiochemicals at EU and/or national level need to be simplified without compromising safety standards (http://orgprints.org/11518/). In this regard, several publications dealing with the legal frameworks involved are available (Aguilar, 2001; Gaudillière, 2001; Van Overwalle, 2005; Le Buanec, 2006; Moreira *et al.*, 2006). Unfortunately, the rules governing the use of products for organic agriculture vary between countries and not all products may be legally used in every country, and even in different states of the same country (Dayan *et al.*, 2009). Additionally, the regulations for inclusion or exclusion are not always based on scientific rationale. The use of synthesized natural compounds is generally not permitted in organic agriculture, despite the development and demonstration of economy of scale, resources and purity.

26.6 FUTURE PERSPECTIVES

The increased demand for replacing current, not always environmentally harmless compounds with novel, more ecologically acceptable ones, highlights the future of natural plant extracts. As the technology develops and our understanding of the mode of actions of such molecules increases, new applications emerge. Technological advancement in the field of nanotechnology allows improved mode of application encompassing the development of microencapsulation and slow release of plant extracts such as EOs. However, the important combination of proven, existing methods of protection with new antimicrobial agents is of interest. Results recently reported by Varona *et al.* (2009) state that lavandin (*Lavandula hybrida*) EO is applied as an alternative to synthetic chemical biocides. In this study, an encapsulation of the oil in a biodegradable polymer (polyethylenglycol) with *n*-octenyl succinic anhydride (OSA) starches as surfactants has been achieved using two high-pressure precipitation techniques. Results showed that encapsulation efficiencies of lavandin oil were higher in polyethylene glycol (PEG) microcapsules obtained using this process.

The use of bioactive packaging where antimicrobial compounds are incorporated into the packaging materials (Devlieghere *et al.*, 2004) is an exciting new field of research. This

results in the manipulation of the gaseous environment to which the food is exposed to control and suppress microbial growth. The active plant extract may be coated onto the surface of the packing material or included in the form of a sachet from where it can be released during storage. Edible films containing the antimicrobial agent may also be applied by dipping or spraying onto the food to enhance contact between them. However, the use of active packaging is currently not in line with European legislation and is therefore restricted in its use (Devlieghere *et al.*, 2004). A further drawback is that the remedial quantities of compounds reaching the fruit surface are often limited.

26.7 CONCLUSIONS

Although the number of reports dealing with applications of plant extracts, particularly EOs, have exploded, very few of these have reached fruition as crop-protective products. Reasons for this could include the tedious task of registration and the expenses involved to comply with new legislation, such as regulations accepted by the EU requiring toxicity testing. Moreover, the lack of clear guidelines regarding the interaction between the components of natural products and poor cooperation between researchers and the food industry discourage industrial development of mycobiocides, resulting in wastage of research investments. Although several compounds of natural origin display great potential for the control of bacterial and fungal pathogens of foods, the addition of these compounds at sufficient concentrations to guarantee effective control may cause unacceptable structural and organoleptic changes to food. In addition, very few plant-derived extracts or compounds offer a sufficiently consistent level of disease control at a level of application that is acceptable. Better understanding of the modes of actions involved in the efficacy of natural antimicrobials as well as the phenomenon of additive and synergistic action between compounds will eventually promote the development of mycobiocides.

In conclusion, it is clear that there is no turning back on the road towards application of natural products in food protection. Results obtained by numerous researchers indicate that natural compounds are valuable as antimicrobials and can no longer be ignored. Exciting developments envisaged for the future include the establishment of slow-release gels and micro-encapsulation of active compounds.

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27 Plant-Based Products as Control Agents of Stored-Product Insect Pests in the Tropics

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Abstract: The unreliable supply of quality food is the major challenge facing the tropics, where production is heavily dependent upon rain-fed agriculture. Food losses due to insect pest attack are the most important constraint on the attainment of self-sufficiency and food security in subsistence agriculture. Although effective synthetic insecticides are available, smallholder farmers in the tropics are yet to fully integrate them into their stored-product insect management practices. This is due to prohibitive costs, inaccessibility in rural areas, toxicity (health and safety) concerns, erratic efficacies, adulteration and insect resistances to synthetic pesticides. Conversely, a literature survey indicates that most smallholder farmers in the rural areas control pests in storage using plant-based products. They have used botanicals for generations, making them compatible with existing farming conditions, accessible, trusted, acceptable and inexpensive. However, the efficacy ratings of plant-based products are either unknown or unavailable. This chapter covers the importance of storage and associated storage systems in food security, major insect pests of stored food commodities and insect pest control strategies. It places special emphasis on the potential of plant-based products (powders, extracts, essential oils, etc.) as cost-effective alternatives to synthetic insecticides. The chapter also discusses their low priority in agricultural policy, biosafety and intellectual property rights issues, among other challenges, that are serious bottlenecks to commercialization and standardization of botanical pesticides.

Keywords: botanical pesticides; post-harvest losses; stored-product insect pests; subsistence agriculture

27.1 INTRODUCTION

Food security of citizens is a leading basic priority of any nation in the tropics and is of immense socioeconomic importance, particularly in sub-Saharan Africa, where agriculture is often limited by the unpredictable weather. According to the UN Food and Agriculture Organization (1996), food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food for a healthy and active life.

Smallholder farmers produce 90% of sub-Saharan Africa's food supply yet they make up 50% of the food-insecure population. Subsistence farming in the region is challenged by high

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population growth, low land productivity, low performance of rain-fed agriculture, huge postharvest losses caused by pests and diseases, inadequate food storage and preservation techniques and weak science and technological advancement (Economic Commission for Africa, 2002; Kimenyi, 2002). The post-harvest losses in the tropics stand at 30–40% with arthropod and vertebrate pests accounting for 80–90% of the total food grain losses. Among the factors responsible for this high level of post-harvest food loss in the tropics are the prevailing climatic conditions (high temperature and humidity), which significantly contribute to the development and proliferation of storage pests and microbial diseases (Obeng-Ofori and Amiteye, 2005). Additionally, the adoption of improved high-yielding cereal and legume grains, known to be more susceptible to pests, has exposed subsistence farmers in the tropics to increased insect pest attack causing quantitative and qualitative post-harvest losses varying in magnitude from 10 to 60% depending on grain type, insect pest species, duration of storage and pest control method employed (Golob *et al*., 1996; Shaaya *et al*., 1997; Lee *et al*., 2003; Ogendo *et al*., 2003a; Obeng-Ofori and Amiteye, 2005) (Figure 27.1). Production and preservation of stored grains is nearly synonymous to ensuring food security because grains constitute 90% of the dietary carbohydrates and proteins for human consumption and are also used as animal feed (Baba, 1994; Ferdu *et al*., 2001). Secure food storage and improved post-harvest handling of grains are therefore critical contributors to food security in the tropics.

Prudent post-harvest handling and the control of stored-product pests is principally undertaken to prevent or minimize grain losses (quantitative, nutritional and economic) and contamination of human food. For cost-effectiveness, such control measures must be administered before pest infestations reach levels that could cause economic injury. A number of pest-control options can be sampled by farmers, but the use of synthetic pesticides, though it produces quick results, has not been fully integrated in crop production and storage systems in the tropics due to cost and infrastructural limitations. Owing to environmental concerns, toxicity to non-target organisms, rising cases of pest resistance and prohibitive costs, modern integrated pest management (IPM) emphasizes the use of non-chemical pest-control options with judicious use of pesticides. Therefore, there has been a global paradigm

Figure 27.1 Beans damaged by bean bruchid (*A. obtectus* Say). Photograph courtesy of the US Department of Agriculture. GIPSA Webmaster.

shift and attention is rapidly shifting to non-synthetic, safer options. Use of indigenous knowledge to preserve stored agricultural products has existed in the tropics for generations and the knowledge is being documented. Despite the enormous potential of the natural indigenous pest-management practices, these approaches remain largely unexploited in the dispensation of agricultural development with little local research intervention and few resources being committed. Evidence of efficacy of natural pesticides against pests has lately rekindled a hope of development of suitable, simple and natural anti-pest products which has provided impetus for the scientific rationalization, improvement and packaging of the existing indigenous knowledge base and practices.

Plant-based products, also referred to as botanical insecticides, are derived from plants such as pyrethrum, neem, *Lantana*, etc. (Balwa and Shaefers, 1997; Keita *et al*., 2000). From such plants compounds such as pyrethrins, nicotine and rotenone are extracted, which have proven lethal to insects through different modes of action on the physiology of insects.

Despite having different bioactive principles, botanical insecticides are target-specific, relatively safe and cheaper, as they are readily available. In the past three decades, pest-management research has focused on cultural, host-plant resistance, biological and botanical control measures. This chapter discusses the importance of grain preservation in storage, the economic importance of insect pests and their biology and ecology. The chapter also samples key control options of these pests. In-depth analysis of plant-based products and the principles of their bioactivity is discussed. In view of the need for food security in the new dispensation of unstable agriculture, prospects for possible approaches to commercialization of plant-based derivatives in pest control on different scales of storage are also discussed.

27.2 COMMON INSECT PESTS OF STORED FOOD GRAINS IN THE TROPICS

The grain weevils (*Sitophilus* spp.), angoumois grain moth (*Sitotroga cerealella* Olivier), bostrichid beetles (*Prostephanus truncatus* Horn and *Rhyzopertha dominica* F.), bean bruchid (*Acanthoscelides obtectus* Say), cowpea beetle (*Callosobruchus maculatus* F.), Mexican bean weevil (*Zabrotes subfasciatus* Boheman) and groundnut borer (*Caryedon serratus* Olivier) have been identified as the major primary insect pests of stored cereal and legume grains in the tropics (Raja *et al*., 2001; Lee *et al*., 2003; Ogendo *et al*., 2003a; Gomez, 2004; Obeng-Ofori and Amiteye, 2005). The rust-red flour beetle (*Tribolium castaneum* Herbst), the saw-toothed grain beetle (*Oryzaephilus surinamensis* L.), *Cryptolestes* spp., *Trogoderma granarium* Everts and *Cadra cautella* are the major secondary insect pests of food grains in sub-Saharan Africa and the tropics at large (Haines, 1991; Ferdu *et al*., 2001; Lee *et al*., 2003; Boeke *et al*., 2004a). The description, distribution, damage and biology of individual insect pests of stored foods and major crop types are discussed below.

27.2.1 Primary insect pests of stored cereals

*27.2.1.1 Maize weevil (*Sitophilus zeamais *Motschulsky)*

The adult maize weevil (*S. zeamais* Motschulsky) (Figure 27.2a) is about 3–4 mm long, brownish-black in colour with a characteristic snout or rostrum. The larvae are 4 mm long, curved in shape, legless and are dirty white in colour with biting/chewing mouthparts. They are normally found in tunnels and chambers bored into cereal grains. Pupae occur in the grains

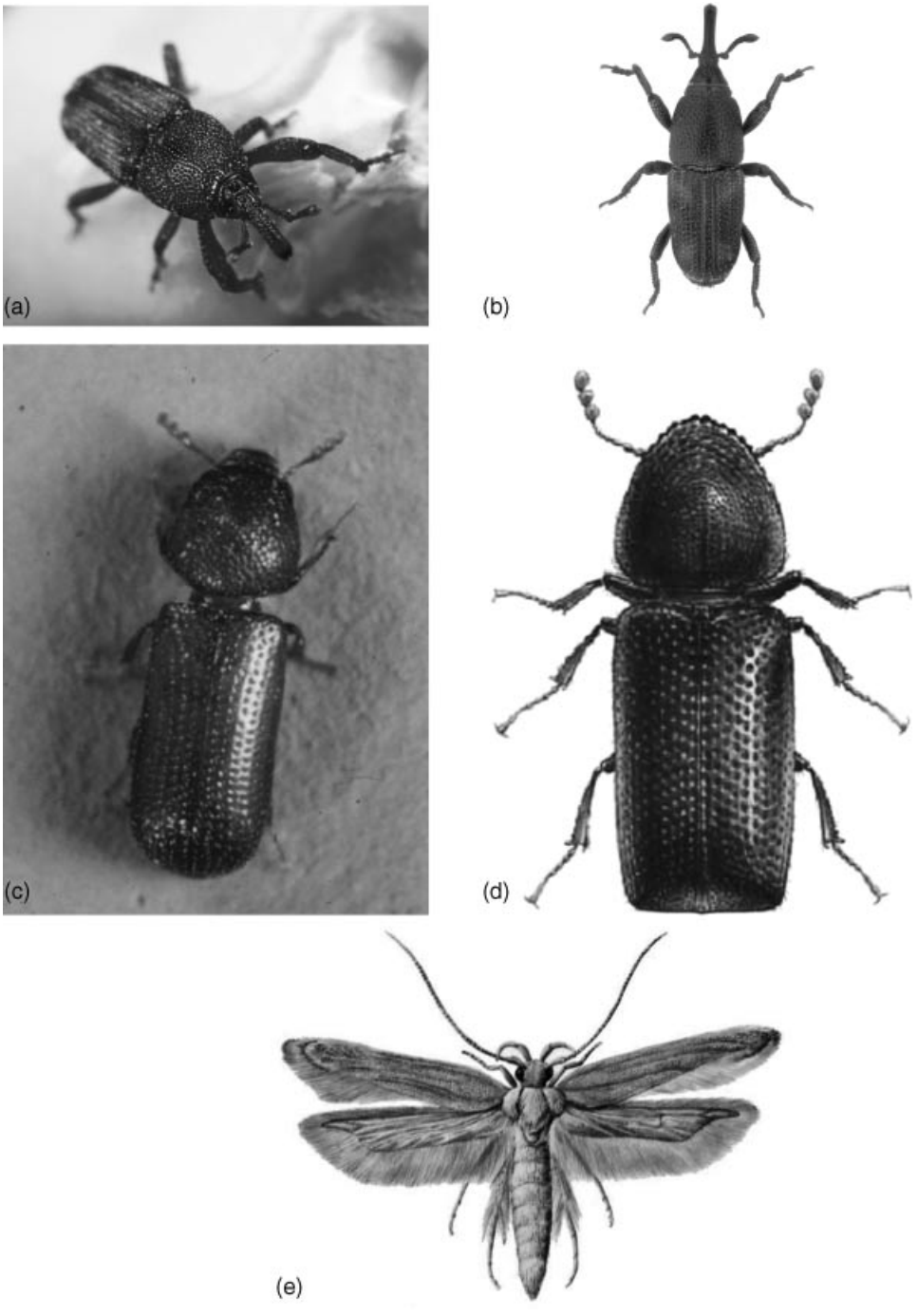

Figure 27.2 Common Coleopteran (a–d) and Lepidopteran (e) primary insect pests of stored cereal grains in tropical agriculture. (a) Maize weevil (*S. zeamais*). Photograph courtesy of Wikimedia.org. (b) Rice weevil (*S. oryzae*). Clemson University, USDA Cooperative Extension Slide Series, Bugwood.org. (c) Lesser grain borer (*R. dominica*). Photograph courtesy of the US Department of Agriculture. GIPSA Webmaster. (d) Larger grain borer (*P. truncatus*). Photograph courtesy of the US Department of Agriculture. GIPSA Webmaster. (e) Angoumois grain moth (*S. cerealella*). Photograph courtesy of the US Department of Agriculture. GIPSA Webmaster.

and do not move from one grain to another (Longstaff, 1981; Haines, 1991). The females bore holes into the grain and lay white oval eggs (150–300 per female throughout adult life) inside and seal the holes unnoticeably with waxy or gummy secretions. The maize weevil is a cosmopolitan pest of stored food products (Longstaff, 1981) and is the most important primary pest on stored maize and rice in Africa and other warmer parts of the world.

27.2.1.2 *Rice weevil (*Sitophilus oryzae *L.)*

The adult rice weevil (*S. oryzae* L.) (Figure 27.2b) is 2.5–4.5 mm in size, long-lived (several months to 1 year) and is characterized by a narrow snout-like forward extension of the head which carries mouthparts. The adult female lays up to 150 eggs throughout adult life, at temperatures of 15–35°C (optimum 25°C) and at grain moisture contents above 10%, in tiny holes in the grain and seals the cavity with an egg plug. The developmental period (egg to adult) ranges from 33–41 days under optimal conditions to over 110 days in unfavourable conditions (Haines, 1991). It is found in all warm-temperate and tropical climates of the world (Haines, 1991) as a primary insect pest of economic importance to stored cereal grains, mainly wheat.

27.2.1.3 *Lesser grain borer (*Rhyzopertha dominica *F.)*

The lesser grain borer, *R. dominica* F. (Figure 27.2c), is a wood-boring insect from the family Bostrichidae. Adult *R. dominica* are small in size (2–3 mm) with a cylindrical body and a head that is ventral to the prothorax (deflected) and held beneath the prothorax, making it obscure when viewed from above. The pronotum has rasp-like teeth or hooks and antennae are straight and have a loose three- to four-segmented club; the tarsi are five-segmented. The number of eggs laid by an adult female is temperature-dependent with means of 244 and 418 per female at 25 and 34°C, respectively (Haines, 1991). The pre-adult stages of *R. dominica* develop within cereal grains but larvae may bore out of one grain and into another (Natural Research Institute, NRI, 2001). After pupation the newly developed adult escapes from the grain by chewing its way out but continues to bore through grains. *R. dominica* are adapted to rather higher temperatures and lower moisture content than *Sitophilus* spp. and are therefore a dominant pest in areas which are hot, dry, or both (NRI, 2001). It is a serious primary pest of a wide range of cereal grains, mostly wheat, paddy rice and dried cassava roots, in the tropical, subtropical and warm temperate zones (Haines, 1991; NRI, 2001).

27.2.1.4 *Larger grain borer (*Prostephanus truncatus *Horn)*

The larger grain borer, *P. truncatus* Horn (Figure 27.2d), an indigenous pest of stored maize in Central America, was accidentally introduced into Tanzania (Dunstan and Magazini, 1981) and Togo (Harnisch and Krall, 1984), and has since spread widely, becoming the most destructive pest of stored maize in both East and West Africa (Hodges *et al.*, 1983; Pantenius, 1988). Adult *P. truncatus* tunnels extensively in the grain, feeding directly on it and excavating side chambers, off the main tunnel, where the females lay batches of four to eight eggs. On hatching, the larvae tunnel into the grain within which the eggs are laid (Li, 1988).

27.2.1.5 *Angoumois grain moth (*Sitotroga cerealella *Olivier)*

Adults of the angoumois grain moth, *S. cerealella* Olivier (Figure 27.2e), are silvery grey or brownish grey, slightly less than 12 mm long, with a long fringe of hairs on the wings. The

forewing is yellow-brown and the hindwing tapers abruptly to a point, with black spots on the distal half of the wings. The wing span is approximately 10–18 mm. The minimum life cycle is 28 days at 30°C and 75% relative humidity. Females lay 40–150 eggs on the surface of the grain. Upon hatching, the 10 mm-long white caterpillars bore into the grain where they feed and develop entirely inside the kernels. Before pupation, they cut a characteristic circular exit hole (1 mm diameter) in the seed coat for adult emergence. The adult moth is a short-lived (7–14 days) and non-feeding flying stage (Haines, 1991). It is a primary colonizer of maize, paddy and sorghum in temperate and tropical regions worldwide. It infests grains both pre- and post-harvest conditions, exposing seeds to infestation by other insects, bacteria and fungi (NRI, 2001).

27.2.2 Primary insect pests of pulses

*27.2.2.1 Bean bruchid (*Acanthoscelides obtectus *Say)*

The bean bruchid (family Bruchidae), *A. obtectus* Say (Figure 27.3a), is a primary pest of *Phaseolus* beans in temperate to subtropical regions worldwide. It sometimes attacks other legumes, although it is seldom a serious pest of these (NRI, 2001). The pest originated in tropical South America but has spread to warm and hot regions of the world, except Australia. The potential for damage to stored grains by this pest is great because the insect infests grains during both pre- and post-harvest periods and several larvae can develop in one seed. Adults are 3.2–4.0 mm long with a conical prothorax and posterior femora bearing a strong notched tooth. They are grey, brown or greyish-brown in colour. The forewings and prothorax surface is covered by yellow or yellowish-grey hairs with darker brown patches. The beetle develops mainly on bean, and more rarely on soya and lentil. Adults hibernate inside the seeds, with each being able to contain several individuals. It starts to move around in the seed in storehouses or in fields once the temperature reaches 11°C, and flies in dry and sunny weather (21°C). The eggs are deposited in clusters of two to 20 on the pods or inside them, or directly on the seeds, and females have an average lifetime fecundity of 40–60 eggs (Haines, 1991). The eggs are usually milky white. After hatching from eggs, the first instar larvae penetrate the seed coat, develop inside the seed and go through four instars before pupation. The first-instar larva has a yellow head and long legs; the second-instar larva is apodous and white with a brownish head (NRI, 2001). Feeding by the last instar produces the first characteristic circular 'windows' in the seed that become visible externally as insect development progresses. The newly formed adults may remain in the cell for several days before pushing out through a window and exiting the seed. Mating occurs immediately, and egg-laying follows. The life cycle is completed in 28 days and adult longevity is about 7–12 days. Adult bruchids do not feed; only the larvae cause damage (NRI, 2001). The minimum development period is about 22.5 days at optimum conditions (30°C, 70% relative humidity).

*27.2.2.2 Cowpea beetle (*Callosobruchus chinensis *L.)*

The cowpea beetle, *C. chinensis* L. (Figure 27.3b), is a beetle in the family Bruchidae and adults are of the same general form as *A. obtectus* but usually smaller. Males have pectinate to strongly pectinate antennae whereas females are subserrate. Each hind femur bears two longitudinal ridges, each carrying a terminal spine. Adults are generally 2.5–3.5 mm in length with typical white markings on the scutellum. The females lay many eggs, up to 115 per adult at the optimum temperature of 30–35°C. Upon hatching the larva bites

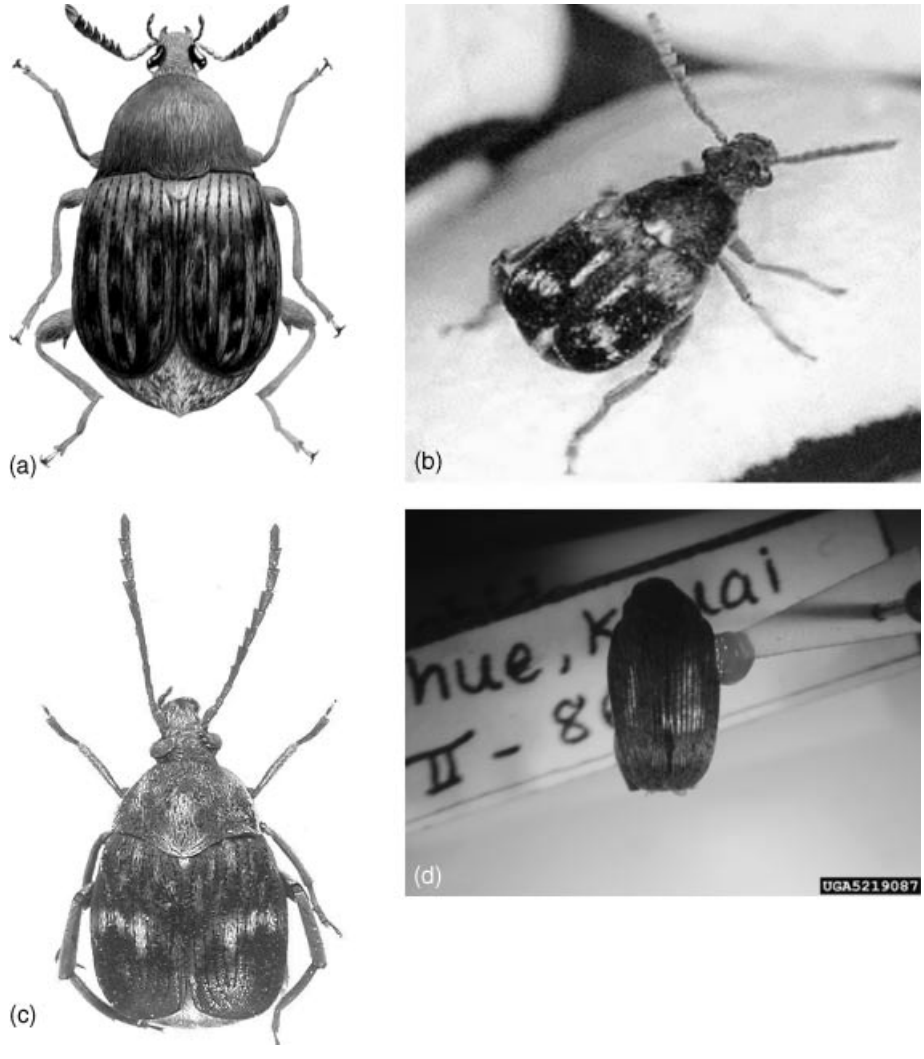

Figure 27.3 Common Coleopteran primary insect pests of stored legume grains. (a) Bean bruchid (*A. obtectus*). Photograph courtesy of the US Department of Agriculture. GIPSA Webmaster. (b) Cowpea beetle (C. *chinensis* L). Forest & Kim Starr, Starr Environmental, Bugwood.org. (c) Mexican bean weevil (*Z. subfasciatus*). Frank Peairs, Colorado State University, Bugwood.org. (d) Groundnut borer (C. *serratus*). Forest & Kim Starr, Starr Environmental, Bugwood.org.

through the testa of the seed and into the cotyledons (Haines, 1991). The larva feeds entirely within a single seed, excavating a chamber within the cotyledons as it grows. Under optimum conditions (32°C and 90% relative humidity) the development (egg to adult) period of *C. chinensis* is 21 days. *C. chinensis* is an important primary pest of pulses (such as cowpeas, pigeon peas, chickpeas, adzuki beans, peas and grams) in tropical and subtropical climates (Haines, 1991; NRI, 2001; Semple *et al.*, 1992). It occasionally attacks soybeans but not kidney beans or butter beans (*Phaseolus* spp.). The other species of cowpea bruchid are *Callosobruchus maculatus* and the Rhodesian bean weevil, *Callosobruchus rhodesianus*.

27.2.2.3 *Mexican bean weevil (*Zabrotes subfasciatus *Boheman)*

The Mexican bean weevil, *Z. subfasciatus* (Figure 27.3c), of the family Bruchidae, is 2–2.5 mm long and has elytra that are rather square and broad with strong white markings on a dark background (NRI, 2001). The life cycle and size are similar to those of *Callosobruchus* but, unlike other bruchids infesting stored food products, *Z. subfasciatus* has two moveable spines on each hind tibia. *Z. subfasciatus* is a primary pest of kidney beans and butter beans, and seldom attacks other legumes. It originated in tropical America, but is now distributed widely in many tropical and subtropical regions, especially Central and East Africa, Madagascar, the Mediterranean and India (NRI, 2001).

27.2.2.4 *Groundnut borer (*Caryedon serratus *Olivier)*

The groundnut borer, *C. serratus* Olivier (Figure 27.3d), of the family Bruchidae, is a large (3.5–6.8 mm-long) reddish-brown beetle with dark irregular markings on the elytra. Each hind femur bears a characteristic conspicuous comb of spines. Adult females glue their eggs to groundnut shells or onto the testa after decortications. The larvae bore into the seeds, but may leave one seed and attack another. Pupation takes place outside the seeds in a paper-like cocoon spun by the larva. It is a major primary pest of groundnuts and tamarind (*Tamarindus indica*) found mainly in parts of Asia, Africa, the West Indies and parts of Central, South and North America (NRI, 2001).

27.2.3 Secondary insect pests of stored cereals and pulses

27.2.3.1 *Rust-red flour beetle (*Tribolium castaneum *L.)*

The rust-red flour beetle (*T. castaneum* L.) is a red-brown tenebrionid beetle (2.5–4.5 mm in length) (Figure 27.4a) that is parallel-sided and partially flattened dorsoventrally. The males possess a hairy puncture on the ventral surface of the anterior femur. Adult females lay sticky eggs loosely among their food throughout their lives. The number of eggs laid depends upon temperature, with averages of 2.5 and 11 eggs each day at 25 and 32.5°C, respectively (Haines, 1991). Development (egg to adult) can be very quick (20–30 days), leading to rapid population growth. It is found throughout all tropical, subtropical and warm temperate areas of the world. It is a secondary pest of a range of commodities especially cereals but also groundnuts, spices, coffee, cocoa, dried fruit and occasionally pulses (NRI, 2001).

27.2.3.2 *Saw-toothed grain beetle (*Oryzaephilus surinamensis *L.)*

The saw-toothed grain beetle, *O. surinamensis* L. (family Silvanidae) (Figure 27.4b) is a moderately small (2.5–3.5 mm long), rather flat, parallel-sided beetle, distinguished by six large tooth-like projections on each side of the prothorax. The two common species of the genus *Oryzaephilus*, *O. surinamensis* and *Oryzaephilus mercator* are similar in appearance but differ biologically. The former develop more quickly and are tolerant to high temperatures and humidity (35°C and 90% relative humidity) and are more successful on starchy cereal diets, whereas the latter prefer diets with high oil content (NRI, 2001). *O. surinamensis* is a secondary pest of cereal, cereal products, oil seeds, spices, nuts and dried fruits in the tropics.

27.2.3.3 *Khapra beetle (*Trogoderma granarium *Everts)*

Adult Khapra beetles (*T. granarium* Everts; Figure 27.4c) are small (1.75–3.5 mm-long) oval beetles, the females of which are larger than the males. The mid-brown or irregularly mottled elytra are slightly closed with yellowish hairs (Semple *et al*., 1992). Larval diapause occurs

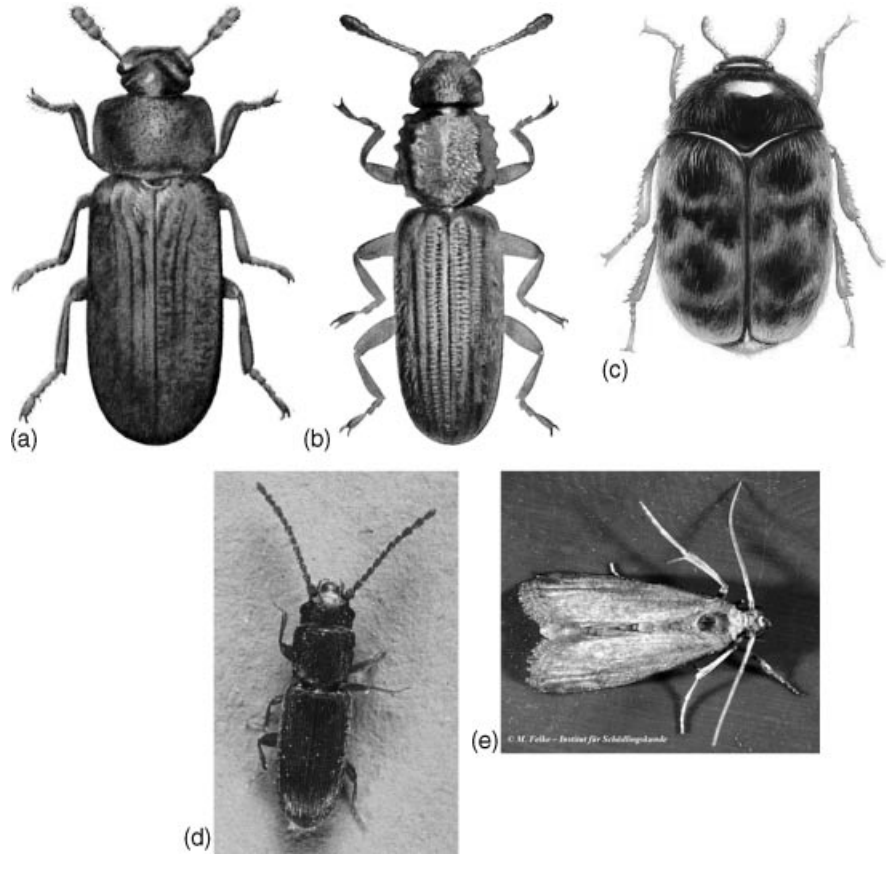

Figure 27.4 Common secondary insect pests of stored food products. (a) Rust-red flour beetle (*T. castaneum*). Photograph courtesy of the US Department of Agriculture. GIPSA Webmaster. (b) Saw-toothed grain beetle (*O. surinamensis*). Photograph courtesy of the US Department of Agriculture. GIPSA Webmaster. (c) Khapra beetle (*T. granarium*). Photograph courtesy of the US Department of Agriculture. GIPSA Webmaster. (d) Flat grain beetle (*Cryptolestes pusillus*). Charles Olsen, USDA APHIS PPQ, Bugwood.org. (e) Warehouse moth (*C. cautella*). Charles Olsen, USDA APHIS PPQ, Bugwood.org.

when physical conditions are unfavourable and they are able to survive several years under such adverse conditions. Under diapause, the pest is difficult to control using residual insecticides. *T. granarium* is widespread in the Indian subcontinent, adjacent areas and many hot dry regions around the world but is never found in humid zones. It is a very serious secondary pest of cereal grains and oil seeds and many countries have specific quarantine regulations against its importation. The densely hairy adults and larval skins are a serious health hazard to storage workers and consumers.

*27.2.3.4 Flat grain beetles (*Cryptolestes *spp.)*

Adults of the flat grain beetle, *Cryptolestes* spp. (family Cucujidae) (Figure 27.4d), are small (2–2.5 mm-long), elongate and very flat, and light-coloured. The head and prothorax are large and account for half of the body length. The small larvae may enter cereal grains at points of minor damage and preferentially attack the cereal grain embryo. *Cryptolestes* spp. prefer food with high moisture content (NRI, 2001). *Cryptolestes* spp., are common in mills and stores as secondary pests of cereals, nuts, oilcakes, dried fruits and other commodities.

27.2.3.5 *Tropical warehouse moth* (Cadra cautella*; formerly* Ephestia cautella*)*

Adult *C. cautella* (Figure 27.4e) are fairly short-lived (7–14 days), and non-feeding with a wing span of 11–28 mm. Newly emerged adults have greyish-brown forewings, which turn dull grey with age due to the loss of scales. The females lay eggs loosely on the surface of the commodity. Upon hatching, larvae move extensively through the commodity as they feed and spin copious quantities of silk, called webbing. The webbing from heavy infestations forms a mat which renders the commodity unfit for human consumption. The final-instar larvae wander around until they pupate in sites such as cracks, crevices and gaps between grain bags (NRI, 2001).

27.3 ADVANCES IN STORED-PRODUCT INSECT PEST CONTROL IN THE TROPICS

27.3.1 Cultural control

Cultural control practices involve manipulating the storage environment in such a way as to render it unfavourable to pests. Although effective cultural control measures combine preventative, sanitation, monitoring and mechanical removal of insect pests (Donahaye, 2000), there have been limited modern research efforts. Prevention consists of routine store inspection, adequate ventilation, suitable storage structure and product type/form and the minimization of insect pest entry. Good sanitation practices entail timely removal of all spilled or old infested produce, treatment of any infested products and clearing of areas surrounding the store to reduce sources of infestation. Although good ventilation minimizes mould problems, protection against insects and rodents needs use of pesticides. Shelled produce can be stored in bags in warehouses or in bulk in silos. Warehouses and silos must be cleaned thoroughly of old infested produce before the new harvest is brought in. Produce in bags should be separately stacked on pallets and stand free of walls and ceilings. Food stores should be swept out weekly and the sweepings burned immediately. The storage structures should be closed off to prevent entry by pests. For example, airtight silos with good thermal insulation offer the best protection by depriving insects and other living organisms of oxygen supply. The lowest oxygen concentration below which insects cannot survive is about 2%. For airtight storage on a small scale, oil drums or plastic bags may be used. In addition, stock rotation, based on a 'first in first out' (FIFO) procedure, is another way of preventing insect infestation. Ventilation is also important in reducing or keeping the grain moisture content low.

27.3.2 Monitoring of pest populations

The monitoring of pest populations forms an important component of stored-product pest-management programmes that leads to earlier detection of infestations. Monitoring information can be used to justify reduction in pesticide use and as an indicator of IPM programme effectiveness. Monitoring traps, such as pheromone lure traps, are used to monitor insect pests in stores.

27.3.3 Grain moisture content control

For long-term storage, food grains must be dried to remove moisture and reduce biodeterioration due to insect infestations, mould and bacterial development. All grains must be kept dry, secure and free from insect infestations before and during storage. In the

tropics, most stored-product insects are unable to survive and reproduce in grains with a moisture content below 9%. Each staple food grain has maximum moisture content for safe storage; for instance, sorghum, wheat, maize and rice (13%), groundnuts (7%) and legumes (15%).

Drying in most farming communities in the tropics is largely weather-dependent and often done on bare ground. This results in contamination from soil and foreign matter, mould infection and discoloration of produce. Hence, artificial drying (sun and air-drying, solar dryers and modern cribs) is often necessary.

27.3.4 Biological control

Although the principle of biological control in field crops is an important management strategy for smallholder farmers in the tropics (van Emden, 1990; Godfrey *et al*., 2005), it is limited by factors such as low plant diversity, elimination and low population of natural enemies, and the inability to catch up with pests (Holst and Meikle, 2003). Biological control can be extended to stored-product insect pests because storage structures resemble glass houses, where it has been successful. Since consumer tolerance for the presence of insect pests in food is zero, a biological control agent is not an acceptable contaminant. In spite of this zero tolerance for insects of any kind, there are successful biological control agents against stored-product pests. Probably the most widely released predator has been the histerid beetle *Teretriosoma nigrescens* against a bostrichiid beetle *P. truncatus* (LGB) (Holst and Meikle, 2003).

27.3.5 Synthetic chemical control

For the protection of stored food grains, synthetic pesticides are often admixed with the produce to provide short- to long-term protection of treated grains. Such pesticides are expected to be of low mammalian toxicity, to be easily biodegradable and to offer an acceptable level of persistence after treatment. Insecticides may also be used for spraying (bagged produce, walls, floors and ceilings of warehouses or storerooms) to prevent infestations, to kill latent infestations or to delay re-infestation of insect-free produce. Besides contact insecticides, fumigation is the best way to disinfect produce, warehouses or storerooms because synthetic fumigants penetrate the grain mass and kill all insects and microorganisms. To avoid re-infestation, use of contact synthetic insecticides or insect-proof silos or containers is recommended. The choice of synthetic pesticides depends upon the insect species present, method of storage, type of stored produce, availability in local market and affordability, shelf life, legal restrictions, toxicity and knowledge of the user, among other basic considerations.

Fumigation, on the other hand, is still one of the most effective methods for the prevention of stored-product losses from insect pests, especially in bulk storage. It plays a major role in insect-pest elimination from stored products. Currently, phosphine and methyl bromide are the two common fumigants used for stored-product protection globally. Insect resistance to phosphine is a global issue now and control failures have been reported in field situations in some countries (Taylor, 1989; Collins *et al*., 2002). Methyl bromide, a broad-spectrum fumigant, has been declared an ozone-depleting substance and therefore is being phased out completely. In view of the problems with the current synthetic fumigants, there is global interest in alternative control strategies including non-chemical options such as controlled atmospheres, integration of physical methods and exploitation of botanical pesticides (MBTOC, 2002).

27.4 ADVANCES IN DEVELOPMENT OF BOTANICAL PESTICIDES IN THE TROPICS

27.4.1 Botanical insecticides

Plant derivatives (ashes, powders and extracts) were commonly used in the tropics for insect control before the advent of synthetic pesticides (Balwa and Shaefers, 1997; Keita *et al.*, 2000). It is important to note that some of the earliest insecticides, like nicotine, pyrethrins and rotenone, were derived from plants. However, the commercial exploitation of pyrethrins through synthetic options has rendered these insecticides largely uneconomic in subsistence agriculture. Although collection and utilization of natural products may be expensive in terms of time and labour, these resources are often more available than hard cash (Bajwa and Shaefers, 1997). Effective control of stored-grain insect pests with minimal use of insecticides require an IPM approach combining sanitation, monitoring, preventive and curative (control) measures. Therefore, there is renewed scientific effort aimed at seeking cost-effective and environment friendly alternative stored-product insect control strategies based on indigenous knowledge and practices of the target users.

Natural products are well known to have a range of useful biological properties against insect pests. However, it is clear from recent history that synthetic insecticides have effectively relegated botanicals from an important role in agriculture to an essentially trivial position in the market for crop protectants.

At present there are four major types of botanical products used for insect control, namely pyrethrin, rotenone, azadirachtin and essential oils and their constituents. The toxic, repellent, antifeeding and reproductive inhibition effects of these products against stored-product insects have already been demonstrated (Su and Scheffrahn, 1990; Dunkel and Sears 1998).

27.4.1.1 Pyrethrins

The insecticidal action of the pyrethrins is characterized by a rapid knock-down effect, particularly in flying insects, and hyperactivity and convulsions in most insects. These symptoms are a result of the neurotoxic action of the pyrethrins which block voltage-gated sodium channels in nerve axons. Pyrethrins are especially labile in the presence of the UV component of sunlight, greatly limiting their use outdoors (Isman, 2005).

27.4.1.2 Azadirachtin

Neem seeds contain azadirachtin and more than a dozen azadirachtin analogues, and seed extracts also have considerable quantities of other triterpenoids, notably salannin, nimbin and derivatives thereof. The role of these other natural substances has been controversial, but most evidence points to azadirachtin as the most important active principle (Isman *et al.*, 1996). Azadirachtin has two profound effects on insects. At the physiological level, azadirachtin blocks the synthesis and release of moulting hormones (ecdysteroids) from the prothoracic gland, leading to incomplete ecdysis in immature insects. In adult female insects a similar mechanism of action leads to sterility. In addition, azadirachtin is a potent antifeedant to many insects. In practice, reliable efficacy is linked to the physiological action of azadirachtin as an insect growth regulator. What is clear is that azadrichtin is considered non-toxic to mammals (rat oral acute LD_{50} is less than 5000 mg/kg), fish and pollinators (Newman, 1996). The

influence of azadirachtin on natural enemies is highly variable (Lowery and Isman, 1995; Spollen and Isman, 1996) and, like the pyrethrins, azadirachtin is rapidly degraded by sunlight.

27.4.1.3 Rotenone

Rotenone is one of several bioflavonoids produced in the roots or rhizomes of the tropical legumes *Derris*, *Lonchocarpus* and *Tephrosia* spp. Rotenone is a mitochondrial poison, which blocks the electron transport chain and prevents energy production (Hollingsworth, 1994) and as an insecticide is considered a stomach poison. Pure rotenone is comparable to DDT and other synthetic insecticides in terms of its acute toxicity to mammals (rat oral LD_{50} is 132 mg/kg) although it is much less toxic at the levels seen in formulated products. The safety of rotenone has recently been called into question in formulated products.

27.4.1.4 Sabadilla

Sabadilla is a botanical insecticide obtained from the seeds of the southern-US lily *Schoenocaulon officinale*. Its pure active principles, cevadine-type alkaloids, are extremely toxic to mammals (rat oral LD_{50} is approximately 13 mg/kg) but commercial preparations typically contain less than 1% active ingredients, providing a margin of safety. The mode of action of these alkaloids is remarkably similar to that of the pyrethrins, despite their lack of structural similarly.

27.4.1.5 Nicotine

Nicotine, like pyrethrum and rotenone, is an alkaloid obtained from the foliage of tobacco plants (*Nicotiana tabacum*) and related species. It has a long history of being used as an insecticide. Nicotine causes poisoning characteristics similar to those seen with organophosphate and carbamate insecticides (Hayes, 1982). Owing to the extreme toxicity of pure nicotine to mammals (rat oral LD_{50} is 50 mg/kg) and its rapid dermal absorption in humans, nicotine has seen declining use, primarily as a fumigant in greenhouses against soft-bodied insect pests. However, there remains some interest in preparing stable nicotine fatty acid soaps, presumably with reduced bioavailability and toxicity to humans (Casanova *et al.*, 2002).

27.4.2 Essential oils

Volatile terpene hydrocarbons and corresponding oxygenated derivatives, known as essential oils or secondary metabolites, have been used for a long time in folk medicine and therapeutics, and in traditional and alternative medicine. The volatile oils of many plants are known to have antimicrobial (Bhattacharjee *et al.*, 2004) and insecticidal (Shaaya and Kostjukovsky, 1998; Huang *et al.*, 2000) properties. Essential oils are naturally occurring terpenic mixtures in plants whose bioactivity against specific pests and pathogenic microorganisms have recently received research attention (Dafetera *et al.*, 2003; Ketoh *et al.*, 2005). Most essential oil constituents are monoterpenoids which are secondary plant metabolites with little metabolic importance. They are believed to be allelopathic agents or irritants that protect plants from predation by insects and infestation by parasites (Huang *et al.*, 2000).

The toxic, repellent, reproduction inhibition and antifeedant effects of a large number of essential oils and their chemical constituents have been evaluated against insect pests of

several stored products. Essential oils obtained from *Elletaria cardmomum* (L.) Maton., *Lavandula hybrida*, *Rosmarinus officinalis*, *Mentha microphylla*, *Mentha viridis* and *Eucalyptus globulus* have shown insecticidal activity against *Sitophilus* spp., *Callosobruchus* spp., *T. castaneum* Herbst, *Lasioderma serricorne* and *Acanthoscelides obtectus* Say (Huang *et al*., 2000; Papachristos and Stamopoulos, 2002a, 2002b). Pungitore *et al.* (2005) reported that five triterpenoids from *Junellia aspera* (Gillies ex. Hook) (Verbenaceae) exhibited feeding deterrence activity and were highly toxic to adults of the rice weevil, *S. oryzae* L. Several studies involving terpenoids, such as linalool, cineole, *p*-cymene, camphor, terpineol, α-pinene and eugenol, showed significant toxicity against insect pests of a number of stored products and inhibited reproduction (Regnault-Roger and Hamroui, 1995; Lee *et al*., 2003; Park *et al*., 2003; Sundufu and Shoushan, 2004; Ogendo *et al.*, 2008).

The intraspecies and interplant variations in essential oil compositions provide the scientific principles for differential bioactivity responses elicited in stored-product insects pests. On the basis of physiological activities on insects, Jacobson (1982) conveniently classified the plant essential oils in relation to effects on insects into groups, namely repellents, feeding deterrence/antifeedants, toxicants, and growth and development inhibitors.

27.4.2.1 Repellents

Repellents from plant origins are considered safe in pest-control operations because they minimize pesticide residues, ensuring safety of people, food, environment and wildlife (Talukder, 2005). Plant extracts, powders and essential oil from different bioactive plants have been reported as repellent against different economically important stored-product insects (Talukder *et al*., 2004; Ogendo, 2008; Ogendo *et al*., 2008; Oyewole *et al*., 2008). The essential oil of *Artemisia annua* was found as repellent against *T. castaneum* and *C. maculatus* (Tripathi *et al*., 2000). Similarly essential oils extracted from aerial parts of *Ocimum americana*, *Lantana camara* and *Tephrosia vogelli* and the monoterpene constituent eugenol have also been found to have concentration-, exposure-time-, species- (plant and insect) and plant-part-dependent instant and residual repellent potency against adults of *T. castaneum*, *R. dominica*, *S. oryzae* and *C. chinensis*. Repellents are desirable chemicals as they offer protection with minimal impact on the ecosystem, driving away insect pests from treated materials by stimulating insect olfactory or other receptors (Ogendo, 2008). Therefore the discovery of a repellent of plant origin to be used to control stored-product insect pests will be a holy grail to plant protection experts.

27.4.2.2 Antifeedants

Antifeedants are of great value in protecting stored commodities from insects. Insects remain on treated food indefinitely and eventually starve to death without feeding (Talukder *et al*., 2004; Pungitore *et al*., 2005). Some naturally occurring antifeedants that have been characterized include glycosides of steroidal alkaloids, aromatic steroids, hydroxylated steroid meliantriol, triterpene hemizectal and others (Isman, 2008). However, on the other hand, not a single crop-protection product based unequivocally on feeding or oviposition deterrence has been commercialized. Two main problems face the use of antifeedants in agriculture: these are interspecific variation in response and behavioural plasticity; insect pests can rapidly habituate to feeding deterrents, rendering them ineffective in a matter of hours (Isman, 2005).

27.4.2.3 Toxicants

There have been various reports from around the world on the toxicity of different plant products to stored-product insects (Lee *et al*., 2003; Talukder *et al.*, 2004; Rozman *et al*., 2007). Research studies on plant essential oils and their constituents as fumigants – compounds in the vapour or gaseous phase acting against target stored-product insects – have been reviewed. Fumigant toxicity tests conducted with essential oils of plants (mainly belonging to Apiaceae, Lamiaceae, Lauraceae and Myrtaceae) and their components (cyanohydrins, monoterpenoids, sulphur compounds, thiocyanates and others) have largely focused on beetle pests such as *T. castaneum*, *R. dominica*, *S. oryzae* and *S. zeamais* but little or no attention has been paid to moths such as *Corcyra cephalonica* and *S. cerealella* (Lee *et al.*, 2003; Rajendran and Sriranjini, 2008). Furthermore, essential oils from *L. camara*, *O. americana* and *T. vogelli* have exhibited contact and fumigant toxicity against *S. zeamais*, *T. castaneum*, *R. dominica*, *S. oryzae* and *C. chinensis*. Insect mortality depended upon rate, formulation, exposure period and plant part used, confirming the existence of moderate to strong intraspecies- and interplant-dependent instant and residual contact toxicity and reproductive inhibitory effects of essential oils (Ogendo, 2008).

27.4.2.4 Growth and development inhibitors

Many researchers have also reported essential oils or extracts mixed with grain to reduce insect oviposition, egg hatchability, post-embryonic development and progeny production (Rajendran and Sriranjini, 2008). Plant extracts showed deleterious effects on the growth and development of insects and reduced larval and pupal adult weight significantly, lengthened the larval and pupal periods and reduced pupal recovery and adult eclosion. The crude extract also retarded growth, development and caused mortality of larvae, cuticle melanization and high mortality in adults. It has also been reported that grains coated with plant extracts completely inhibited the development of insects like *S. oryzae*. Plant derivatives also reduce the survival rates of larvae and pupae, and adult emergence. Development of egg and immature stages inside grain kernels were also inhibited by plant derivatives (Tripathi *et al*., 2000).

However, in spite of the wide recognition that many plants possess insecticidal, repellent, antifeedant and reproductive inhibition properties, only a handful of pest-control products directly obtained from plants are presently in use because of issues surrounding the sustainability of the botanical resource, standardization of chemically complex extracts and regulatory approval (Isman, 2008). However, research on the insecticidal properties of such plants as *L. camara* var. *aculeate*, *T. vogelii* and *Ocimum* species have recently received attention, as demonstrated in the following case studies.

27.4.3 Case studies on control of stored-grain insect pests using essential oils

27.4.3.1 Lantana camara *var.* aculeate

Hiremath *et al*. (1997) reported that Indian *L. camara aculeate* (Verbenaceae) leaves yielded 16–19% methanol extracts which produced less than 40% mortality against *Nilaparvata lugens*. Studies with 0.02–0.04% of essential oils extracted from *L. camara* leaves produced 90–100% mortality against the fourth-instar larvae of three mosquito species (Adebayo *et al*., 1999). The oils at 0.02% were more toxic, causing 100% mortality in 24 h, to *Culex pipiens fatigans* and *Aedes aegypti* than *Anopheles gambiae* (Adebayo *et al*., 1999). Recent local

studies using crude ground powders showed that *L. camara* has strong insecticidal and repellent effects against the maize grain weevil *S. zeamais* (Ogendo *et al.*, 2003b).

27.4.3.2 Tephrosia vogelii

Preliminary field studies in Zambia showed that crude aqueous leaf extracts (10% w/v) of *T. vogelii* produced a promising acaricidal effect comparable to synthetic chemicals by protecting cattle from tick infestation for up to 10 days (Berger, 1994). In the same study aqueous fresh leaf and crushed pod extracts (15% w/v) reduced the infestation of maize stem borers, maize streak virus and termites, possibly through antifeedant effects. Kambewa *et al.* (1997) reported that aqueous extracts of *T. vogelii* caused 100% mortality in adult ticks and fleas after 24 and 12 h, respectively. Similarly, Mathias (1997), using a 16% (w/v) aqueous extract plus soap as a wetting agent, reported significant insecticidal activity against American bollworm in cotton.

In recent years, research attention has been directed towards studying the bioactivity of crude extracts/slurries, powders and essential oils of *T. vogelii* against the major insect pests of stored food grains. Studies conducted in Congo showed that *Tephrosia* leaf powder admixed with groundnut seeds at a ratio of 1:40 caused 98.8% mortality of the groundnut borer *C. serratus* after 13 days (Delobel and Malonga, 1987). Similarly in Kenya, crude powders from a mixture of leaves, succulent stems and pods caused 100% adult insect mortality and 85–93% repellence against the maize grain weevil *S. zeamais* after 21 days and 4 h, respectively (Ogendo *et al.*, 2003b). Boeke *et al.* (2004a, 2004b) reported that powders and slurries obtained from *T. vogelii* showed significant repellent and reproduction inhibition effects against the cowpea beetle *C. maculatus*.

Like many plants in the Leguminosae family, *T. vogelii* contains organic chemicals in sufficient quantity to be economically and commercially exploited as a phytochemical in the pesticide industry (Ogendo, 2008). The toxic principle in this plant is based on the presence of rotenoids known to be mitochondrial chain inhibitors, inhibiting cellular respiration in almost every living organism including insects and mammals. These compounds block the enzymes glutamate- and succino-dehydrogenase and thus the transport of hydrogen ions (Neuwinger, 2004). Although not quantified, the presence of rotenone and other rotenoid compounds, such as tephrosine, has notably been reported in *T. vogelii*, *Tephrosia candida* and other species, and the compounds have insecticidal, piscidal and repellent properties (Delobel and Malonga, 1987; Boeke *et al.*, 2004a, 2004b; Niassy *et al.*, 2005).

27.4.3.3 Ocimum species

Chemical analyses of the essential oils from *O. americanum* have revealed that the yields and the principal compounds, like other species, are dependent upon the chemical race, climate, soil, geographical location (origin), plant part or tissue sampled, maturity, time of harvest and extraction method (Hegarty *et al.*, 2001; Vasconcelos-Silva *et al.*, 2003; Djibo *et al.*, 2004; Katzer, 2004).

Several *Ocimum* species, such as *Ocimum suave*, *Ocimum basilicum* and *Ocimum kilimandscharicum*, have reportedly been used in various forms as pesticides. Their leaves and seeds are rich in essential oils, which are toxic, repellent or have growth-inhibitory effects against many field and storage insect pests. *O. americanum* is among the six most commonly known repellent plants in western Kenya, particularly through direct burning (Seyoum *et al.*, 2003) and that use of potted plants or burning of *O. americanum* (Labiatae) and *L. camara*

(Verbenaceae) repelled an average of 39.7 and 32.4% of the mosquitoes, respectively (Seyoum *et al*., 2003). Field studies have shown that fresh *O. canum* significantly repelled (63.6%) mosquitoes in Guinea Bissau (Pålsson and Jaenson, 1999).

Phytotoxicity studies with various formulations from *Ocimum* species have shown significant potencies against field and storage insect pests. For instance, Umerie *et al*. (1998) reported that leaf extract aerosol and mosquito coil from *O. basilicum* had potencies of 93 and 95%, respectively, against mosquitoes and that the efficacy of formulation was dependent upon duration of fumigation. Chemical analyses of essential oils from *O. suave* identified eugenol as the principal compound with strong repellent effects against mosquitos and inhibitory effects on growth of many fungi (Chogo and Crank, 1981; Garg and Siddiqui, 1992). Similarly, another terpenoid, linalool, found in dried leaves of hoary basil (*Ocimum canum*), was reportedly the source of toxic effects against the bruchid *Zabrotes subfasciatus* and other storage pests (Weaver *et al*., 1991). Locally, extracts from *O. suave* have shown promising results in laboratory bioassays on maize weevils, *S. zeamais*, and the plant holds promise for future botanical pesticide evaluations (Hassanali and Lwande, 1989).

27.5 PROSPECTS OF BOTANICAL PESTICIDES

The increasing incidence of cases of insect pest resistance to synthetic pesticides and toxicity concerns associated with their indiscriminate use have led to the need for cost-effective and biodegradable pesticides with greater selectivity. Therefore the pesticide of the future must be specific, non-toxic to mammals, biodegradable, less prone to pest resistance and relatively inexpensive. Recent studies have shown that essential oils and their constituents from plants in the families Lamiacae, Verbenaceae and Fabaceae, among others, have good bioactivity against a wide range of stored-product insect pests. Efficacy rates are comparable to synthetic fumigants such as methyl bromide and phosphine. Therefore, there is hope in plant-derived pesticides, which have been used by farmers for many years. Furthermore, plant-derived products are more readily available and biodegradable, and they are less toxic to the environment and mammals. In addition to existing scientific knowledge there is need for genetic and agronomic manipulations of promising pesticidal plants and the products thereof to meet set quality standards for natural pesticides.

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28 Preservation of Plant and Animal Foods: An Overview

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Abstract: Generally, human food consists of resources of either plant or animal origin, which cannot be kept long after harvest or slaughter and starts deteriorating rapidly. Thus, it becomes imperative to find various ways of extending the shelf life of these materials/resources. The nature and characteristics of the material, like environment of the food and the interactions between the food and its environment, should be well understood. Traditional methods of food preservation include cold storage, fermentation, salting, drying, curing and smoking. However, the features of these traditional methods are largely centred on non-controllable processes that rely solely on 'chance effects'. Modern food preservation techniques include dehydration, refrigeration, freezing, industrial fermentation, freeze drying, irradiation, evaporation, concentration, thermal processing, use of chemical preservatives, high-pressure technology, plant-derived food preservation technology, modified-atmosphere packaging, use of bacteriolytic enzymes and a combination of two or more preservative methods (the hurdle concept), which lend themselves to controllable processes and allow for predictable final product quality attributes to be attainable. Traditional and modern food preservation techniques applicable to some of the common food raw materials are discussed in this chapter.

Keywords: food preservation; hurdle effect; modern methods; shelf life; traditional methods

28.1 INTRODUCTION: DEFINITION AND PRINCIPLES

Food preservation refers to methods used for keeping food from getting spoilt. Food spoilage is any adverse change that makes food unfit for human consumption and this process can be due to chemical and physical changes; for example, browning and bruising, growth of unwanted pathogenic microorganisms and infestation by insects or other pests. Thus food preservation is important in increasing/enhancing the shelf life of food and ensuring food safety. Extending the shelf life of foods is based on controlling enzymes or chemically active molecules in food, controlling microbial deteriorative processes and avoiding faulty post-harvest handling practices.

28.2 FOOD PRESERVATION METHODS

Ensuring that harvested commodities are alive with sustained chemical and respiration processes and the need to maintain moisture content and quality of produce during storage and to reduce diseases (Young *et al.*, 1998) are very important steps in post-harvest storage.

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Table 28.1 Examples of some selected storage methods for selected food commodities.

Food commodity	Storage method	References
Tomato	4–7°C Ethanol vapour and 5°C	Yanuriati *et al.* (1996); Decastro *et al.* (2006)
Apple	Controlled atmosphere + fungicide	Anonymous (1992); Errampali (2006)
Peanut, maize, spices	γ-Irradiation	Rizk and Botros (2006)
Shredded cabbage	Packaging films	Ibrahim *et al.* (2005)
Fresh sweetcorn (*Zea mays* L. var. *saccharatta*)	Forced-air cooling	Cortbaoui *et al.* (2006)
Cassava	Sealed polyethylene bags 3–30 ± 2°C	Akingbala *et al.* (2005)
Groundnut seeds	Polythylene lining + $CaCl_2$	Tripathy *et al.* (1996)
Pear (*Pyrus pyrifolia*)	Ethanol treatment	Jihuen and Seunghoo (1997)
Strawberries	Modified-atmosphere packaging (1°C/10 days)	Shamaila *et al.* (1992)

Extension of shelf life of foods can be carried out using refrigeration and freezing (www.fao.org), canning, drying and dehydration, film packaging, smoking, chemicals or food additives, forced-air cooling, modified/controlled atmospheric storage, irradiation and high-pressure food processing. Some examples of selected storage methods for various foods are listed in Table 28.1.

However, it must be noted that in order to extend shelf life and maintain quality of fresh fruits and vegetables, temperature management must be considered as an important factor (Kader and Marris, 1978; Van den Broeck *et al.*, 1998). Markarian *et al.* (2006) developed a mathematical model based on heat transfer, water vapour, temperature and other parameters for horticultural storage facilities and the results obtained correlated with those obtained for a potato storage facility.

Exposure of microorganisms to low temperatures reduces their rates of growth and reproduction. This principle is used in refrigeration and freezing. The microbes are not killed. In refrigerators held at 5°C, foods remain unspoilt. In a freezer at −5°C the crystals formed tear and shred microorganisms. This may kill many microbes but some are able to survive, like *Salmonella* spp. and streptococci. For these types of microorganisms rapid thawing and cooking are necessary. Deep freezing at −60°C forms smaller crystals and which reduces the biochemical activity of microbes.

Blanching of fruits and vegetables by scalding with hot water or steam prior to deep freezing inactivates plant enzymes that may produce a change in colour, etc. Brief scalding prior to freezing also reduces the number of microorganisms on the food surface by up to 99% and enhances the colour of green vegetables.

Refrigeration is the cooling of space and/or material below the general environmental temperature. It is applied to food material for the purpose of preservation. Refrigeration is used to extend the useful life of fresh and processed food that is required to be stored or transported from one place to another. Before the advent of modern refrigeration systems, perishable foods were kept in a cool environment such as cellars or buckets immersed in water. Sometimes ice from ice-making machines was used in cities to preserve foods. The advent of mechanical refrigeration systems significantly simplified the application of refrigeration to food preservation. The first patent for mechanical refrigeration was issued

in 1834 in Great Britain to the American inventor Jacob Perkins. Although this requires access to a regular electricity supply it was one of the easiest methods for preserving food.

Freezing was used commercially for the first time in 1842, but large-scale food preservation by freezing began in the late 19th century with the advent of mechanical refrigeration. Freezing preserves food by preventing microorganisms from multiplying. The process does not kill all types of bacteria, however; those that survive reanimate in thawing food and often grow more rapidly than before freezing. Enzymes in the frozen state remain active, although they work at a reduced rate. Vegetables are blanched or heated in preparation for freezing to ensure enzyme inactivity and thus to avoid degradation of flavour. Blanching has also been proposed for fish to kill cold-adapted bacteria on the outer surface of the fish. Various methods are used to freeze meats depending on the type of meat and the cut. Pork is frozen soon after butchering but beef is hung in a cooler for several days to tenderize the meat before freezing.

Frozen foods have the advantage of resembling the fresh product more closely than the same food preserved by other techniques. Frozen foods also undergo some changes as freezing causes water in food to expand and tends to disrupt the cell structure by forming crystals. In quick freezing the ice crystals are smaller, producing less cell damage than if a product is frozen slowly. The quality of the product, however, may depend more on the rapidity with which the food is prepared and stored in the freezer than on the rate at which it is frozen. Some solid foods that are frozen slowly, such as fish, may, upon thawing, show a loss of liquid called drip, while liquid foods that are frozen slowly, such as egg yolk, may become coagulated.

Consumer-size packages of frozen food generally may weigh up to 0.9 kg. In one type of freezer used for tin packaged foods, the packages are transported mechanically on a conveyor belt through an air blast, which produces temperatures as low as -40°C. Another type of freezing technique, used in the freezing of concentrated orange juice, contains a secondary refrigerant, such as calcium chloride brine, as a spray-on bath for cans at temperatures of -29°C. In a widely used freezer called the plate freezer, the packages are put in contact with hollow metal plates containing a refrigerant and are subjected to pressure in order to increase the rate of freezing. This method of preservation is most widely used for a great variety of foods, including bakery goods, soups and precooked complete meals.

28.2.1 Precooling

Precooling, according to Kader (2002), may be carried out using forced air, water, liquid and vacuum. Vacuum cooling is recommended for hydro cooling and liquid icing cannot be used for highly moisture-sensitive containers (Vigneault and Goyette, 2002) or produce (Kader, 2002). The rate of cooling, further storage, shipping conditions and capital and labour costs are determining factors in choosing any precooling method for some plant products (Sargent *et al.*, 1998; Kader, 2002), Thus, Cortbaoui *et al.* (2006) used forced-air precooling to extend the storage life of fresh sweetcorn (*Zea mays* L. var. *saccharata*) for 21 days with resultant general good quality.

28.2.2 Canning

The process of canning is sometimes called sterilization because the heat treatment of the food eliminates all microorganisms that can spoil the food and those that are harmful to humans, including directly pathogenic bacteria and those that produce lethal toxins. Most commercial canning operations are based on the principle that destruction of bacteria

increases 10-fold for each 10°C increase in temperature. Food exposed to high temperatures for only minutes or seconds retains more of its natural flavour. In the Flash 18 process, a continuous system, the food is flash-sterilized in a pressurized chamber to prevent the superheated food from boiling while it is placed in a container and further sterilizing is not required. Pasteurization combined with microfiltration can be used to extend the shelf life of milk. Milk packed in sterile containers and exposed briefly to temperatures higher than those required for pasteurization may be stored unopened for months without refrigeration.

28.2.3 Drying and dehydration

The terms drying and dehydration are applied to the removal of water from food. To the food technologist, drying refers to natural desiccation, such as by spreading fruit on racks in the sun, and dehydration designates drying by artificial means, such as with a blast of hot air. In freeze drying a high vacuum is maintained in a special cabinet containing frozen food until most of the moisture has sublimed. Removal of water offers excellent protection against the most common causes of food spoilage. Microorganisms cannot grow in a water-free environment, enzyme activity is absent and most chemical reactions are greatly retarded. This last characteristic makes dehydration preferable to canning if the product is to be stored at a high temperature. In order to achieve such protection, practically all the water must be removed. The food then must be packaged in a moisture-proof container to prevent it from absorbing water from the air. For this reason a hermetically sealed can is frequently used to store dry foods. Such a can offers the further advantage of being impervious to external destructive agents such as oxygen, light, insects and rodents.

Vegetables, fruits, meat, fish and some other foods, the average moisture content of which may be as high as 80%, may be dried to one-fifth of their original weight and about one-half of their original volume. The disadvantages of this method of preservation include the time and labour involved in rehydrating the food before eating. Furthermore, reconstituting the dried product may be difficult because it absorbs only about two-thirds of its original water content and this process tends to make the texture tough and chewy.

Drying was used in ancient times to preserve many foods. Large quantities of fruits such as figs have been sun-dried from ancient times to the present day. In the case of meat and fish, other preservation methods, such as smoking or salting, which yielded a palatable product, were generally preferred. Dehydration is confined largely to the production of a few dried foods, such as powdered milk, soup, potatoes, eggs, yeast and powdered coffee, which are particularly suited to the dehydration method.

Present-day dehydration techniques include the application of a stream of warm air to vegetables. Protein foods such as meat are of good quality only if freeze-dried. Liquid food is dehydrated usually by spraying it as droplets into a chamber of hot air, or occasionally by pouring it over a drum that is heated internally by steam.

Dehydration of food can be accompanied by chemical treatment. Raj *et al.* (2006) described a three-stage dehydration process following pretreatment with potassium meta-bisulphite used at 2.5 g/kg and obtained onion rings with good quality characteristics within 6 months of storage.

28.2.4 Packaging methods

Hussein *et al.* (2000), Carlin *et al.* (1990) and Barth *et al.* (1993) noted the usefulness of packaging and low-temperature storage of foods combined with high relative humidity in

increasing shelf life by slowing the growth of spoilage organisms, and reducing physico-chemical and biochemical degradation processes as well as maintaining the nutritional and sensory qualities of minimally processed plant foods. According to Brown (1992) the use of modified atmospheres, gas flushing and vacuum packaging changes the atmosphere surrounding the food (e.g. fresh produce) in a such a way that the food's shelf life is extended. Vacuum packaging is normally used to remove air from a package's headspace and to a limited degree from the food itself to eliminate spoilage of the food by oxidation (Hui, 1992).

28.2.5 Antimicrobial-packaging technology

This method encompasses any packaging technique that can be used to control microbial growth in a given food product (Cha and Chinnam, 2004). Antimicrobial-packaging technology can be combined with lactic acid fermentation technology, making use of the hurdle concept to extend the shelf life of foods without refrigeration. Antimicrobial-packaging technology uses natural agents to control foodborne microorganisms and it will continue to be attractive technologically because of the increase in consumer demand for minimally processed and preservative-free products (Cha and Chinnan, 2001). Furthermore, No *et al.* (2007) also noted that the antimicrobial activity and film-forming properties of chitosan, a modified, natural biopolymer derived by deacetylation of chitin, a major component of the shells of crustaceans, make it potential food preservative or coating material of natural origin.

28.2.6 Smoking

Smoking is used for preserving fish, ham and sausage. The smoke is obtained by burning hickory or similar wood under a low draft. During the process of smoking, the preservative action is provided by bactericidal chemicals like formaldehyde and creosote in the smoke and by the dehydration that occurs in the smokehouse.

28.2.7 Chemical preservatives/food additives

Salt, sugar and benzoate are widely used in the food industry. Salt is a bactericidal agent that can be used to preserve fish or pork, either as dry salt or brine, while sugar is a major ingredient of jams and jellies. Sugar acts in much the same way as salt, inhibiting bacterial growth after the product has been heated. To ensure effective preservation, the total sugar content should at least make up to 65% of the weight of the final product. Vinegar (acetic acid) is used as a preservative in pickling relishes and other foods that have been heated.

Sodium benzoate is used in fruit products to protect against yeasts and moulds and its final concentration should not be more than 0.1%. Nwanekezi and Onyeali (2005) used sodium metabisulphite (100 ppm) and sodium benzoate (30 ppm) to extend the shelf life of bottled intermediate-moisture tomato paste; the product was stored at 33–38°C for 40 weeks without loss of quality.

Calcium propionate may be added to baked foods to inhibit moulds. Sulphur dioxide, permitted for use in some countries, can be added to dehydrated foods for colour retention. Preservation of fat- and lipid-containing foods from the development of objectionable colours and flavour and from the formation of decomposition products that can be toxic has been documented (Sims and Fioriti, 1977).

Adegoke *et al.* (1998) have also described the selection and classification of antioxidants for use in the food industry. Falola *et al.* (2008) used crude antioxidant extract from the spice *Aframomum danielli* (family Zingiberaceae) to extend the shelf life of *akara*, a cowpea paste with very poor keeping quality, for about 2 weeks. Some characteristics of *akara* have been described elsewhere (Hung and McWatters, 1990). Adegoke *et al.* (2000) used 200 ppm of antioxidant extract of *A. danielli* to stabilize soybean oil against oxidation for 28 days. Adegoke *et al.* (2004) also used antioxidant extract of *A. danielli* in combination with packaging to control lipid oxidation and fungal infestation of roasted peanut (*Arachis hypogea*) stored at 30°C for 35 days. The antioxidant extracts obtained from *A. danielli* have been reported to be more potent than butylated hydroxytoluene and butylated hydroxyanisole (Adegoke and Gopakrishna, 1998).

28.2.8 Shelf-life extension using additives of plant origin

Non-toxic and relatively cheap plant materials have been used to preserve foods (Pruthi, 1980; Charterjee, 1990; Adegoke and Sagua, 1993). Adegoke and Odesola (1996) used the powder and essential oil of lemon grass (*Cymbopogon citratus*) to preserve maize and cowpea for 10 days at 26±2°C. Furthermore, Adegoke *et al.* (2002) used the powder of *A. danielli* to protect maize (*Z. mays*) and soybeans (*Glycine max*) against mouldiness and insect infestation for 15 months with no loss of nutritive quality.

28.2.9 Food irradiation

Food irradiation can help to reduce high rates of food losses, especially with respect to cereals, root crops and dried foods (International Atomic Agency, 1990). Irradiation delays ripening of fruits and vegetables, inhibits sprouting in bulbs and tubers, disinfects grains, cereal products, fresh dried fruits and vegetables of insects, and destroys bacteria in fresh meats. Irradiation can extend the shelf life of several types of seafood stored at low temperatures (Licciardello and Ronsivaki 1982) and optimal radiation doses of 0.75–2.5 kGy can extend storage life by 2–6 weeks at 0–5°C (Angel *et al.*, 1986; Przybylski *et al.*, 1989). On a commercial basis, peppers are generally irradiated at doses of 5–10 kGy. Hayashi *et al.* (1994) used 5 kGy for the irradiation of black and white peppers stored at 3°C for 6 months.

28.2.10 High-pressure food processing

High-pressure treatment is a preservation method which does not involve high temperatures, and avoids undesirable alterations caused by thermal treatment of food such as vitamin loss, reduced bioavailability of essential amino acids, loss of flavour and modification of taste and colour (Butz *et al.*, 2003). Major advantages of using high pressure are inactivation of microorganisms (Hoover *et al.*, 1989) and quality retention and shelf-life extension (Shigeshisa *et al.*, 1991). High-pressure treatment is used for fruit jams, fruit juices, guacamole, sauces, oysters and packaged cured ham (Butz *et al.*, 2003).

28.2.11 Modified gas atmosphere

Prince (1989) defined modified-atmosphere packaging as 'the initial alteration of the gaseous environment in the immediate vicinity of the product, permitting the packaged product interactions to naturally vary their immediate gaseous environment.' Han *et al.*

(1985) and Kader *et al.* (1989) noted that development of a modified atmosphere within polymeric film can be used for extending the shelf life of fruits and vegetables. Farber (1991) reported that many modified atmospheres contain moderate to high concentrations of carbon dioxide. Proper sanitation and refrigeration can be used together with modified atmosphere to extend the shelf life of fresh red meats by reducing microbial load and retarding enzymic spoilage (Young *et al.*, 1988). Modified-atmosphere packaging which involves the use of gas mixtures including oxygen and carbon dioxide has been found to maintain the desired colour and inhibit undesirable microorganisms in red meat (Hotchkiss and Galloway, 1989).

A controlled atmosphere can be used for apple storage for more than 6 months to preserve fruit quality. However, to reduce the incidence of blue mould in apples that are stored for long periods of time under controlled-atmosphere storage in Canada, Australia and the USA, a pre-storage chemical control has been recommended (Koffman and Penrose, 1987; Eckert and Ogawa, 1988). Thus, after harvesting and before cold and controlled-atmosphere storage, the post-harvest use of thiabendazole (with or without application of antioxidant and diphenylamine, an anti-scalding agent) has been found to be useful for controlling storage rot and superficial scalding of apples (Anonymous, 1992). Selected storage methods that can be used for some food commodities are shown in Table 28.1.

28.3 CONCLUSION

As plant foods are good sources of essential nutrients for the human population, careful handling and storage of fruits and vegetables is of utmost importance because extended storage, high temperatures, low relative humidity, physical damage and chilling injury (Lee and Kader, 2000) can contribute to loss of essential nutrients. Synthetic chemicals are coming under very close scrutiny and consumers are interested in food that has undergone less thermal processing, so it is possible that more attention will now be focused on: (i) plant-derived food preservation technology encompassing use of natural plant products for antimicrobial packaging of plant and animal foods; and (ii) lactic acid fermentation technology. Whichever method is employed for storage of food commodities, the importance of careful handling procedures cannot be overemphasized.

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